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IM. PIASTÓW ŚLĄSKICH WE WROCŁAWIU

Klinika Urologii Małoinwazyjnej i Robotycznej

Uniwersyteckie Centrum Urologii

Badanie ekspresji nowych markerów immunohistochemicznych oraz ocena ich wartości klinicznej i prognostycznej u pacjentów leczonych z powodu raka gruczołu krokowego z obecnością przerzutów w węzłach chłonnych

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ROZPRAWA DOKTORSKA

Cykl publikacji powiązanych tematycznie

PROMOTOR

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Dziękuję.

Spis treści

1. Wykaz publikacji stanowiących rozprawę doktorską

Publikacja 1. **Novel Histopathological Biomarkers in Prostate Cancer: Implications and Perspectives.** Kiełb P, Kowalczyk K, Gurwin A, Nowak Ł, Krajewski W, Sosnowski R, Szydełko T, Małkiewicz B. *Biomedicines*. 2023; 11(6):1552

DOI: 10.3390/biomedicines11061552

Współczynnik wpływu (IF) = 4,7

Punktacja MEiN = 100

Publikacja 2. **Comparative analysis of GOLPH3 expression in lymph node-positive prostate cancer: immunohistochemistry staining patterns and clinical significance.** Kiełb P, Kaczorowski M, Kowalczyk K, Piotrowska A, Nowak Ł, Krajewski W, Gurwin A, Dudek K, Dzięgiel P, Hałoń A, Szydełko T and Małkiewicz B. *Frontiers in Oncology*. 2023; 13:1265788.

DOI: 10.3389/fonc.2023.1265788

Współczynnik wpływu (IF) = 4,7

Punktacja MEiN = 100

Publikacja 3. **Role of IL-17A and IL-17RA in Prostate Cancer with Lymph Nodes Metastasis: Expression Patterns and Clinical Significance.** Kiełb P, Kaczorowski M, Kowalczyk K, Piotrowska A, Nowak Ł, Krajewski W, Chorbińska J, Dudek K, Dzięgiel P, Hałoń A, Szydełko T, Małkiewicz B. *Cancers*. 2023; 15(18):4578.

DOI: 10.3390/cancers15184578

Współczynnik wpływu (IF) = 5,2

Punktacja MEiN = 200

Sumaryczna wartość punktowa IF = 14,6

Sumaryczna wartość punktów MEiN = 400

2. Wykaz stosowanych skrótów

AMACR	Racemaza alpha-methylacyl-CoA
Appl1	Adaptor protein containing a pleckstrin-homology domain, phosphotyrosine binding domain, and leucine zipper motif 1
BCR	Wznowa biochemiczna (ang. *biochemical recurrence*)
BMI	Body mass index
EAU	Europejskie Towarzystwo Urologiczne (ang. *European Association of Urology*)
ECE	Naciek pozatorebkowy (ang. *extracapsular extension*)
GOLPH3	Golgi phosphoprotein 3
IF	Współczynnik wpływu (ang. *Impact Factor*)
IL-17	Interleukina 17
ISUP	Międzynarodowe Towarzystwo Urologii Patologicznej (ang. *International Society of Urological Pathology*)
LIMK-1	LIM domain kinase 1
LVI	Inwazja naczyń chłonnych (ang. *lymphovascular invasion*)
MEiN	Ministerstwo Edukacji i Nauki
MMP9	Metaloproteinaza 9 macierzy (ang. *matrix metalloproteinases 9*)
mTOR	Szlak ssaczego celu rapamycyny (ang. *mammalian target of rapamycin*)
PCa	Rak gruczołu krokowego/prostaty (ang. *prostate cancer*)
PSA	Swoisty antygen sterczowy (ang. *prostate specific antigen*)
PSAD	Gęstość PSA (ang. *PSA density*)
PSA-DT	Czas podwojenia stężenia PSA (ang. *PSA-doubling time*)
PSMA	Swoisty antygen błonowy gruczołu krokowego (ang. *prostate-specific membrane antygen*)
STAT3	signal transducer and activator of transcription proteins 3

3. Streszczenie w języku polskim

Wprowadzenie: Rak gruczołu krokowego (*prostate cancer* – PCa) jest drugim najczęściej rozpoznawanym nowotworem złośliwym wśród mężczyzn na świecie Częstość występowania PCa rośnie wraz z wiekiem, co razem z wydłużającą się spodziewaną długością życia pozwala zakładać że PCa stanowić będzie w przyszłości jeszcze istotniejszy problem, wpływający na zdrowie społeczeństwa. W celu oceny rokowania i prognozowania przebiegu PCa wykorzystywane są różne czynniki prognostyczne. Zaliczamy do nich m.in. klasyfikację histopatologiczną PCa w skali Gleasona, stopień zaawansowania klinicznego wg klasyfikacji TNM, stężenie swoistego antygenu sterczowego (*prostate specific antigen* – PSA), klasyfikację pacjenta do danej grupy ryzyka wystąpienia wznowy biochemicznej po leczeniu radykalnym wg Europejskiego Towarzystwa Urologicznego (*European Association of Urology* – EAU) czy modele statystyczne takie jak nomogram Brigantiego. Obecność przerzutów węzłowych jest bardzo istotnym czynnikiem ryzyka negatywnie wpływającym na przeżycie oraz na ryzyko wznowy po leczeniu pierwotnym pacjentów z PCa. Wykrycie przerzutów w węzłach chłonnych po leczeniu pierwotnym wpływa na dalszy proces terapeutyczny (np. poprzez konieczność leczenia adjuwantowego) oraz wymusza ścisły nadzór nad pacjentem w celu wczesnego wykrycia ewentualnej wznowy. Pomimo ciągłego rozwoju technik diagnostyki obrazowej ich zdolność do wykrywania obecności przerzutów węzłowych PCa jest ciągle zdecydowanie mniejsza niż, aktualnie uważanej za złoty standard, rozszerzonej limfadenektomii miednicznej. Wyżej opisane klasyczne czynniki prognostyczne stanowią istotną pomoc w planowaniu leczenia pacjenta, w tym kwalifikacji do limfadenektomii, jednak ich dokładność określania ryzyka zajęcia węzłów chłonnych jest wciąż ograniczona. Limfadenektomia jest inwazyjną procedurą istotnie wydłużającą zabieg radykalnej prostatektomii, zwiększającą ryzyko powikłań około i pooperacyjnych jednocześnie nie wpływającą korzystnie na poprawę przeżycia pacjentów. Wyniki licznych badań sugerują potencjalne zastosowanie tzw. nowych markerów immunohistochemicznych w celu poprawy diagnostyki, oceny rokowania lub progresji PCa. Ich wykorzystanie w praktyce klinicznej wymaga potwierdzenia ich użyteczności w dalszych badaniach ale wstępne wyniki są obiecujące. Mogą stanowić niezależny parametr oceniany w materiale pobranym podczas biopsji prostaty lub w materiale po zabiegu operacyjnym. Oprócz tego są potencjalnym uzupełnieniem dla opisanych wyżej klasycznych czynników na których aktualnie opiera się prognozowanie przebiegu PCa. Obiecujące wyniki w tym zakresie uzyskano oceniając ekspresję fosfoproteiny 3 aparatu Golgiego (*Golgi phosphoprotein 3* - GOLPH3). Dokładna rola GOLPH3 w patogenezie PCa

pozostaje ciągle nie do końca określona. Dotychczas opisano kilka proponowanych mechanizmów działania GOLPH3 w komórkach PCa takich jak wpływ na proces różnicowania komórek (przez aktywację szlaku mTOR), a przez to stymulowanie transformacji z hormonowrażliwego PCa w opornego na kastrację PCa lub stymulację wydzielania metaloproteinazy 9 macierzy (MMP9) a przez to udział w formowaniu przerzutów PCa. Innym białkiem, uznawanym za potencjalny nowy marker immunohistochemiczny PCa, jest interleukina 17A (IL-17A) oraz jej receptor IL-17RA. Zwiększoną ekspresję IL-17A i IL-17RA stwierdzano u pacjentów z bardziej agresywnym przebiegiem PCa również w warunkach kastracyjnych. Dokładne mechanizmy onkogennego działania IL-17 nie zostały do tej pory jednoznacznie określone. Na podstawie dostępnych danych sugeruje się że IL-17 stymuluje proliferację komórek PCa oraz angiogenezę przy równoczesnym hamowaniu apoptozy komórek nowotworowych. Pomimo obserwowanej korelacji pomiędzy ekspresją GOLPH3, IL-17A i IL-17RA a progresją PCa, w tym procesie formowania przerzutów, do tej pory nie badano ekspresji tych markerów w przerzutowych węzłach chłonnych.

Cel pracy: Usystematyzowanie aktualnej wiedzy na temat potencjalnych nowych biomarkerów histopatologicznych raka gruczołu krokowego oraz przeprowadzenia badania ekspresji wybranych markerów immunohistochemicznych (GOLPH3, IL-17A oraz IL-17RA) u pacjentów leczonych z powodu raka gruczołu krokowego z obecnością przerzutów w węzłach chłonnych a następnie ocena ich wartości klinicznej i prognostycznej.

Materiały i metody: W Publikacji 1 dokonano przeglądu narracyjnego dostępnych danych literaturowych na temat potencjalnych nowych biomarkerów histopatologicznych raka gruczołu krokowego oraz ich możliwego wykorzystania w praktyce klinicznej. Prace zostały wyselekcjonowane na podstawie analizy dwóch baz danych elektronicznych: PubMed i Scopus. W Publikacji 2 oraz Publikacji 3 przeprowadzono ocenę ekspresji wybranych czynników immunohistochemicznych w materiale pooperacyjnym pacjentów poddanych radykalnej prostatektomii z limfadenektomią z powodu raka gruczołu krokowego oraz obecnymi przerzutami w węzłach chłonnych. W tym celu przygotowano mikromacierze tkankowe z materiału histopatologicznego guza pierwotnego oraz przerzutowego węzła chłonnego każdego z pacjentów, a następnie przeprowadzono barwienie immunohistochemiczne preparatów. W kolejnym etapie przeprowadzono ocenę poziomu ekspresji przy użyciu skali immunoreaktywności barwionych tkanek – ostateczny wynik poziomu ekspresji był wynikiem oceny dwóch cech tj. oceny odsetka komórek raka prostaty z obecnym barwieniem oraz intensywność ich barwienia. Następnie przeprowadzono analizę statystyczną uzyskanych

wyników w celu wykrycia ewentualnych korelacji pomiędzy poziomem ekspresji a danymi klinicznymi pacjentów oraz cechami patologicznymi guza pierwotnego i przerzutów węzłowych. Dodatkowo przeprowadzono analizę porównawczą pomiędzy grupą wysokiej i niskiej ekspresji. W Publikacji 2 oceniono ekspresję GOLPH3 w materiale pooperacyjnym 78 pacjentów, a w celu przeprowadzenia barwienia immunohistochemicznego wykorzystano monoklonalne przeciwciało anty-GOLPH3. W Publikacji 3 oceniono ekspresję IL-17A oraz IL-17RA w materiale pooperacyjnym 77 pacjentów, a w celu przeprowadzenia barwienia immunohistochemicznego wykorzystano poliklonalne przeciwciało anty-IL-17A oraz monoklonalne przeciwciało anty-IL-17RA.

Wyniki: W Publikacji 1 szczegółowo opisano możliwe wykorzystanie w procesie diagnostyki, prognozowania i leczenia raka prostaty takich markerów histopatologicznych jak: makrofagi CD169, neuropilina-1, CD15, interleukina 17, kofilina-1, STAT3, LIMK-1, AMACR, PSMA, Appl1, Sortylina, Syndekan-1 oraz p63. Wstępne wyniki prac badawczych badających rolę tych markerów w patogenezie raka prostaty, pozwalają zakładać że w przyszłości mogą stanowić istotną pomoc w procesie diagnostyki oraz prognozowania przebiegu choroby. W Publikacji 2 i Publikacji 3 zaprezentowano wyniki oceny ekspresji GOLPH3, IL-17A oraz IL-17RA. Ekspresję GOLPH3 stwierdzono we wszystkich badanych preparatach zarówno w tkankach z guza pierwotnego gruczołu krokowego jak również w przerzutowych węzłach chłonnych. Poziom ekspresji GOLPH3 był wyższy w przerzutowych węzłach chłonnych niż w gruczole krokowym, jednak nie były to różnice istotne statystycznie ($p=0.056$). Stwierdzono dodatnią korelację pomiędzy poziomem ekspresji GOLPH3 w guzie pierwotnym gruczołu krokowego a przerzutowymi węzłami chłonnymi ($rho=0,294$, $p<0.05$). Zaobserwowano istotną statystycznie dodatnią korelację pomiędzy poziomem ekspresji GOLPH3 w przerzutowych węzłach chłonnych a odsetkiem zajętych węzłów chłonnych ($p=0.036$). Nie stwierdzono istotnej statystycznie korelacji pomiędzy poziomem ekspresji GOLPH3 a takimi zmiennymi jak BMI, grupa ryzyka EAU czy przedoperacyjne stężenie PSA. Po przeprowadzeniu szczegółowej analizy stwierdzono że u pacjentów z wyższym odsetkiem komórek wykazujących ekspresję GOLPH3 częściej stwierdzano inwazję naczyń chłonnych (*lymphovascular invasion* – LVI; $p=0.02$). Dodatnią korelację stwierdzono również pomiędzy intensywnością barwienia w kierunku GOLPH3 w przerzutowym węźle chłonnym a grupą ryzyka EAU. Dodatkowo analizując dane pooperacyjne pacjentów stwierdzono istotną negatywną korelację pomiędzy ekspresją GOLPH3 a efektywnością radykalnej prostatektomii definiowaną jako stężenie PSA < 0.1 ng/ml w pierwszym pomiarze po operacji (zazwyczaj po

6 tygodniach). Ekspresję IL-17A stwierdzono w 98,7% preparatów guza pierwotnego gruczołu krokowego oraz w 100% przerzutowych węzłów chłonnych. Poziom ekspresji IL-17A w gruczole krokowym dodatnio korelowała z BMI pacjentów (p=0.028). W przerzutowych węzłach chłonnych poziom ekspresji IL-17A dodatnio korelował z odsetkiem zajętych węzłów chłonnych (p=0.006) oraz grupą ryzyka EAU (p=0.001). Dodatkowo w grupie pacjentów z wysoką ekspresją IL-17A w przerzutowych węzłach chłonnych częściej stwierdzano przekraczanie torebki gruczołu krokowego przez guz (*extracapsular extension* – ECE; p=0.033). Ekspresję IL-17RA stwierdzono w 90,9% preparatów guza pierwotnego gruczołu krokowego oraz w 93,5% przerzutowych węzłów chłonnych. Ekspresja IL-17RA była istotnie wyższa w guzie pierwotnym gruczołu krokowego niż w przerzutowych węzłach chłonnych (p=0.009). Poziom ekspresji IL-17RA w przerzutowych węzłach chłonnych dodatnio korelował z grupą ryzyka EAU (p=0.045). W grupie pacjentów z wysoką ekspresją IL-17RA w przerzutowym węźle chłonnym istotnie częściej stwierdzano przekraczanie torebki węzła chłonnego przez raka gruczołu krokowego (p=0.009).

Wnioski:

1. Guz pierwotny gruczołu krokowego oraz przerzutowe węzły chłonne wykazują ekspresję GOLPH3, IL-17A i IL-17RA, a poziomy ekspresji pomiędzy tymi miejscami wykazują dodatnią korelację.

2. Wraz ze wzrostem poziomu ekspresji GOLPH3 w zajętych węzłach chłonnych rośnie odsetek zajętych węzłów chłonnych, częściej występuje inwazja naczyń chłonnych przez PCa oraz tym wyższa grupa ryzyka EAU pacjentów.

3. Im wyższy odsetek komórek wykazujących ekspresję GOLPH3 w gruczole krokowym tym niższa efektywność (radykalność) radykalnej prostatektomii, poza tym poziom ekspresji GOLPH3 nie koreluje z innymi cechami patologicznymi oraz wynikami pooperacyjnymi pacjentów.

4. Ekspresja IL-17A w przerzutowych węzłach chłonnych wykazuje dodatnią korelację z negatywnymi czynnikami prognostycznymi PCa takimi jak wyższa grupa ryzyka EAU, częstsze przekraczanie torebki prostaty przez guz czy wyższy odsetek zajętych węzłów chłonnych, natomiast poziom ekspresji w guzie pierwotnym prostaty rośnie wraz z BMI pacjenta.

5. Ekspresja IL-17RA w przerzutowych węzłach chłonnych jest niższa niż w guzie pierwotnym prostaty, natomiast jej poziom dodatnio koreluje z negatywnymi czynnikami prognostycznymi PCa takimi jak wyższa grupa ryzyka EAU oraz częstsze przekraczanie torebki węzła chłonnego przez PCa.

6. Wartość prognostyczna oznaczanej w materiale pooperacyjnym ekspresji GOLPH3, IL-17A i IL-17RA jest ograniczona w porównaniu do klastycznych czynników predykcyjnych raka gruczołu krokowego.

7. Zastosowanie potencjalnych nowych biomarkerów immunohistochemicznych może poprawić proces diagnostyczno-terapeutyczny pacjentów z rakiem gruczołu krokowego, jednak do tej pory jest zbyt mało badań oceniających możliwość ich wykorzystania w praktyce klinicznej.

4. Streszczenie w języku angielskim

Introduction: Prostate cancer (PCa) is the second most frequently diagnosed malignant tumor in men worldwide. The incidence of PCa increases with age, which, together with increasing life expectancy, allows us to assume that PCa will constitute an even more significant problem in the future, affecting society. Various prognostic factors are used to assess prognosis and predict the course of PCa. These include, among others, histopathological grade expressed in Gleason score, clinical stage according to the TNM classification, prostate-specific antigen (PSA) concentration, classification of the patient into a given risk group for biochemical recurrence after radical treatment according to the European Association of Urology (EAU), or statistical models such as the Briganti nomogram. The presence of nodal metastases is an important risk factor that negatively affects survival and the risk of recurrence after primary PCa treatment. The detection of metastases in lymph nodes after primary treatment affects further therapeutic processes (e.g., the need for adjuvant therapy) and requires close supervision of the patient to detect possible recurrence early. Despite the continuous development of diagnostic imaging techniques, their ability to detect the presence of PCa nodal metastases remains much lower than that of extended pelvic lymphadenectomy, which is currently considered the gold standard. The classic prognostic factors described above constitute an important aid in planning the patient's treatment, including qualification for lymphadenectomy, but their accuracy in determining the risk of lymph node involvement is still limited. Lymphadenectomy is an invasive procedure that significantly prolongs the radical prostatectomy procedure, increases the risk of peri- and postoperative complications, and does not improve patient survival. The results of numerous studies suggest the potential use of new immunohistochemical markers to improve diagnosis and assess the prognosis or progression of PCa. Their use in clinical practice requires confirmation of their usefulness in further research; however, the preliminary results are promising. They can be an independent parameter assessed in materials taken during prostate biopsy or in materials after surgery. In addition, they are potential complements to the classical factors described above, on which the prediction of the course of PCa is currently based. Promising results were obtained by assessing Golgi phosphoprotein 3 (GOLPH3) expression. However, the exact role of GOLPH3 in PCa pathogenesis remains unclear. Several proposed mechanisms of action of GOLPH3 in PCa cells have been described, such as influencing the cell differentiation process (by activating the mTOR pathway), thereby stimulating the transformation from hormone-sensitive PCa into castration-resistant PCa, or stimulating the secretion of matrix metalloproteinase 9 (MMP9),

which is involved in the formation of PCa metastases. Another protein considered a potential new immunohistochemical marker for PCa is interleukin 17A (IL-17A) and its receptor IL-17RA. Increased expression of IL-17A and IL-17RA was observed in patients with a more aggressive course of PCa also under castration conditions. The exact mechanisms underlying the oncogenic action of IL-17 have not been clearly determined. Based on the available data, it is suggested that IL-17 stimulates PCa cell proliferation and angiogenesis, while inhibiting tumor cell apoptosis. Despite the observed correlation between the expression of GOLPH3, IL-17A, and IL-17Ra and the progression of PCa, including the process of metastasis formation, the expression of these markers in metastatic lymph nodes has not yet been studied.

Aim: To summarize the current knowledge regarding potential new histopathological biomarkers of prostate cancer. Examine the expression of specific immunohistochemistry markers (GOLPH3, IL-17A, and IL-17RA) in prostate cancer patients with lymph node metastases, and then evaluate their clinical and prognostic relevance.

Materials and methods: Publication 1 presents a narrative review of the available literature on potential new histopathological biomarkers of prostate cancer and their possible use in clinical practice. Studies were selected based on the analysis of two electronic databases: PubMed and Scopus. In Publications 2 and 3, the expression of selected immunohistochemical factors was assessed in the postoperative material of patients who underwent radical prostatectomy with lymphadenectomy due to prostate cancer and the presence of metastases in the lymph nodes. For this purpose, tissue microarrays were prepared from the histopathological material of the primary tumor and metastatic lymph node of each patient, and immunohistochemical staining of the preparations was performed. In the next stage, the expression level was assessed using a scale of immunoreactivity of stained tissues, and the final result of the expression level was the result of the assessment of two features: the percentage of prostate cancer cells with staining and the intensity of their staining. Then, statistical analysis of the obtained results was performed to detect possible correlations between the expression level and the patients' clinical data and pathological features of the primary tumor and nodal metastases. Additionally, a comparative analysis was performed between high- and low-expression groups. In Publication 2, GOLPH3 expression in the postoperative material of 78 patients was assessed, and a monoclonal anti-GOLPH3 antibody was used for immunohistochemical staining. In Publication 3, the expression of IL-17A and IL-17RA was assessed in the postoperative material of 77 patients, and a polyclonal anti-IL-17A antibody and monoclonal anti-IL-17RA antibody were used to perform immunohistochemical staining.

Results: Publication 1 describes in detail the possible use of such histopathological markers in the diagnosis, prognosis, and treatment of prostate cancer: macrophages CD169, neuropilin-1, CD15, interleukin 17, cofilin-1, STAT3, LIMK-1, AMACR, PSMA, Appl1, Sortilin, Syndecan-1, and p63. Preliminary results of research examining the role of these markers in the pathogenesis of prostate cancer allow us to assume that, in the future, they may provide significant assistance in the process of diagnosis and prognosis of the disease course. Publications 2 and 3 present the results of the assessment of GOLPH3, IL-17A, and IL-17RA expression, respectively. GOLPH3 expression was detected in all tested preparations, both in tissues from the primary prostate tumor and metastatic lymph nodes. The expression level of GOLPH3 was higher in the metastatic lymph nodes than in the prostate, but these differences were not statistically significant ($p=0.056$). A positive correlation was found between the level of GOLPH3 expression in primary prostate tumor and metastatic lymph nodes ($rho=0.294$, $p<0.05$). A statistically significant positive correlation was observed between the level of GOLPH3 expression in metastatic lymph nodes and the percentage of involved lymph nodes ($p=0.036$). There was no statistically significant correlation between the level of GOLPH3 expression and variables such as BMI, EAU risk group, or preoperative PSA level. A detailed analysis revealed that patients with a higher percentage of cells expressing GOLPH3 were more likely to have lymphovascular invasion (LVI; $p=0.02$). A positive correlation was also found between the intensity of GOLPH3 staining in the metastatic lymph nodes and the EAU risk group. Additionally, when analyzing patients' postoperative data, a significant negative correlation was found between GOLPH3 expression and the effectiveness of radical prostatectomy, defined as PSA concentration < 0.1 ng/ml in the first measurement after surgery (usually after 6 weeks). IL-17A expression was detected in 98.7% of primary prostate tumor specimens and in 100% of metastatic lymph nodes. The expression level of IL-17A in the prostate positively correlated with patient BMI ($p=0.028$). In metastatic lymph nodes, the level of IL-17A expression was positively correlated with the percentage of involved lymph nodes ($p=0.006$) and the EAU risk group ($p=0.001$). Additionally, in patients with high IL-17A expression in metastatic lymph nodes, tumor extracapsular extension (ECE) in the prostate was more common ($p=0.033$). IL-17RA expression was found in 90.9% of primary prostate tumor specimens and 93.5% of metastatic lymph nodes. IL-17RA expression was significantly higher in primary prostate tumor than in metastatic lymph nodes ($p=0.009$). The expression level of IL-17RA in the metastatic lymph nodes was positively correlated with EAU risk group ($p=0.045$). In the group of patients with high IL-17RA expression in a metastatic lymph node, prostate cancer ECE of the lymph node was significantly more frequent ($p=0.009$).

Conslusions:

1. The primary prostate tumor and metastatic lymph nodes expressed GOLPH3, IL-17A, and IL-17RA, and the expression levels between these sites showed a positive correlation.

2. As the level of GOLPH3 expression in metastatic lymph nodes increases, the total percentage of involved lymph nodes increases, the incidence of lymphatic vessels invasion by PCa increases, and the higher the EAU risk group of patients.

3. The higher the percentage of cells expressing GOLPH3 in the prostate, the lower the effectiveness (radicality) of radical prostatectomy, and the level of GOLPH3 expression does not correlate with other pathological features or postoperative results of patients.

4. The expression of IL-17A in metastatic lymph nodes is positively correlated with negative prognostic factors of PCa, such as a higher EAU risk group, more frequent extracapsular extension of the prostate by the tumor, or a higher total percentage of involved lymph nodes, whereas the expression level in the primary prostate tumor increases with the patient's BMI.

5. The expression of IL-17RA in metastatic lymph nodes is lower than that in the primary prostate tumor, while its level positively correlates with negative prognostic factors of PCa, such as a higher EAU risk group and more frequent extracapsular extension of the lymph node by PCa.

6. The prognostic value of the expression of GOLPH3, IL-17A, and IL-17RA determined in the postoperative material is limited compared with the classical predictors of prostate cancer.

7. The use of potential new immunohistochemical biomarkers may improve diagnostic and therapeutic processes in patients with prostate cancer; however, very few studies have assessed the possibility of their use in clinical practice.

5. Omówienie rozprawy doktorskiej

5.1 Wstęp

Rak gruczołu krokowego (rak prostaty; *prostate cancer* – PCa) jest drugim najczęściej rozpoznawanym nowotworem złośliwym wśród mężczyzn na świecie (1). Według danych Krajowego Rejestru Nowotworów, PCa jest najczęściej rozpoznawanym nowotworem u mężczyzn w Polsce oraz odpowiada za ok. 5700 zgonów rocznie (2). Częstość występowania PCa rośnie wraz z wiekiem, co razem z wydłużającą się spodziewaną długością życia pozwala zakładać że PCa stanowić będzie w przyszłości jeszcze istotniejszy problem, wpływający na zdrowie społeczeństwa (3,4).

Najczęstszym histologicznym typem PCa jest rak gruczołowy prostaty (*adenocarcinoma prostatae)* (5). W celu określenia stopnia złośliwości histologicznej (*grading*) wykorzystuje się skalę Gleasona, której wynik jest jednym z najważniejszych parametrów wpływających na rokowanie pacjenta oraz wybór leczenia (6). Na podstawie skali Gleasona każdy przypadek PCa jest klasyfikowany do jednej z 5 grup wg Międzynarodowego Towarzystwa Urologii Patologicznej (*International Society of Urological Pathology grade group* – ISUP GG). Im wyższa grupa wg ISUP tym wyższy stopień złośliwości histologicznej PCa (7). Innym ważnym czynnikiem jest stopień zaawansowania klinicznego (*staging*), do opisania którego używana jest klasyfikacja TNM. Określenie stopnia zaawansowania miejscowego oraz obecność lub braku przerzutów (do węzłów chłonnych oraz innych narządów) jest kluczowe dla wyboru postępowania terapeutycznego. Istotną rolę w diagnostyce oraz planowaniu leczenia PCa ma stężenie swoistego antygenu sterczowego (*prostate specific antigen* – PSA), oraz inne wynikające z niego zmienne takie jak czas podwojenia stężenia PSA (*PSA-doubling time*; PSA-DT) czy gęstość PSA (*PSA-density; PSAD*). W celu oszacowania ryzyka progresji PCa wykorzystywane są różne klasyfikacje oraz nomogramy. Jedną z podstawowych, wykorzystywanych w praktyce klinicznej, jest klasyfikacja Europejskiego Towarzystwa Urologicznego (*European Association of Urology* – EAU), uwzględniająca takie zmienne jak stopień zaawansowania klinicznego, wynik w skali Gleasona oraz stężenie PSA (8). Na podstawie tych danych klinicznych pacjent jest klasyfikowany do różnych grup ryzyka wystąpienia wznowy biochemicznej (*biochemical recurrence* – BCR) po leczeniu radykalnym tj. radykalnej prostatektomii lub radioterapii. BCR najczęściej definiowana jest jako co najmniej dwukrotne stwierdzenie stężenia PSA równego lub większego niż 0.2 ng/ml po prostatektomii radykalnej lub wzrostu stężenia PSA o co najmniej 2 ng/ml w stosunku do najniższego stężenia uzyskanego po radioterapii radykalnej . Wystąpienie BCR jest

negatywnym czynnikiem rokowniczym sugerującym wystąpienie wznowy miejscowej lub wystąpienia przerzutów PCa (9) – jednak nie u każdego pacjenta ryzyko jest takie samo. W aktualnych wytycznych EAU zaleca się stratyfikację pacjentów z BCR po radykalnej prostatektomii na dwie grupy ryzyka progresji PCa – ryzyko niskie (PSA-DT >1 rok i ISUP GG <4) oraz ryzyko wysokie (PSA-DT <1 rok lub ISUP GG 4-5). Podział wg grupy ryzyka umożliwia lepszą kwalifikację pacjentów do leczenia uzupełniającego (związanego również ryzykiem wystąpienia powikłań) oraz prognozowania dalszego przebiegu choroby (9).

Dodatkowymi narzędziami służącymi do określenia ryzyka występowania przerzutów do węzłów chłonnych są różnego rodzaju modele statystyczne bazujące głównie na wyżej wymienionych danych kliniczno-patologicznych – najpopularniejsze z nich to nomogram Brigantiego czy tabele Partina. Narzędzia te stanowią istotną pomoc w planowaniu leczenia pacjenta, jednak ich dokładność określania ryzyka zajęcia węzłów chłonnych jest wciąż niewystarczająca (10–12).

Powyżej opisane dane kliniczne oraz klasyfikacje można zaliczyć do tzw. klasycznych czynników prognostycznych PCa, tj. takich które są wykorzystywanych w praktyce klinicznej od wielu lat, ich skuteczność w prognozowaniu przebiegu PCa była wielokrotnie potwierdzona w dużych badaniach klinicznych oraz ich stosowanie jest rekomendowane przez wytyczne towarzystw urologicznych (8).

Obecność przerzutów węzłowych jest bardzo istotnym czynnikiem ryzyka negatywnie wpływającym na przeżycie oraz na ryzyko wznowy po leczeniu pierwotnym pacjentów z PCa (13,14). Wykrycie przerzutów w węzłach chłonnych po leczeniu pierwotnym wpływa na dalszy proces terapeutyczny (np. poprzez konieczność leczenia adjuwantowego) oraz wymusza ścisły nadzór nad pacjentem w celu wczesnego wykrycia ewentualnej wznowy. Pomimo ciągłego rozwoju technik diagnostyki obrazowej ich zdolność do wykrywania obecności przerzutów węzłowych PCa jest ciągle zdecydowanie mniejsza niż, aktualnie uważanej za złoty standard, rozszerzonej limfadenektomii miednicznej (15). U pacjentów u których ryzyko zajęcia węzłów chłonnych przed leczeniem pierwotnym jest ocenione jako średnie lub wysokie (na podstawie klasyfikacji EAU), zalecane jest wykonanie rozszerzonej limfadenektomii miednicznej podczas zabiegu radykalnej prostatektomii (8). W tym miejscu należy podkreślić że limfadenektomia jest inwazyjną procedurą stanowiącą dodatkowy element do wyjściowo bardzo skomplikowanego i trudnego technicznie zabiegu jakim jest radykalna prostatektomia. Dodatkowo, aktualnie dostępne dane wskazują na brak korzystnego wpływu limfadenektomii na przeżycie pacjentów (16,17) równocześnie istotnie zwiększając ryzyko powikłań zabiegu

takich jak np. zwiększona utrata krwi, dłuższy czas zabiegu i hospitalizacji czy zwiększone ryzyko rozwoju torbieli chłonnych w okresie pooperacyjnym (15).

Pomimo ciągłego rozwoju w zakresie leczenia PCa, wybór najlepszej metody dla danego pacjenta ciągle pozostaje wyzwaniem, a ostateczny rezultat leczenia jest niepewny. Jako przyczynę tego postuluje się zbyt małą precyzję aktualnie wykorzystywanych metod służącym prognozowaniu przeżycia, wystąpienia przerzutów czy ryzyka progresji po pierwotnym leczeniu pacjentów z PCa. Obserwowane różnice w przebiegu choroby oraz odpowiedzi na leczenie pomiędzy pacjentami mogą wynikać również z heterogeniczności PCa na poziomie komórkowym, co może wyrażać się poprzez różnice w poziomie ekspresji różnych białek. Po skorelowaniu danych klinicznych pacjentów z określonym poziomem ich ekspresji, białka te mogą być traktowane jako potencjalne markery służące do oceny agresywności przebiegu czy ryzyka wznowy PCa.

Wyniki licznych badań sugerują potencjalne zastosowanie tzw. nowych markerów immunohistochemicznych w celu poprawy diagnostyki, oceny rokowania lub progresji PCa. Ich wykorzystanie w praktyce klinicznej wymaga potwierdzenia ich użyteczności w dalszych badaniach ale wstępne wyniki są obiecujące. Mogą stanowić niezależny parametr oceniany w materiale pobranym podczas biopsji prostaty lub w materiale po zabiegu operacyjnym. Oprócz tego są potencjalnym uzupełnieniem dla opisanych wyżej klasycznych czynników na których aktualnie opiera się prognozowanie przebiegu PCa. Dodatkowo, w przyszłości, badania nad ekspresją różnych czynników immunohistochemicznych w komórkach PCa mogą stanowić ważny element w rozwoju nowych metod leczenia (18–20). Należy jednak podkreślić, że aktualne wytyczne towarzystw urologicznych dotyczące postępowania w PCa nie zalecają ich rutynowej oceny ze względu na brak dostatecznych dowodów na zastosowanie w praktyce klinicznej.

Dostępne wyniki badań sugerują możliwość wykorzystania licznych cząsteczek jako potencjalnych nowych markerów immunohistochemicznych PCa. Obiecujące wyniki w tym zakresie uzyskano oceniając ekspresję fosfoproteiny 3 aparatu Golgiego (*Golgi phosphoprotein 3* - GOLPH3). GOLPH3 odpowiada m.in. za utrzymanie siateczkowatej struktury aparatu Golgiego oraz bierze udział w wewnątrzkomórkowym transporcie pęcherzykowym (21,22). Dokładna rola GOLPH3 w patogenezie PCa pozostaje ciągle nie do końca określona. Dotychczas opisano kilka proponowanych mechanizmów działania GOLPH3 w komórkach PCa. Jednym z nich jest aktywacja szlaku ssaczego celu rapamycyny (*mammalian target of rapamycin* – mTOR), mogąca wpływać na proces różnicowania komórek, a przez to

stymulować transformację z hormono-wrażliwego PCa w opornego na kastrację PCa, co istotnie pogarsza rokowanie pacjentów (23,24). Innym sugerowanym mechanizmem jest stymulujący efekt GOLPH3 na sekrecję metaloproteinazy 9 macierzy (*matrix metalloproteinase 9* – MMP9) a przez to udział w formowaniu przerzutów PCa (25). Oprócz PCa związek pomiędzy zwiększoną ekspresją GOLPH3 a agresywnością nowotworu złośliwego zaobserwowano również między innymi u pacjentów z czerniakiem, gruczolakorakiem jelita grubego czy glejakiem mózgu (24,26,27). Inne proponowane mechanizmy onkogennego działania GOLPH3 obejmują m.in. wzmożenie transportu z aparatu Golgiego do błony komórkowej, destabilizację struktury genomu czy zaburzenie regulacji endocytozy (28,29). Pomimo obserwowanej korelacji pomiędzy ekspresją GOLPH3 a progresją PCa, do tej pory nie badano ekspresji tego markera w przerzutowych węzłach chłonnych.

Innym białkiem, uznawanym za potencjalny nowy marker immunohistochemiczny PCa, jest interleukina 17 (IL-17) oraz jej receptory. IL-17 stanowi rodzinę cytokin składającą się z sześciu ligandów (nazwane kolejno od IL-17A do IL-17F) oraz pięciu receptorów (oznaczonych kolejno od IL-17RA do IL-17RE) wydzielanych głównie przez limfocyty T pomocnicze i inne komórki układu odpornościowego (30,31). Liczne badania wykazały zwiększoną ekspresję ligandu IL-17A i receptora IL-17RA u pacjentów u których obserwowano bardziej agresywny przebieg PCa. Stymulujący efekt IL-17 na progresję i powstawanie przerzutów PCa obserwowano również w warunkach kastracyjnych (32–34). Dokładne mechanizmy onkogennego działania IL-17 nie zostały do tej pory jednoznacznie określone, a dostępne wyniki badań bywają sprzeczne. Na podstawie dostępnych danych sugeruje się że IL-17 stymuluje proliferację komórek PCa oraz angiogenezę przy równoczesnym hamowaniu apoptozy komórek nowotworowych. Dodatkowo badania wskazują również na potencjalny udział IL-17 w tworzeniu mikro środowiska sprzyjającemu rozwojowi przerzutów nowotworów złośliwych (31). Zwiększoną ekspresję IL-17 obserwowano również w przebiegu bardziej agresywnych form raka piersi, trzustki czy jelita grubego (18). Podobnie jak w przypadku GOLPH3, do tej pory nie badano ekspresji IL-17A oraz IL-17RA w węzłach chłonnych z obecnymi przerzutami PCa.

5.2 Cel badań

Celem badań Rozprawy Doktorskiej była analiza ekspresji wybranych markerów immunohistochemicznych oraz ocena ich wartości klinicznej i prognostycznej u pacjentów leczonych z powodu raka gruczołu krokowego z obecnością przerzutów w węzłach chłonnych.

Cele szczegółowe:

1. Usystematyzowanie aktualnej wiedzy na temat potencjalnych nowych biomarkerów histopatologicznych raka gruczołu krokowego oraz analiza dostępnych wyników badań nad ich zastosowaniem w praktyce klinicznej.
2. Ocena ekspresji GOLPH3 w materiale tkankowym raka gruczołu krokowego pacjentów ze stwierdzonymi przerzutami do węzłów chłonnych po radykalnej prostatektomii z limfadenektomią.
3. Analiza korelacji poziomu ekspresji z danymi klinicznymi pacjentów w celu oceny wartości klinicznej GOLPH3 jako markera prognostycznego raka gruczołu krokowego.
4. Ocena ekspresji IL-17A oraz IL-17RA w materiale tkankowym raka gruczołu krokowego pacjentów ze stwierdzonymi przerzutami do węzłów chłonnych po radykalnej prostatektomii z limfadenektomią.
5. Analiza korelacji poziomu ekspresji z danymi klinicznymi pacjentów w celu oceny wartości klinicznej IL-17A i IL-17RA jako markerów prognostycznych raka gruczołu krokowego.

5.3 Materiały i metody

W niniejszym podrozdziale zaprezentowano zarys metodologii przeprowadzonych badań. Szczegółowy opis zawarty jest w załączonych publikacjach.

Publikacja 1. **Novel Histopathological Biomarkers in Prostate Cancer: Implications and Perspectives.** Kiełb P, Kowalczyk K, Gurwin A, Nowak Ł, Krajewski W, Sosnowski R, Szydełko T, Małkiewicz B. *Biomedicines*. 2023; 11(6):1552

W Publikacji 1 dokonano przeglądu narracyjnego dostępnych danych literaturowych na temat potencjalnych nowych biomarkerów histopatologicznych raka gruczołu krokowego oraz ich możliwego wykorzystania w praktyce klinicznej. Prace zostały wyselekcjonowane na podstawie analizy dwóch baz danych elektronicznych: PubMed i Scopus. Uwzględniono jedynie artykuły anglojęzyczne opublikowane do grudnia 2022 roku. Ostatecznie wybrano 211 artykułów na podstawie których przygotowano publikację.

Publikacja 2. **Comparative analysis of GOLPH3 expression in lymph node-positive prostate cancer: immunohistochemistry staining patterns and clinical significance.** Kiełb P, Kaczorowski M, Kowalczyk K, Piotrowska A, Nowak Ł, Krajewski W, Gurwin A, Dudek K, Dzięgiel P, Hałoń A, Szydełko T and Małkiewicz B. *Frontiers in Oncology*. 2023; 13:1265788.

W Publikacji 2 oceniono ekspresję GOLPH3 w materiale pooperacyjnym 78 pacjentów poddanych radykalnej prostatektomii z limfadenektomią z powodu raka gruczołu krokowego oraz obecnymi przerzutami w węzłach chłonnych. W tym celu przygotowano mikromacierze tkankowe z materiału histopatologicznego guza pierwotnego oraz przerzutowego węzła chłonnego każdego z pacjentów, a następnie przeprowadzono barwienie immunohistochemiczne preparatów wykorzystując monoklonalne przeciwciało anty-GOLPH3. W kolejnym etapie przeprowadzono ocenę poziomu ekspresji GOLPH3. W tym celu wykorzystano skalę oceny immunoreaktywności barwionych tkanek – ostateczny wynik poziomu ekspresji był wynikiem oceny dwóch cech tj. oceny odsetka komórek raka prostaty z obecnym barwieniem oraz intensywność ich barwienia. Następnie przeprowadzono analizę statystyczną uzyskanych wyników w celu wykrycia ewentualnych korelacji pomiędzy poziomem ekspresji GOLPH3 a danymi klinicznymi pacjentów oraz cechami patologicznymi guza pierwotnego i przerzutów węzłowych. Dodatkowo przeprowadzono analizę porównawczą pomiędzy grupą wysokiej i niskiej ekspresji GOLPH3. Uzyskane wyniki zaprezentowano w postaci tabel, wykresów oraz rycin.

Publikacja 3. **Role of IL-17A and IL-17RA in Prostate Cancer with Lymph Nodes Metastasis: Expression Patterns and Clinical Significance.**
Kiełb P, Kaczorowski M, Kowalczyk K, Piotrowska A, Nowak Ł, Krajewski W, Chorbińska J, Dudek K, Dzięgiel P, Hałoń A, Szydełko T, Małkiewicz B. *Cancers*. 2023; 15(18):4578.

W Publikacji 3 oceniono ekspresję IL-17A oraz IL-17RA w materiale pooperacyjnym 77 pacjentów poddanych radykalnej prostatektomii z limfadenektomią z powodu raka gruczołu krokowego oraz obecnymi przerzutami w węzłach chłonnych. W tym celu przygotowano mikromacierze tkankowe z materiału histopatologicznego guza pierwotnego oraz przerzutowego węzła chłonnego każdego z pacjentów, a następnie przeprowadzono barwienie immunohistochemiczne preparatów wykorzystując poliklonalne przeciwciało anty-IL-17A oraz monoklonalne przeciwciało anty-IL-17RA. W kolejnym etapie przeprowadzono ocenę poziomu ekspresji IL-17A i IL-17RA. W tym celu wykorzystano skalę oceny immunoreaktywności barwionych tkanek – ostateczny wynik poziomu ekspresji był wynikiem oceny dwóch cech tj. oceny odsetka komórek raka prostaty z obecnym barwieniem oraz intensywność ich barwienia. Następnie przeprowadzono analizę statystyczną uzyskanych wyników w celu wykrycia ewentualnych korelacji pomiędzy poziomem ekspresji IL-17A i IL-17RA a danymi klinicznymi pacjentów oraz cechami patologicznymi guza pierwotnego i przerzutów węzłowych. Dodatkowo przeprowadzono analizę porównawczą pomiędzy grupą

wysokiej i niskiej ekspresji IL-17A oraz IL-17RA. Uzyskane wyniki zaprezentowano w postaci tabel, wykresów oraz rycin.

5.4 Podsumowanie wyników

Publikacja 1. **Novel Histopathological Biomarkers in Prostate Cancer: Implications and Perspectives.** Kiełb P, Kowalczyk K, Gurwin A, Nowak Ł, Krajewski W, Sosnowski R, Szydełko T, Małkiewicz B. *Biomedicines*. 2023; 11(6):1552

W Publikacji 1 został szczegółowo opisany aktualny stan wiedzy na temat potencjalnych nowych markerów histopatologicznych raka prostaty. Dokonano narracyjnego przeglądu dostępnej literatury a następnie szczegółowo opisano możliwe wykorzystanie w procesie diagnostyki, prognozowania i leczenia raka prostaty takich markerów histopatologicznych jak: makrofagi CD169, neuropilina-1, CD15, interleukina 17, kofilina-1, STAT3, LIMK-1, AMACR, PSMA, Appl1, Sortylina, Syndekan-1 oraz p63. Większość prac nad wykorzystaniem wyżej wymienionych białek jako markerów raka prostaty jest na wstępnym etapie a dostępne wyniki badań aktualnie nie są wystarczające by jednoznacznie ocenić ich wartość w zastosowaniu klinicznym. Niemniej jednak, wstępne wyniki prac badawczych nad rolą tych markerów w patogenezie raka prostaty, pozwalają zakładać że w przyszłości mogą stanowić istotną pomoc w procesie diagnostyki oraz prognozowania przebiegu choroby.

Publikacja 2. **Comparative analysis of GOLPH3 expression in lymph node-positive prostate cancer: immunohistochemistry staining patterns and clinical significance.** Kiełb P, Kaczorowski M, Kowalczyk K, Piotrowska A, Nowak Ł, Krajewski W, Gurwin A, Dudek K, Dzięgiel P, Hałoń A, Szydełko T and Małkiewicz B. *Frontiers in Oncology*. 2023; 13:1265788.

Publikacja 2 jest pracą badawczą w której oceniono ekspresję GOLPH3 w materiale pooperacyjnym 78 pacjentów z rakiem gruczołu krokowego po radykalnej prostatektomii z limfadenektomią oraz ze stwierdzonymi przerzutami do węzłów chłonnych. Badania przeprowadzono na materiale z guza pierwotnego gruczołu krokowego oraz, po raz pierwszy, w przerzutowych węzłach chłonnych.

Ekspresję GOLPH3 stwierdzono we wszystkich badanych preparatach zarówno w tkankach z guza pierwotnego gruczołu krokowego jak również w przerzutowych węzłach chłonnych. Poziom ekspresji GOLPH3 był wyższy w przerzutowych węzłach chłonnych niż w gruczole krokowym, jednak nie były to różnice istotne statystycznie ($p=0.056$). Stwierdzono dodatnią korelację pomiędzy poziomem ekspresji GOLPH3 w guzie pierwotnym gruczołu krokowego

a przerzutowymi węzłami chłonnymi (rho=0,294, p<0.05). Zaobserwowano istotną statystycznie dodatnią korelację pomiędzy poziomem ekspresji GOLPH3 w przerzutowych węzłach chłonnych a odsetkiem zajętych węzłów chłonnych (p=0.036). Następnie przeprowadzono analizę korelacji pomiędzy poziomem ekspresji GOLPH3 a danymi klinicznymi pacjentów. Nie stwierdzono istotnej statystycznie korelacji pomiędzy poziomem ekspresji GOLPH3 a takimi zmiennymi jak BMI, grupa ryzyka EAU czy przedoperacyjne stężenie PSA. Po przeprowadzeniu szczegółowej analizy stwierdzono że u pacjentów z wyższym odsetkiem komórek wykazujących ekspresję GOLPH3 częściej stwierdzano inwazję naczyń chłonnych (*lymphovascular invasion* – LVI; p=0.02). Dodatnią korelację stwierdzono również pomiędzy intensywnością barwienia w kierunku GOLPH3 w przerzutowym węźle chłonnym a grupą ryzyka EAU. Dodatkowo analizując dane pooperacyjne pacjentów stwierdzono istotną negatywną korelację pomiędzy ekspresją GOLPH3 a efektywnością radykalnej prostatektomii definiowaną jako stężenie PSA < 0.1 ng/ml w pierwszym pomiarze po operacji (zazwyczaj po 6 tygodniach).

Publikacja 3. **Role of IL-17A and IL-17RA in Prostate Cancer with Lymph Nodes Metastasis: Expression Patterns and Clinical Significance.**
Kiełb P, Kaczorowski M, Kowalczyk K, Piotrowska A, Nowak Ł, Krajewski W, Chorbińska J, Dudek K, Dzięgiel P, Hałoń A, Szydełko T, Małkiewicz B. *Cancers*. 2023; 15(18):4578.

Publikacja 3 jest pracą badawczą w której oceniono ekspresję IL-17A oraz IL-17RA w materiale pooperacyjnym 77 pacjentów z rakiem gruczołu krokowego po radykalnej prostatektomii z limfadenektomią oraz ze stwierdzonymi przerzutami do węzłów chłonnych. Badania przeprowadzono na materiale z guza pierwotnego gruczołu krokowego oraz, po raz pierwszy, w przerzutowych węzłach chłonnych.

Ekspresję IL-17A stwierdzono w 98,7% preparatów guza pierwotnego gruczołu krokowego oraz w 100% przerzutowych węzłów chłonnych. Poziom ekspresji IL-17A w gruczole krokowym dodatnio korelowała z BMI pacjentów (p=0.028). W przerzutowych węzłach chłonnych poziom ekspresji IL-17A dodatnio korelował z odsetkiem zajętych węzłów chłonnych (p=0.006) oraz grupą ryzyka EAU (p=0.001). Dodatkowo w grupie pacjentów z wysoką ekspresją IL-17A w przerzutowych węzłach chłonnych częściej stwierdzano przekraczanie torebki gruczołu krokowego przez guz (*extracapsular extension* – ECE; p=0.033).

Ekspresję IL-17RA stwierdzono w 90,9% preparatów guza pierwotnego gruczołu krokowego oraz w 93,5% przerzutowych węzłów chłonnych. Ekspresja IL-17RA była istotnie wyższa w guzie pierwotnym gruczołu krokowego niż w przerzutowych węzłach chłonnych (p=0.009). Poziom ekspresji IL-17RA w przerzutowych węzłach chłonnych dodatnio korelował z grupą ryzyka EAU (p=0.045). W grupie pacjentów z wysoką ekspresją IL-17RA w przerzutowym węźle chłonnym istotnie częściej stwierdzano przekraczanie torebki węzła chłonnego przez raka gruczołu krokowego (p=0.009).

5.5 Wnioski

1. Ekspresję GOLPH3 stwierdzono w guzie pierwotnym gruczołu krokowego oraz w przerzutowych węzłach chłonnych. Przerzuty węzłowe wykazują wyższą ekspresję GOLPH3 niż guz pierwotny gruczołu krokowego a pomiędzy stwierdzonymi poziomami ekspresji występuje dodatnia korelacja.

2. Wraz ze wzrostem poziomu ekspresji GOLPH3 w przerzutowych węzłach chłonnych rośnie odsetek zajętych węzłów chłonnych. Im wyższy odsetek komórek wykazuje ekspresję GOLPH3 tym częściej występuje inwazja naczyń chłonnych (LVI) przez raka gruczołu krokowego. Intensywność barwienia GOLPH3 w przerzutowym węźle chłonnym dodatnio koreluje z grupą ryzyka EAU pacjenta.

3. Im wyższy odsetek komórek wykazujących ekspresję GOLPH3 w gruczole krokowym oraz im intensywniejsze barwienie komórek w przerzutowych węzłach chłonnych tym niższa efektywność (radykalność) radykalnej prostatektomii. Poziom ekspresji GOLPH3 nie koreluje z innymi cechami patologicznymi oraz wynikami pooperacyjnymi pacjentów.

4. Ekspresję IL-17A oraz IL-17RA wykryto w guzie pierwotnym gruczołu krokowego oraz w przerzutowych węzłach chłonnych, a pomiędzy poziomami ekspresji występuje dodatnia korelacja.

5. Poziom ekspresji IL-17A w guzie pierwotnym gruczołu krokowym dodatnio koreluje z BMI pacjentów. Im wyższy poziom ekspresji IL-17A w przerzutowym węźle chłonnym tym wyższa grupa ryzyka EAU pacjenta oraz wyższy odsetek zajętych węzłów chłonnych. W grupie pacjentów z wysoką ekspresją IL-17A w przerzutowych węzłach chłonnych częściej występuje przekraczanie torebki gruczołu krokowego przez guz (ECE).

6. Poziom ekspresji IL-17RA jest wyższy w guzie pierwotnym gruczołu krokowego niż w przerzutowych węzłach chłonnych. Im wyższy poziom ekspresji IL-17RA w przerzutowym węźle chłonnym tym wyższa grupa ryzyka EAU pacjenta. W grupie pacjentów z wysoką

ekspresją IL-17RA w przerzutowych węzłach chłonnych częściej występuje przekraczanie torebki węzła chłonnego przez raka gruczołu krokowego (ECE).

7. Wartość prognostyczna oznaczanej w materiale pooperacyjnym ekspresji GOLPH3, IL-17A i IL-17RA jest ograniczona w porównaniu do klastycznych czynników predykcyjnych raka gruczołu krokowego.

8. Zastosowanie potencjalnych nowych biomarkerów immunohistochemicznych może poprawić proces diagnostyczno-terapeutyczny pacjentów z rakiem gruczołu krokowego, jednak do tej pory jest zbyt mało badań oceniających możliwość ich wykorzystania w praktyce klinicznej.

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7.1 Novel Histopathological Biomarkers in Prostate Cancer: Implications and Perspectives.

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Review

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Review

Novel Histopathological Biomarkers in Prostate Cancer: Implications and Perspectives

Paweł Kiełb [1,*,†], Kamil Kowalczyk [1,†], Adam Gurwin [1], Łukasz Nowak [1], Wojciech Krajewski [1], Roman Sosnowski [2], Tomasz Szydełko [1] and Bartosz Małkiewicz [1,*]

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Abstract: Prostate cancer (PCa) is the second most frequently diagnosed cancer in men. Despite the significant progress in cancer diagnosis and treatment over the last few years, the approach to disease detection and therapy still does not include histopathological biomarkers. The dissemination of PCa is strictly related to the creation of a premetastatic niche, which can be detected by altered levels of specific biomarkers. To date, the risk factors for biochemical recurrence include lymph node status, prostate-specific antigen (PSA), PSA density (PSAD), body mass index (BMI), pathological Gleason score, seminal vesicle invasion, extraprostatic extension, and intraductal carcinoma. In the future, biomarkers might represent another prognostic factor, as discussed in many studies. In this review, we focus on histopathological biomarkers (particularly CD169 macrophages, neuropilin-1, cofilin-1, interleukin-17, signal transducer and activator of transcription protein 3 (STAT3), LIM domain kinase 1 (LIMK1), CD15, AMACR, prostate-specific membrane antigen (PSMA), Appl1, Sortilin, Syndecan-1, and p63) and their potential application in decision making regarding the prognosis and treatment of PCa patients. We refer to studies that found a correlation between the levels of biomarkers and tumor characteristics as well as clinical outcomes. We also hypothesize about the potential use of histopathological markers as a target for novel immunotherapeutic drugs or targeted radionuclide therapy, which may be used as adjuvant therapy in the future.

Keywords: prostate cancer; prognostic markers; IL-17; STAT3; NRP1; LIMK1; Cofilin-1; CD169; PSMA; AMACR

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1. Introduction

Prostate cancer (PCa) is the second most commonly diagnosed cancer in men worldwide (with 1,414,259 new cases in 2020) and the seventh most frequent cause of death due to cancer, accounting for 375,304 deaths in 2020 [1]. Since life expectancy is increasing worldwide and PCa incidence is correlated with increasing age, we expect a rise in the number of men newly diagnosed with this type of cancer in the near future [2]. Fortunately, cancer-specific survival (CSS) in patients with PCa has also increased in the past few years. This is probably due to the development of more effective tools for screening and diagnosing PCa at an early stage and improved protocols for adjuvant therapy. However, despite the advances in adjuvant therapy, which treatment would be most beneficial for a given patient remains unknown. Moreover, there is still a lack of tools for predicting survival prognosis and the risk of cancer dissemination in patients after prostatectomy. Tissue markers represent tumor heterogeneity, which in clinical practice denotes different responses to certain types of adjuvant therapy, which we will further describe in this review.

Currently, there are no guidelines recommending the use of markers in decision-making regarding treatment. However, in the future, incorporating these additional data into treatment may establish the foundation for a more personalized and effective approach to treating PCa patients. They could also serve as an additional prognostic factor to be used alongside clinicopathologic parameters, such as prostate-specific antigen level, histological grade group, and clinical stage. In this review, we focus on specific histopathological markers, particularly CD169 macrophages, neuropilin-1, cofilin-1, interleukin-17, signal transducer and activator of transcription protein 3 (STAT3), LIM domain kinase 1 (LIMK1), prostate-specific membrane antigen (PSMA), Appl1, Sortilin, Syndecan-1, AMACR, CD15, and p63, which in the future may help to establish the prognosis of patients with PCa as well as assist in choosing the most beneficial adjuvant therapy.

2. Evidence Acquisition

We used the PUBMED/Scopus database and gray literature to conduct a thorough search for original and review articles published up to December 2022 for the purposes of this narrative review. The search was limited to the English-language literature. The following terms were combined in our search: prostate cancer; biomarkers; IL-17; STAT3; NRP1; LIMK1; Cofilin-1; PSMA; AMACR; CD15; Appl1; Sortilin; Syndecan-1, and p63. Ultimately, 275 articles were selected for this review. Based on the authors' agreement, 211 studies with the strongest level of evidence and relevance to the subject were selected.

3. Biomarkers

Histopathological markers can be roughly divided into two groups according to their localization. Extracellular biomarkers comprise surface antigens involved in the identification of cell type as well as cell differentiation (CD antigens) and transmembrane receptors acting as signal transducers, i.e., for vascularization, which is crucial in tumor progression. Intracellular biomarkers include transcription activators, cytoskeleton-bind proteins, intracellular receptors, cytokines, etc.

In Table 1, we summarize the characteristics of the novel biomarkers described in this review.

3.1. Extracellular Biomarkers

3.1.1. CD169

One promising marker is the surface antigen CD169, which can be found on macrophages located in the sinuses of the lymph nodes. Various cells residing in the lymphatic system are known to be involved in anti-tumor activity, playing a pivotal role in the immune response against cancer cells. Macrophages found in regional lymph nodes absorb tumor-derived antigens, which are then presented to lymphocytes, including CD8+ T cells and natural killer (NK) cells responsible for cytotoxic responses. It has been suggested that CD169 is significant not only in PCa but also in melanoma, bladder cancer, endometrial tumors, and colorectal tumors [3,4]. The anti-tumor activity of these specific lymph node sinus macrophages has already been shown in animal model studies. Komohara et al. demonstrated that in colorectal and endometrial tumors, a high density of CD169 macrophages is associated with a higher number of infiltrating CD8-positive lymphocytes and NK cells responsible for a direct cytotoxic response. Further, a high number of CD169-positive macrophages in the tumor environment is also correlated with better overall survival and is independent of gender or age [3]. Some highly metastatic cancers are suspected of producing specific factors that precondition the tumor environment to promote their growth and metastasis. Such factors may potentially suppress the activity of macrophages via antigen CD169 expression in tumor-draining lymph nodes. Asano et al. showed that, in patients with bladder cancer, the abundance of CD169+ macrophages is correlated with a low T stage and a higher number of cytotoxic CD8+ cells. Moreover, the group with a high CD169 score had longer cancer-specific survival (five-year cancer-specific survival rate: 83.3% vs. 31.3%) [5]. Strömvall et al. found that PCa patients with low CD169 im-

munostaining in metastasis-free regional lymph nodes had worse outcomes (significantly shorter survival time) than patients with high CD169 scores—200 (95% CI 178–221) vs. 232 (95% CI 226–239) months [4]. However, the CD169 scores were not related to PSA relapse, although they were associated with an increased risk of death from PCa [4]. Figure 1 shows the proposed mechanism underlying the anticancer activity of CD169 macrophages.

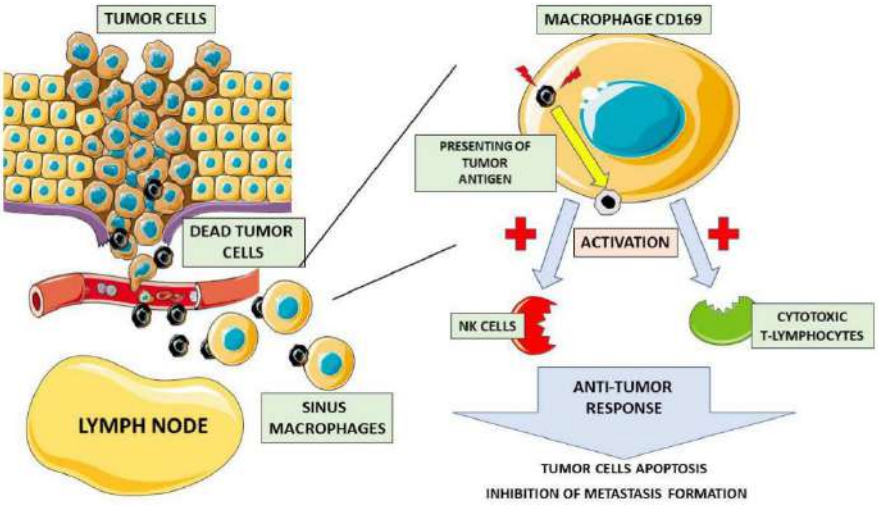

Figure 1. Role of CD169 macrophages in anticancer response. Dead cancer cells travel through the bloodstream to the lymph nodes, where they are phagocytized by CD169 sinus macrophages. Then, CD169 macrophages present cancer cell antigens on their surface, activating natural killer cells (NK cells) and cytotoxic T-lymphocytes. Upon activation, an anti-tumor response takes place, leading to cancer cell apoptosis and inhibiting the metastatic process.

3.1.2. Neuropilin-1 (NRP1)

Another example of an extracellular biomarker is neuropilin-1 (NRP1), which acts as a transmembrane co-receptor and interacts with vascular endothelial growth factor (VEGF) [6]. The tumor microenvironment is distinguished by enhanced metabolic activity, which results in hypoxia-triggering hypoxia-inducible factors (HIFs). VEGF is one of the transcripts controlled by HIF-1 that supports each phase of the vessel formation cascade. HIF-1 consists of two different subunits, α and β [7]. When the oxygen level in tissue is not sufficient, subunit α of HIF-1 remains in its non-hydroxylated form inducing numerous gene products that act proangiogenic [8]. Interestingly accumulation of non-hydroxylated HIF-1α does not only occurs under extended exposure to hypoxic conditions. Numerous DNA mutations in cancer cells may result in genetic modifications, which hinder the ubiquitination and proteasomal degradation of HIF-1α [9,10]. Due to angiogenesis promotion, VEGF enables tissues to adapt to a hypoxic environment. By interacting as a transmembrane co-receptor with VEGF, NRP1 seems to be crucial for vessel development. Several studies have demonstrated that neuropilin is elevated in tumors such as breast cancer, neuroblastoma, colon cancer, and lung cancer, highlighting its role in neovascularization [11–14]. However, NRP1 overexpression in tumor cells is associated not only with angiogenesis but also with PCa cells developing resistance to androgen-targeted therapy and progression to metastatic castration-resistant prostate cancer (mCRPC). This information may help some patients, after radical prostatectomy, to avoid potentially inefficacious androgen-targeted therapy and to choose more effective options for the management of the disease. Tse et al. demonstrated that NRP1 expression in cancer tissue is positively

correlated with increased Gleason grade and pathological T scores, positive lymph node status, and the failure of primary therapy. Flow cytometric tests have found rather low NRP1 levels in benign prostate epithelial cell lines and demonstrated elevated NRP1 levels in tumor cells with increased metastatic potential, with the highest expression found in mCRPC cells. Furthermore, one study reported a significantly lower chance of relapse-free survival in patients with NRP1 overexpression [15]. NRP1 may also serve as a predictor of adjuvant radiotherapy failure. Patients diagnosed with pT3 or positive margin disease with higher levels of the neuropilin-1 co-receptor were found to develop a post-RT biochemical recurrence (BCR) more often than those with lower marker expression. Furthermore, multivariate analysis has revealed high NRP1 expression to be an independent predictor of BCR. Finally, after radical prostatectomy, higher levels of the NRP1 marker are present in patients with distant metastasis than no metastasis, establishing NRP1 expression as a significant independent predictor of metastatic progression [15]. It is worth noting that NRP1 is expressed not only on cancer cells but also on immune cells such as regulatory T cells. Its expression levels appear correlated with the chemotherapy response in cervical cancer patients, as highlighted in a study by Battaglia et al., who observed a reduced number of regulatory T cell lymphocytes expressing NRP1 after neoadjuvant therapy (platinum-based CR) [16]. Moreover, decreases in NRP1 levels were directly correlated with tumor mass reduction. The authors hypothesized that this decrease might have been caused by stress signals released from disintegrating tumor cells, leading to the differentiation of regulatory/suppressor T cells into effector types. Interestingly, NRP1 seems to be not only a biomarker, which may help identify post-RP patients at risk of metastasis but also a potential target of immunotherapeutic agents. The idea of targeting the neuropilin-1 receptor has already been highlighted in two studies, which found that NRP1 blockade suppresses angiogenesis and medulloblastoma regression, decreases the rate of metastasis, and leads to better oncological outcomes in treated mice [17,18].

3.1.3. CD15 (Lewis X/Lex)

Lastly, extracellular biomarkers are CD15 (Lewis X/Lex) and CD15s (a sialyl form of CD15), which are the fucosyl (3-fucosily-N-acetyl-lactosamine) moieties found on the surface of various cancer cells. These malignancies include gastrointestinal system cancers, breast cancer, hematological malignancies, lung cancer, gliomas, and melanoma [19–25]. The expression of CD15 and CD15s has also been confirmed in urological malignancies. These antigens have been demonstrated to occur on the surfaces of the bladder, renal, and PCa cells, while they are not usually detected in healthy tissue, except occasionally in umbrella cells [26–29]. In fact, in bladder and renal cancer, the diagnostic and prognostic value of CD15 and CD15s is not unequivocal [30]. Nevertheless, their value seems to be clearer in PCa, in which overexpression of these antigens had already been demonstrated in the 1990s [28,31,32]. The upregulation of CD15s is associated with hormonal resistance, aggressive disease, and poor prognosis [28,32]. The high expression of CD15 and CD15s may influence PCa progression through several mechanisms. First, CD15 and CD15s play a crucial role in the adhesion of cancer cells to the blood vessel endothelium [33–36]. The initial step of cell adhesion (tethering) between CD15s and selectins, a family of cell adhesion molecules located on the surface of endothelial cells, is essential for cancer metastasis [37,38]. Second, CD15 and CD15s can change the structure of prostate cell mucins (membrane-bound proteins) that enable cancer cells to hide from destructive NK cells [39]. Third, it has been established that CD15s is the specific ligand of leukocyte-expressed L-selectin [40] that allows the binding of cancer cells and leukocytes, facilitating the spread of metastases through the circulatory system. Recently, Munkley et al. found CD15s expression on PCa cells to be regulated by androgens [41]. This might be a valuable factor that explains why androgens play a crucial role in the development and progression of PCa and why androgen deprivation therapy (ADT) is usually the first-line treatment in metastatic disease. ADT affects the CD15s antigen via the modulation of androgen receptors (AR). The latter are responsible for regulating the biosynthesis of related glycans, such

as CD15s, and it has been demonstrated that ADT reduces glycan production associated with PCa progression. Furthermore, a study used the newly developed anticancer drug compound 1 on androgen-independent PCa cell lines with the overexpression of CD15s on their surfaces [42]. The percentage of CD15s-positive cells was significantly lower after treatment. Targeting CD15 and CD15s in PCa treatment deserves attention and should therefore be further analyzed by researchers. CD15 and CD15s antigens were also recently observed on the surface of PSA from cancer cells [43]. The authors suggested that the CD15s expressed on PSA is a good candidate for a prognostic marker.

3.2. Intracellular Biomarkers

3.2.1. Interleukin 17 (IL-17)

The IL-17 family is an example of a very broad intracellular biomarker group whose precise mechanisms of action and clinical application in cancer disease are still not fully known. The Il-17 cytokine family consists of six ligands (IL-17A to IL-17F) and five receptors (IL-17RA to IL-17RE). In most studies, the ligands IL-17A and IL-17E were investigated due to their unique biological functions in tumors [44,45]. IL-17F acts similarly to IL-17A, and studies have shown that these two ligands are crucial proinflammatory cytokines in inflammatory and autoimmune diseases [46,47]. Il-17E is an important cytokine in the pathogenesis of asthma and atopic diseases [48]. Cytokines of the IL-17 family have been reported to show ambiguous effects on tumor development: with some ligands showing pro- and others anti-tumor effects [49]. The studies to date show that IL-17 may promote cancer development in several types of non-urological cancers, such as colon cancer [50–53], skin cancer [54,55], lung cancer [56,57], and breast cancer [58]. The role of Il-17 in renal cancer development is primarily pro-tumor, but further investigation is required to determine its ultimate character [59]. The results of a complex study on the immunoreactivity of the ligands and receptors of the IL-17 family in patients with bladder cancer showed significantly elevated IL-17A, IL-17F, and IL-17RC immunoreactivity in the bladder cancer group but decreased IL-17E, IL-17RA, and IL-17RB immunoreactivity. The researchers concluded that changed patterns of the expression of IL-17 cytokine family ligands and receptors may contribute to the occurrence and development of bladder cancer [60]. The role of IL-17 in PCa development is still unclear. Elevated levels of mRNAs encoding IL-17A and IL-17RA were detected in PCa and benign prostate hyperplasia (BPH) tissue [61,62]. A more detailed study investigated the expression of a wide spectrum of IL-17 types and their receptors with a comparison between normal prostate, PCa, and BPH tissues, demonstrating that IL-17A, acting through the IL-17RA receptor, may contribute to the development of PCa and BPH. In contrast, the interaction of IL-17E with the IL-17RB receptor demonstrated an anti-tumor effect [63]. Other studies have shown that the expression of IL-17RC is significantly increased in castrate-resistant prostate cancer (CRPC) compared with hormone-sensitive prostate cancer [64,65]. Results from studies on mouse models suggest that IL-17 promotes the growth of prostate adenocarcinoma, even in castrate conditions [66,67], and may contribute to the metastasizing process [68]. Using a mouse model, Zang et al. described the molecular mechanism underlying the pro-tumor effect of IL-17 [69]. They designed their study based on the known phenomenon of the increased IL-17 and matrix metalloproteinase 7 (MMP7) expression in PCa tissue. The results of their study showed that IL-17 promotes prostate carcinogenesis through MMP7, which induces the epithelial-to-mesenchymal transition (EMT). In conclusion, the authors indicated that the IL-17–MMP7–EMT axis is a potential target of new PCa treatments [69]. A schematic of this mechanism is presented in Figure 2.

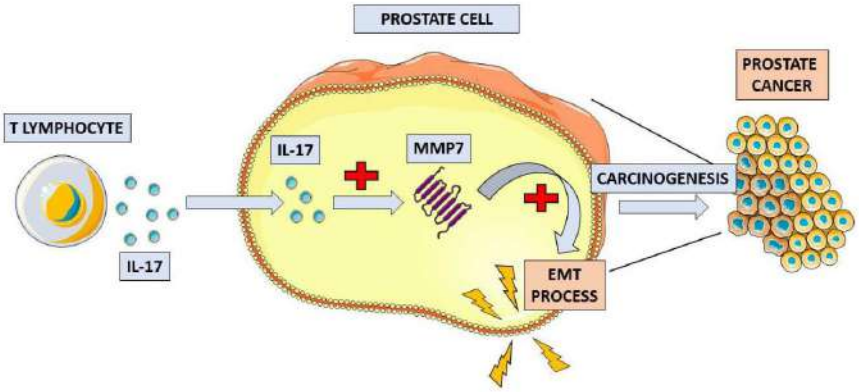

Figure 2. Schematic overview of the proposed mechanism of IL-17 in prostate cancer pathogenesis. IL-17 increases the concentration of matrix metalloproteinase 7 (MMP7) in prostate tissue. MMP7 induces the process of epithelial-to-mesenchymal transition (EMT), which leads to the development of prostate cancer.

In one of the most recent studies, Janiczek et al. investigated the expression of IL-17 in the prostates of patients treated for PCa (including Gleason score stratification) and BPH. The findings of this study showed that IL-17A and IL-17F, acting through the IL-17RC receptor, were involved in the pathogenesis of PCa and BPH. Moreover, compared with IL-17A, expression of IL-17F was more often observed in PCa with a higher histological grade. Expression of the IL-17RA receptor was not detected in either PCa or BPH tissue. The authors concluded that the inflammatory process was more severe in BPH than in PCa. Additionally, the authors observed that a lower Gleason score for PCa was associated with higher expression of selected IL-17 types. These findings may suggest that the inflammatory process is more important in the carcinogenesis of lower grade PCa [70]. Although the role of the IL-17 cytokine family and their receptors in the pathogenesis of PCa currently remains unclear, the results of recent studies are promising. Future studies that investigate the expression of IL-17 in PCa may assist with determining the role of inflammation in carcinogenesis and, potentially, future immunotherapy.

3.2.2. Cofilin-1 (CFL1)

Another mechanism in which intracellular biomarkers are involved is cancer cell migration and its ability to invade adjacent stroma as well as distant tissues. This process is reliant on constant cytoskeleton remodeling, which is regulated, inter alia, by cofilin-1 (CFL1), a protein that binds actin monomers and plays a key role in pseudopod formation and cytokinesis of cells [71]. CFL1 overexpression has been linked with the particular aggressiveness and migration rates of not only PCa cells but also breast, ovarian, and colorectal cancer cells [72–76]. It seems that CFL1 expression also has an impact on cancer cell resistance to systemic therapy. In a study by Xiao et al., knocking down the protein expression of cofilin-1 in PCa cells enhanced the anticancer effect of docetaxel [71,72]. Furthermore, docetaxel itself suppressed the expression of CFL1 in cancer cells [71]. In the future, this protein may serve as a target of immunotherapy prior to the administration of chemotherapy. Apart from its association with metastasis and chemoresistance, CFL1 is also associated with the clinicopathological characteristics of PCa. In a study by Lu et al., significantly higher expression of cofilin-1 (50% vs. 86.9%) was observed in patients with post-operative Gleason scores (GS) ≥ 7 than those with GS < 7, and CFL1 was considered an independent predictor of high GS. Furthermore, the increased expression of CFL1 was also observed in patients with lymph node metastasis (100 vs. 62.9%), although cofilin-1 status was independent of PSA level or age. The important takeaway from the study is that CFL1

seems to be specific to tissue samples positive for PCa: it was expressed in 70.3% of PCa specimens but not in any samples from the control group (patients with BPH) [77]. Chen et al. demonstrated, in their study [78], that cofilin-1 promoted cytoskeleton remodeling and activates the migration and invasion of PCa cells, significantly contributing to their metastatic potential. In the same study, the researchers found that cofilin-1 overexpression was linked with higher resistance to adriamycin, which was evidenced by higher IC50 values of the drug being found in CFL1 (+) cells [78].

3.2.3. STAT3

Intracellular biomarkers are also important agents in the development of the pre-metastatic niche, and one of their most important representatives is the signal transducer and activator of transcription protein 3 (STAT3). The important role of STAT3 in tumor development has been well described [79]. Activated STAT3 in tumor cells is a crucial mediator of oncogenesis [79–86] and the cancer-related inflammation process. Active STAT3 promotes tumor cell proliferation, angiogenesis, immunosuppression, and tumor invasion; all of these factors create a pro-carcinogenic microenvironment [87] and facilitate inflammation. Via this process, a pre-metastatic niche is created, changing the environment to one of a future metastatic site for cancer cells [82,88]. Further, studies have shown that STAT3 may promote tumor growth and hamper the effects of treatment by influencing mitochondrial metabolism [89] or epigenetic regulation [90] or by inducing drug resistance [91,92]. Due to its important role in tumor development, STAT3 has been investigated as a potential therapeutic target for new drugs, and the results are promising [93–97]. The role of STAT3 overexpression in tumor tissue remains inconclusive. In patients with PCa [98], as well as with some other urological [99] and non-urological solid tumors such as ovarian cancer [100], hepatocellular cancer [101,102], pancreatic cancer [103,104], and renal cell carcinoma [105], STAT3 overexpression is linked with poor survival. Regarding patients with colorectal cancer [106–108], lung cancer [109–113], gastric cancer [114–117], and melanoma [118,119], the results of studies are ambiguous—STAT3 overexpression is found to be a factor of favorable prognosis in some while being related to poor outcomes in others. To date, research has unequivocally shown that STAT3 overexpression in breast cancer [120–122] tissue may be beneficial in terms of patient outcomes. In the most comprehensive meta-analysis to date, conducted by Pin Wu et al. [123], the authors evaluated the prognostic value of STAT3 expression (for different phosphorylation states) and its correlation with the clinical outcomes of patients with solid tumors, including PCa. The authors found that STAT3 overexpression was associated with worse three- and five-year overall and disease-free survival. Elevated expression of STAT3 (especially its phosphorylated form, pSTAT3) in PCa, similarly to most investigated solid tumors, was found to be associated with poor prognosis, with the exception of a better prognosis found only in the case of breast cancer. Additionally, a meta-analysis on STAT3 expression in PCa, conducted by Tam L. et al. in 2007 [98], suggested that the activated STAT3 pathway might induce progression to the hormone-refractory type of PCa and, simultaneously, that it was a potential target for new drugs.

3.2.4. LIM Domain Kinase 1 (LIMK1)

Another intracellular biomarker involved in cancer cell migration, the metastatic process, and androgenic signaling is LIM domain kinase 1 (LIMK1). LIMK1 belongs to the LIM kinase protein family, and it is particularly involved in reorganization of the actin cytoskeleton, which is crucial for cell migration and metastasis [124,125]. Cytoskeleton remodeling is made possible by phosphorylation and inactivation of the cofilin that binds actin [126]. Further, LIMK1 seems to also be involved in intracellular androgen receptor signaling, which is especially promising in terms of developing new therapies targeting AR in CRPC patients, which could serve as alternatives to docetaxel [127]. This may contribute to avoiding the severe side effects of docetaxel therapy, such as cytopenia and pneumotoxicity. While reports on LIM domain kinase 1 (LIMK1) in PCa specimens are limited, it may

be a promising marker, as in many other cancers are LIMK1-positive, including colorectal cancer [128], lung cancer [129], osteosarcoma [130], and breast cancer [131], which also has clinical implications. In colorectal cancer tissue specimens, LIMK1 upregulation was found to be associated with lower overall survival rates as well as increased lymph node metastasis potential [126]. In a study by Huang et al. [132], which strictly focused on PCa patients, LIMK1 expression was linked with worse clinicopathological characteristics as well as worse disease dissemination and oncological outcomes. The authors compared LIMK1 upregulation between PCa specimens and a control group of BPH tissues (77.1% vs. 26.0%). Interestingly, LIMK1 staining was higher in lymph node metastasis samples than in PCa tissue collected from the same patient. Furthermore, high LIMK1 expression was correlated with prostate volume, PSA, PSA density, Gleason score, T stage, lymph node metastases, extracapsular extension, seminal vesicle invasion, and positive surgical margins, though no association with patient age was found. Finally, in the multivariate analysis, an elevated LIMK1 level was an independent risk factor for lymph node metastasis and biochemical recurrence after prostatectomy. The idea of targeting LIMK1 in treating PCa patients was comprehensively investigated in a study by Mardilovich et al. [127]. In their study, a LIMK selective small molecule inhibitor (LIMKi) was used as a potential agent against PCa cells. LIMKi had a negative effect on cell movement as well as proliferation. Further, LIMKi increased the percentage of PCa cells with sub-G1 DNA content, which is an indicator of apoptosis. Interestingly, these effects were more pronounced in androgen-dependent than androgen-independent cells. LIMKi led to a reduction in AR nuclear transport and transcriptional activity, eventually causing decreased cell proliferation. Furthermore, the authors found clinical implications of LIMK1 expression in PCa specimens after radical prostatectomy: non-metastatic patients with high levels of LIMK1 presented significantly worse survival compared with the metastatic group. Additionally, the increased LIMK1 staining was correlated with lymphovascular invasion, which is considered an independent prognostic factor for biochemical recurrence [133] and is connected with poor outcomes [134]. However, no association between LIMK1 upregulation and patient age, Gleason score, or PSA level was found. In general, the findings suggested that LIMK1 regulateds AR function, leading to disease progression in AR-dependent cells and that it was a promising target for novel drugs, especially those used in the treatment of CRPC patients with increased intracellular AR activity [135].

3.2.5. AMACR

The tumor microenvironment is characterized by the increased generation of reactive oxygen species (ROS) and a disturbed redox balance [136], which lead to DNA damage in cells by peroxides. One of the enzymes involved in this process is alpha-methylacyl-CoA racemase (AMACR), which is an enzyme that plays a significant role in lipid and drug metabolism. It is found in peroxisomes and mitochondria and is involved in the b-oxidation-mediated degradation of branched fatty acids [137,138]. Elevated AMACR levels have been linked to many cancer types, including renal cell carcinoma [139], gastric cancer [140], ovarian cancer [141], and hepatocellular carcinoma [142]. Combined with high molecular weight cytokeratin (HMW-CK) and p63, AMACR can also be used in histopathology staining as a negative marker of a benign prostate gland [143]. Its sensitivity as a prostatic adenocarcinoma-specific marker has been found to range from 80 to 100% [144–148]. It can be used together with positive markers for basal cells in prostatic glands, such as HMW-CK and p63, to increase the level of confidence in establishing a final diagnosis [149]. A comprehensive meta-analysis [150], which included 22 studies with 4385 participants, linked the positive expression of AMACR in prostatic tissue with the increased diagnosis of PCa (OR = 76.08; 95% CI, 25.53–226.68; P, 0.00001). The meta-analysis comprised studies from various geographic regions, demonstrating that marker expression in cancer did not differ significantly between Asians and Caucasians. However, there is no evidence for a statistically significant correlation between AMACR positivity and the Gleason score [149,151]. Since the first reports on its possible role as a diagnostic marker [152], AMACR has been

thoroughly examined as a novel drug target [153,154]. Shan Zha et al. reported a fourfold increase in AMACR enzymatic activity in PCa cells compared with normal prostate cells. Small interference RNA (siRNA) knockdown of AMACR disturbed the proliferation of the androgen-responsive PCa cell line. In that same study, the authors also studied a treatment involving the combination of an AMACR inhibitor with anti-androgen therapy, which resulted in increased cell growth inhibition compared to treatment with either in isolation [152]. Interestingly, AMACR also plays a significant role in the progression of PCa from a hormone-sensitive to a hormone-refractory state. Although acquiring resistance to hormonotherapy is a complex process, Takahara et al. found much lower expression of AMACR in androgen-independent than in androgen-dependent cell lines. Furthermore, inhibiting AMACR expression using siRNA resulted in the increased expression of the androgen receptor as well as the decreased expression of insulin-like growth factor I and platelet-derived growth factor alpha (growth factors that regulate cell proliferation and blood vessel formation), which results in the reduced viability of cancer cells in androgen-depleted serum compared with untreated cells. The authors hypothesized that future therapies focusing on AMACR inhibition could theoretically convert PCa cells from being hormone-independent to hormone-dependent [155,156]. AMACR has also been evaluated as a novel diagnostic marker to detect PCa during screening tests. In a recent study by Xin et al. [157], the authors, after conducting digital rectal examinations, collected first-catch urine samples from patients who also underwent prostate biopsy. The urinary exosomal AMACR (UE-A) was then measured using ELISA. The authors concluded that the level of the examined marker was higher in PCa and clinically significant PCa (csPCa)—Gleason score $\geq$ 7—than in BPH ($p < 0.001$). Furthermore, UE-A helped to differentiate between PCa and BPH and between BPH plus non-significant prostate cancer (nsPCa) and csPCa with area under the ROC curve (AUC) values of 0.832 and 0.78 obtained, respectively. Testing UE-A was also superior to PSA ($p = 0.0054$), PSAD ($p = 0.008$), and f/t PSA ($p = 0.056$) in distinguishing PCa from BPH. The clinical utility of UE-A was also evaluated in a multi-center cohort of patients at initial biopsy: by establishing 95% sensitivity as a cutoff, 27.57% of unnecessary biopsies (which was significantly higher than 13.24% when using PSA) were avoided using UE-A, with only four (1.47%) csPCa patients being missed. AMACR combined with PCA3 has also been evaluated as a diagnostic marker of csPCa based on transcript detection in total urine RNA. A study by Kotova et al. [158] found a significant difference in the AMACR score between csPCa and nsPCa, in both the prebiopsy and pre-RP cohorts. Theoretically, UE-A may enhance decision-making regarding prostate biopsy alongside PSA, serving as another easy-to-obtain and non-invasive marker.

3.2.6. Prostate-Specific Membrane Antigen (PSMA)

Prostate-specific membrane antigen (PSMA), which belongs to a class of integral membrane proteins first described in 1987, is a protein expressed in the cytoplasm of normal and malignant prostate tissue, including metastatic specimens [159]. In addition to PCa, the role of PSMA as a biomarker has been studied in other malignant tumors, such as renal cell carcinoma [160] and glioblastoma [161]. PSMA expression is significantly higher in primary PCa than in benign tissue and in distant metastases and metastatic lymph nodes than in primary tumors [162]. PSMA has a well-established, significant application in the detection, management, and follow-up of PCa patients. For example, in the case of biochemical recurrence after radical treatment, 68Ga-PSMA PET/CT can be performed to detect potential local recurrence or distant metastases [163]. It is important to note that the accuracy of this test depends on the level of PSA (detection rates around 50% with PSA levels $\leq$ 0.5 ng/mL and around 90% with PSA levels > 3 ng/mL) [164]. Additionally, during radio-guided salvage surgery, the intraoperative use of 99mTc-PSMA via a gamma probe may result in increased detection of metastatic sites [165]. PSMA expression is heterogeneous and has been found, through immunohistochemistry, to be negative in approximately 10% of primary PCa cases [166]. A recent study found that negative PSMA expression in the primary lesion was associated with the absence of PSMA expression

in metastasis sites in a patient with a castration-resistant disease [167]. Another recent study found that PSMA-negative PCa was associated with negative PSMA-PET scans –even in patients with very high PSA levels. Importantly, this finding may influence the post-treatment surveillance of PSMA-negative patients and potentially help avoid negative PET-PSMA scans [168]. The association of PSMA overexpression in PCa tissues after radical prostatectomy with a poor biochemical recurrence-free survival rate and higher Gleason score has been established [169]. A study by Hupe et al. assessed PSMA expression in prostate biopsy samples. It was found that high PSMA expression was an independent prognostic marker in biopsy at the time of initial diagnosis. The five-year recurrence-free survival rates were 88.2% and 26.8% for patients with no and high PSMA expression on biopsy, respectively [170]. In another study, PSMA was used as one of five markers in a panel used to assess PCa and normal prostate tissue. The results showed that using a panel of several biomarkers (including PSMA) improved the detection of PCa compared with using each marker alone—especially in the case of the low expression of p504s, where the assessment was facilitated by positive PSMA [171]. Similar studies using immunohistochemical staining of PSMA in PCa tissue have found improved detection of aggressive PCa and prediction of therapy failure [172,173]. Further, more sensitive detection of PCa bone metastases was achieved with the use of PSMA (with NKX3.1) than of PSA [174]. Another very promising field is the use of PSMA as a target for targeted radionuclide therapy, especially alpha-particle radiation therapy. In targeted alpha therapy, radionuclides are used that release extremely energetic alpha particles chelated to monoclonal antibodies or small molecules designed to bind to PSMA. Although preclinical studies have demonstrated the potential effectiveness of this therapy, especially in metastatic PCa, further research is still needed to develop better methods for the synthesis of alpha-emitters and to then test these in clinical trials [175–177]. In a novel study by Allelein et al., a potential process for detecting PSMA in the urine of PCa patients was described. The researchers isolated prostate-cancer-derived extracellular vesicles from urine and assessed the value of PSMA as a biomarker for detecting PCa in a urine sample. To automate the whole process, they designed a device that detects PSMA-positive extracellular vesicles in urine. The authors concluded that the automated isolation of extracellular vesicles was feasible, but further research was needed before the described method could be used to detect PCa [178].

3.2.7. Appl1

The adaptor protein containing a pleckstrin homology domain, phosphotyrosine binding domain, and leucine zipper motif 1 (Appl1) is one of the proteins localized in early endosomes [179,180]. Appl1 is considered a potentially important factor in the pathogenesis of PCa due to its multifunctional nature. To date, many of Appl1's mechanisms of action have been described. Some of them, such as in controlling the speed of intracellular transport [181] or regulating transcription factors in the Wnt signaling pathway [182,183], are involved in the development and progression of PCa. Further, Appl1 acts through cytokine transforming growth factor-β type I receptor (TβRI), and the Appl1–TβRI complex was found to be associated with more aggressive PCa [184]. In a study by Martini et al., Appl1 was used as one of three immunohistochemical biomarkers on PCa tissues. Intense Appl1 labeling was observed in basal epithelial cells and in poorly formed malignant glands. The biomarker panel containing Appl1, Sortilin, and Syndacan-1 shows great value in improving the pathology assessment of prostate tissue (especially in distinguishing between benign and malignant tissue and in precise Gleason score classification) [185]. Appl1 alone may be used as a marker of aggressive PCa, potentially affecting the prognosis and follow-up patterns of patients treated for PCa. Additionally, it may be used in combination with other markers to support pathological examination and, in the future, improve the process of diagnosing PCa.

3.2.8. Sortilin

Sortilin is a vacuolar protein sorting 10 family members that controls the transport of specific cargo within cells between structures, such as the Golgi apparatus, lysosomes, endosomes, and the plasma membrane [186]. It is particularly important in sugar metabolism and is highly expressed in tissues and cells with high energy demands. When stimulated by insulin, Sortilin helps facilitate transport to the plasma membrane by inducing the formation of vesicles and binding to glucose transporter 4 (GLUT4) and GLUT1 [187,188]. In PCa tissue, Sortilin is found in a granular pattern around the nuclei of cells and is more highly expressed in PCa cells with well-formed glands. Its ability to interact with both GLUT1 and GLUT4 suggests that Sortilin plays an important role in the metabolism of sugars, which, combined with Sortilin's significant expression in PCa cells, suggests the dependence of these cells on sugar metabolism. In the previously mentioned study, Sortilin was one member of the biomarker panel that showed effectiveness in improving the pathological diagnosis of PCa—especially in the differentiation of benign and malignant lesions—by labeling only well-formed malignant glands with specific supranuclear polar patterns [185]. A study by Tanimoto et al. found that Sortilin, by promoting progranulin degeneration, might contribute to delaying the progression of castration-resistant prostate cancer [189]. More research is needed to unambiguously determine the role of Sortilin in PCa pathogenesis as well as its clinical implications.

3.2.9. Syndecan-1

Syndecan-1, also known as CD138, is a transmembrane proteoglycan involved in various processes, such as cell proliferation, migration, and interactions with the extracellular matrix [190,191]. In PCa, Syndecan-1 expression has been observed in tissue samples with advanced cancer morphologies, including poorly formed glands, nests and cords of cells, and cribriform and intraductal carcinoma patterns. Its expression has also been detected in the tissues of patients with higher Gleason grades. Syndecan-1 has a direct role in binding growth factors and promoting cell migration, and its expression has been linked to the biochemical recurrence of PCa after radical prostatectomy [192]. In a previously cited study by Martini et al., Syndecan-1 was used as a marker and, along with two other previously described proteins (Appl1 and Sortilin), showed its usefulness in assessing histopathological prostate samples. Specifically, the use of Syndecan-1 epitope in the study provided reliable labeling of PCa with poorly formed gland morphologies, suggesting that it might have functional significance and potential utility for assessing advanced PCa using immunohistochemistry techniques [185]. A different study showed that cytoplasmic Syndecan-1 immunostaining was a predictor of poor prognosis in PCa and was associated with a higher Gleason grade and higher tumor stage as well as the occurrence of nodal metastases [193]. In [194], Santos et al. studied the expression of the Syndecan family in PCa tissues, finding that the overexpression of Syndecan-1 (as well as Syndecan-3) was associated with more aggressive tumors and a worse prognosis, and more precisely with decreased recurrence-free survival. In contrast, the overexpression of Syndecan-4 was correlated with a better prognosis [194]. Further, another study investigating the clinical implications of circulating Syndecan-1 in PCa patients found high levels (>123 ng/mL) of soluble Syndecan-1 in the serum of more advanced PCa patients, which was correlated with worse overall and cancer-specific survival. The authors suggested that the evaluation of soluble Syndecan-1 levels may be a promising tool for risk-stratification and therapy monitoring [195]. Another study [196] found that high levels of Syndecan-1 in serum were correlated with worse prostate-cancer-specific survival; additionally, they were found to be correlation with shorter survival in CRPC patients treated with docetaxel [196]. Syndecan-1 is a promising marker with many potential applications in diagnosing, treating, and monitoring PCa patients. However, to date, too few studies have been conducted to clearly indicate its clinical use, although the currently available research results are encouraging.

3.2.10. p63

The last biomarker to be described is p63, also known as tumor protein. p63 belongs to the p53 protein family and is known for its role in tumor suppression [197]. It is located in the basal cells of many epithelial tissues and appears to be essential for proper gland development [198]. Upregulation of p63 has been linked with poor prognosis and cancer aggressiveness in ovarian carcinoma [199] and oral squamous cell carcinoma [200]. In contrast to the previously described markers, p63 is mainly used as an additional immunomarker alongside HMW-CK for distinguishing benign prostate hyperplasia from PCa [201]. The differentiation of prostate lesions on the sole basis of morphologic findings is sometimes challenging. Therefore, basal cells, which express HMW-CK and p63, are widely used in differentiating benign glands (in which basal cells are present) from PCa glands (in which basal cells are absent) [202,203]. While HMW-CK staining seems to be more sensitive than p63 in basal cell identification (90.70% vs. 88.37%, $p = 0.7$) [201], p63 still serves as a complementary marker in difficult cases [204]. A study by Kalantari MR et al. [205] stated that although both HMW-CK and p63 presented high specificity and sensitivity in distinguishing true adenocarcinoma from benign lesions (BPH), p63 seemed to be more specific than HMW-CK in distinguishing PCa mimickers (such as adenosis, atrophy, and partial atrophy [206]) from adenocarcinoma. Although nuclear p63 staining, which is a negative marker for neoplasm, is usually observed in normal basal cells, there is a subset of PCa cancer cells for which cytoplasmic p63 staining is found [207,208]. Interestingly, a study found that elevated expression of cytoplasmic p63 is associated with prostate-cancer-specific mortality, reduced levels of apoptosis, and increased cellular proliferation (for which there were high levels of ki67 protein, a proliferation marker [209]). This association persisted after multivariable adjustment for age, year of diagnosis, Gleason score, and stage [210]. Interestingly, a similar correlation has been observed for lung carcinoma [211]. This nucleus to the cytoplasm mislocalization of the p63 protein, which regulates proliferation and apoptosis, is suggestive of its potential oncogenic function.

Table 1. Summary of the characteristics of novel histopathological biomarkers described in this review.

Marker	Localization	Function	Clinical Implications
CD169	Extracellular; surface antigen on macrophages	Tumor immunity [3].	Improved survival [3,4].
Neuropilin-1 (NRP1)	Extracellular; transmembrane co-receptor	Vascularization and progression of cancers [6].	Higher Gleason and T scores. Positive nodal status. Progression to mCRPC [11].
CD15	Extracellular	Adhesion of cancer cells to the blood vessel endothelium [29–32]. Changes the structure of prostate cells mucins—NK cells cannot detect cancer cells [35].	Disease aggressiveness. Hormone-refractory type of PCa [24,28].
Cofilin-1	Intracellular	Monomer binding; cytoskeleton reorganization [67].	Higher Gleason score [73]. Positive nodal status [74]. Chemoresistance [67,68].
Signal transducer and activator of transcription protein 3 (STAT3)	Intracellular	Transcription activator; promotes tumor cell proliferation [75,83].	Worse overall and disease-free survival. Progression to hormone-refractory type of PCa [94].
LIMK1	Intracellular	Reorganization of actin cytoskeleton; intracellular androgen receptor signaling [120–123].	Independent risk factor for lymph node metastasis and biochemical recurrence after prostatectomy [128].
IL-17 family	Intracellular	Induces and mediates proinflammatory responses [42,43].	Unclear, elevated levels of IL-17RC in CRPC [60,61]; higher expression of IL-17F in cancers with higher histological grades [66].

Table 1. *Cont.*

Marker	Localization	Function	Clinical Implications
AMACR	Intracellular; localized in peroxisomes and mitochondria	Degradation of branched fatty acids [133].	Negative marker for benignity of prostate glands [139]. Possible role as a novel non-invasive diagnostic marker [153].
Prostate-specific membrane antigen (PSMA)	Intracellular	Integral membrane protein [155].	Detection of recurrence or metastases in PET/CT [159]. Radio-guided salvage surgery [161]. High expression linked with poor prognosis [166].
Appl1	Intracellular; in early endosomes	Controls intracellular transport speed [174].	Associated with more aggressive PCa [177]. Improves the pathology diagnosis and grade of PCa [178].
Sortilin	Intracellular	Intracellular transport; involved in sugar metabolism [179,180].	Improves the pathology diagnosis and grade of PCa [178]. Delays progression of CRPC [182].
Syndecan-1	Intracellular/transmembrane	Cell proliferation, migration [183,184].	Improves the pathology diagnosis and grade of PCa [178]. Predictor of poor prognosis [186].
p63	Intracellular; usually nuclei of basal cells	Epithelium development, regulation of proliferation and apoptosis [191].	Positive marker for benignity of prostate glands [194].

PCa: prostate cancer; mCRPC: metastatic castration-resistant prostate cancer; CRPC: castration-resistant prostate cancer.

4. New Perspectives for Biomarkers in PCa

Since the population of patients with PCa is increasing worldwide, it is becoming increasingly important to detect all patients with clinically significant PCa, especially those at risk of metastasis, and to either apply adequate primary treatment or offer the most beneficial adjuvant therapy. Some new biomarkers may be very helpful during the primary diagnosis of PCa, with some studies already having indicated their usefulness in the assessment of biopsy material by a histopathologist (Appl1, Sortilin, Syndecan-1, p63), while others have indicated that they may be used in the non-invasive diagnosis of PCa (AMACR, PSMA) in the future. Not only have the roles of the presented markers been demonstrated in laboratory conditions, but they also have real clinical implications. For instance, they have been found to be correlated with increased resistance to chemotherapy and higher Gleason scores in tissue samples (cofilin-1, IL-17), worse oncological outcomes (STAT3, LIMK1, PSMA), improved survival (CD169), and progression to mCRPC (CD15, STAT3, neuropilin-1).

The different mechanisms of action of extra- and intracellular markers are important in the process of forming the pre-metastatic niche, the pro-cancerous environment of future metastasis sites. These particular biomarkers may help with the early detection of PCa in both the subclinical and more advanced metastatic stages and can serve as possible targets for new anticancer drugs.

A promising branch of new anticancer agents includes poly(ADP-ribose) polymerase (PARP) inhibitors, which seem to be effective in PCa patients with germline BRCA2 mutation [212]. Thanks to extensive sequencing efforts, the genomic environment of PCa is now better understood. DNA damage response (DDR) pathways preserve genomic stability by monitoring DNA integrity, activating the DNA repair process, or, if required, causing cell death. Out of all germline and somatic DDR mutations, the most commonly altered gene is BRCA2 [213]. Germline BRCA2 mutation was found to be a negative prognostic factor on CSS in mCRPC patients (17.4 months vs. 33.2 months in non-carriers) [214]. The inactivation of DDR genes affects 19% of patients with localized prostate tumors, and defects in the BRCA2 gene have been identified in 3–5.3% of patients [215,216]. The PARP family of enzymes plays an important role in DNA repair in a process called PARylation, which marks DNA lesions for repair. Targeting the PARP enzyme using specific inhibitors results in the accumulation of chromosomal instability, cell cycle arrest, and subsequent

apoptosis [217]. Currently, ongoing trials evaluate the efficacy of olaparib, rucaparib, niraparib, and talazoparib, either as a monotherapy or part of combination therapy for prostate cancer in different disease stages. The first results suggest a particular response in patients with BRCA1/2 mutations compared to patients with non-BRCA mutations [212].

Despite the lack of high-quality studies on the expression of CD169 macrophages in prostate tissue, we have decided to propose its use as a biomarker due to its potential impact on the pre-metastatic niche in lymph nodes. To date, research focused on the detection of these macrophages in lymph nodes has found that low concentrations of CD169 macrophages are correlated with shorter PCa survival time. Based on the results of studies on CD169 macrophage expression conducted directly on tissues of other urological [5] and non-urological cancers [3], we believe that future studies on the expression of this marker in PCa tissues will also demonstrate its usefulness—especially in predicting the risk of lymph node involvement. In our opinion, any factor that helps to assess the risk of lymph node metastases is valuable, given that the currently available modalities for PCa patients are still imperfect.

Today, the diagnosis of PCa dissemination is based mostly on lymph node examination in post-RP material, yet lymphadenectomy is burdened with complications and does not bring survival benefits. The side effects of extended lymph node dissection include a longer operating time, increased blood loss, longer length of stay, and postoperative complications such as lymphocele [218]. However, to date, there is no better tool to determine the staging and prognosis of patients, and pelvic node dissection should be performed in intermediate- and high-risk PCa patients. The other traditional risk factors of biochemical recurrence after RP include PSA, PSAD, BMI, pathological Gleason score, seminal vesicle invasion, extraprostatic extension, and intraductal carcinoma [219]. The sentinel lymph node technique (SLN) may improve the detection of metastatic disease while limiting the possible complications resulting from extended lymphadenectomy. Despite promising results, its use remains controversial, and it is not recommended as a standard procedure [220,221]. To improve the intraoperative detection of sentinel nodes or other potentially metastatic lymph nodes, new techniques using gamma probes or cameras with fluorescence detection have been used. The use of radiotracers such as 99mTc-PSMA [165], indocyanine green (ICG)-99mTc-nanocolloid, or ICG alone followed by intraoperative fluorescence detection increases the detection of sentinel lymph nodes, and with the development of probe miniaturization technology, it is possible to use them more extensively during minimally invasive operations such as robotic or laparoscopic prostatectomy [222,223]. It is noteworthy that the results of a study by Claps et al. showed that the use of ICG alone allowed for the correct assessment of lymph node status in 98% of patients undergoing surgery. In addition, the authors emphasize the high negative predictive value of this method and suggest that if fluorescence is not detected during the intraoperative assessment, lymphadenectomy can be safely omitted, which significantly reduces the possibility of previously described complications [224].

Another still intensively developed and promising technique aimed at improving the diagnosis and prognosis of prostate cancer (especially in terms of the risk of metastases) is the so-called liquid biopsy (LB). It is a minimally invasive method that detects, for example, circulating cells or the genetic material of prostate cancer or other metabolites associated with active cancer disease in the patient's blood (or urine). The advantage of LB is the ease of collecting material for testing, which can be repeated many times if necessary without significant harm to the patient [225]. A detailed description of this method and the currently studied markers detected during LB goes well beyond the scope of this article. However, considering the promising results of research on some of the detected markers, we would like to point out a few of them. The first is circulating tumor DNA (ctDNA) with a very wide potential application, from supplementing the currently used diagnostic methods by facilitating risk group stratification before treatment to monitoring the effectiveness of treatment and prognosis of the course of prostate cancer (e.g., castration-resistance development) [226]. The next biomarker is a group of microRNAs (miRNA) consisting

of many different subtypes (such as miRNA-21 and miRNA-141) that show significant potential, especially in terms of predicting the occurrence of metastases or castration resistance. Other biomarkers include bone metabolites, such as bone sialoprotein (high levels are associated with a shorter time to bone metastasis formation) and osteopontin (can be used to assess treatment response in patients with crPCa) [227]. In addition to the promising results, the authors emphasize that the LB technique still requires improvement and the development of standards regarding, among others, the collection of samples for testing and their appropriate storage or the detection methods of the proposed biomarkers. An important aspect is the high cost of using this method [226].

A promising idea for improving patient prognosis is the evaluation of additional specific PCa markers in pre-RP biopsy specimens. More effective assessment of the biopsy material, correct differentiation between benign and malignant lesions, and the accurate assessment of the Gleason score by a histopathologist are key initiating steps in the diagnostic and therapeutic path of a patient with PCa. Biomarkers such as Appl1, Sortilin, Syndecan-1, and p63 have already proven their usefulness in this process.

In the future, these biomarkers could be used not only as helpful prognostic factors for patients after radical prostatectomy but also serve as tools for selecting the most beneficial adjuvant therapy; for instance, patients with a high expression of neuropilin-1 are better candidates for adjuvant chemotherapy than for hormonotherapy.

New biomarkers may become targets of the rapidly developing targeted radionuclide therapy, as shown in the example of PSMA. This potentially represents a big step in the further development of new adjuvant therapies for the most advanced PCa patients, giving them a chance at longer survival or greater control of their symptoms, but more clinical studies are required.

Probably, the most promising aspect of the abovementioned markers is their potential use as targets for new immunotherapeutic drugs. Snuderl et al. [17] found that NRP1 plays a major role in the spread of medulloblastoma tumor cells. Targeting the neuropilin-1 receptor resulted in tumor regression, decreased metastasis, and improved survival of mice. Although this study was performed on a murine model and not on PCa, which is our focus, its results are promising. In that study, LIMK1 inhibition also resulted in reduced PCa cell motility and increased cell apoptosis. The inhibition of LIMK1 also affected AR-dependent cell processes and, in the future, could be used in pharmaceuticals, possibly alongside docetaxel or abiraterone in hormone-resistant PCa patients.

Other markers have also been investigated for their use as potential targets in the systemic treatment of prostate cancer, but such research is scarce and in its early stages. For example, the effectiveness of docetaxel in prostate cancer cells was found to be greater when there is reduced expression of CFL1 [71,72]. These results suggest a benefit may be derived from the combined use of immunotherapy and chemotherapy in the treatment of prostate cancer. The results of numerous studies [93–97] on the therapeutic potential of STAT3 blockers are very promising, but there are currently no such studies focusing on prostate cancer. For example, the results of the first in-human trial [94] examining the effects of STAT3 blockade in head and neck cancers are optimistic, suggesting the possible development of new therapies for cancers insensitive to currently used systemic treatments. A study [42] evaluating the effect of thieno [2,3-b] pyridine anticancer compound on breast and prostate cancer stem cells found a significant reduction in the number of CD15s-positive prostate cancer cells after therapy, but these are preliminary—the first results of such studies. Further research is required to evaluate the potential use of this therapy in the treatment of prostate cancer. Currently, there are no studies that have evaluated the use of IL-17 and AMACR as targets of new anticancer drugs, but researchers that have described their mechanisms of action have emphasized their potential use in the development of new therapies (for example, blocking the IL-17–MMP7–EMT axis [69] or inhibiting AMACR's conversion of CRPC to hormone-dependent PCa [155,156]).

Nevertheless, there is a need to conduct further research in order to find new drugs that can target specific tissue markers in PCa patients.

It is also worth noting that new technologies such as artificial intelligence (AI) and machine learning (ML) are emerging as powerful tools in the field of prostate cancer diagnosis and management as well as in the analysis of histopathological samples [228,229]. ML algorithms, a subset of AI, can be trained on large datasets of medical images and clinical data to help clinicians make more accurate and informed decisions regarding treatment options for prostate cancer patients. In recent years, AI-based algorithms have shown promising results in detecting prostate cancer from medical images, including multiparametric magnetic resonance imaging (mpMRI) (e.g., distinguishing between benign and cancer prostate tissue [230] or predicting Gleason score [231,232]) and ultrasound (e.g., detecting high-grade prostate cancer [233]). Additionally, AI-based systems are being developed to analyze histopathological samples of prostate cancer, which may help pathologists make more accurate and reliable diagnoses. Issues such as the classification of biopsy samples using the Gleason scoring system [234–236] and the transformation of examined images into three dimensions in order to increase detection sensitivity and improve tissue material evaluation [237] are just some of the areas where the use of artificial intelligence has provided promising, but still preliminary, results. Currently, the use of ML cannot adequately replace a specialized histopathologist. However, in the future, it is expected to significantly improve their work and thus shorten the time needed to evaluate tissue material and increase the accuracy of their expert conclusions. Introducing new immunohistochemical markers to protocols for the histopathological evaluation of prostate tissues may provide new, valuable data for AI analysis, which may further increase the effectiveness of evaluation. In addition, ML can be used as an additional tool to predict patient mortality and the risk of prostate cancer recurrence based on various clinical, imaging, and histopathological data [238–240]. By combining machine learning with traditional diagnostic methods, AI has the potential to improve the accuracy and efficiency of prostate cancer diagnosis, management, and treatment, ultimately leading to improved patient outcomes. However, more research is needed to fully evaluate the potential benefits and limitations of AI in the context of prostate cancer.

5. Conclusions

To summarize, novel histopathological biomarkers may facilitate the decision-making process with regard to patients with PCa. To date, there have been too few studies on these biomarkers to conclusively assess their clinical application; however, the early results are very promising, especially regarding their use in the assessment of the risk of metastasis or recurrence after radical treatment. More quality research is required to incorporate these markers into clinical practice.

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Abbreviations

AI	artificial intelligence
AMACR	alpha-methylacyl-CoA racemase
Appl1	adaptor protein containing a pleckstrin-homology domain, phosphotyrosine binding domain, and leucine zipper motif 1
AR	androgen receptors
BCR	biochemical recurrence
BMI	body mass index
BPH	benign prostatic hyperplasia
CD15s	sialyl form of CD15
CFL1	cofilin-1
CRPC	castration-resistant prostate cancer
csPCa	clinically significant prostate cancer
CSS	cancer-specific survival
CT	computed tomography
ctDNA	circulating tumor DNA
DDR	DNA damage response
EMT	epithelial-to-mesenchymal transition
GLUT	glucose transporter
GS	Gleason score
HMW-CK	High Molecular Weight-Cytokeratin
HIF	hypoxia-inducible factor
ICG	indocyanine green
IL-17	interleukin 17
LB	liquid biopsy
LIMK1	LIM domain kinase 1
LIMKi	LIM domain kinase 1 inhibitor
mCRPC	metastatic castration-resistant prostate cancer
ML	machine learning
MMP7	matrix metalloproteinase 7
mpMRI	multiparametric magnetic resonance imaging
NK cell	natural killer cell
NRP1	neuropilin-1
nsPCa	non-significant prostate cancer
PARP	poly(ADP-ribose) polymerase
PCa	prostate cancer
PET	positron emission tomography
PSA	prostate-specific antigen
PSAD	prostate-specific antigen density
PSMA	prostate-specific membrane antigen
pSTAT3	phosphorylated signal transducer and activator of transcription proteins 3
ROS	reactive oxygen species
miRNA	microRNA
siRNA	small interference RNA
SLN	sentinel lymph node
STAT3	signal transducer and activator of transcription proteins 3
TβRI	transforming growth factor-β type I receptor
VEGF	vascular endothelial growth factor

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7.2 Comparative analysis of GOLPH3 expression in lymph node-positive prostate cancer: immunohistochemistry staining patterns and clinical significance.

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Comparative analysis of GOLPH3 expression in lymph node-positive prostate cancer: immunohistochemistry staining patterns and clinical significance

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Introduction: Prostate cancer (PCa) is the second most commonly diagnosed cancer in men worldwide. Lymph node metastasis is a poor prognostic factor for PCa. Previous studies have found that Golgi phosphoprotein 3 (GOLPH3) is overexpressed in various cancers, including PCa. We examined GOLPH3 expression in PCa cells from primary tumor and, as the first, also in metastatic lymph nodes to assess its potential as a new risk factor for PCa progression.

Methods: The study included 78 patients diagnosed with lymph node-positive PCa confirmed in the postoperative material. All the patients underwent radical prostatectomy (RP) with extended lymphadenectomy. The clinical data of the patients were retrospectively analyzed, and their histopathological specimens were selected for further analysis. Immunohistochemistry (IHC) staining was performed and the expression of GOLPH3 was assessed by an experienced uropathologist using an immunoreactive scale (IRS). A correlational analysis of the obtained data with the clinicopathological data of patients was performed.

Results: A positive IHC reaction for GOLPH3 was observed in all samples. IRS score for GOLPH3 expression was higher in the metastatic lymph nodes than in the prostate (not statistically significant; $p=0.056$). Several significant correlations were identified in connection with GOLPH3 expression levels in the prostate and metastatic lymph node tissues. No significant correlations were found between GOLPH3 expression and patient characteristics (e.g. BMI, EAU risk group, or preoperative PSA level), pathological features, or postoperative outcomes. However, we found that lymphovascular invasion (LVI) tended to be more common in patients with a higher percentage of GOLPH3-positive cells ($p=0.02$). We also found a positive association between the intensity of GOLPH3 staining in metastatic lymph nodes and the EAU classification. Finally, we found a significant negative correlation between the GOLPH3 expression and

the efficacy of RP – the higher the expression of GOLPH3, the lower the efficacy of RP was ($p<0.05$).

Conclusion: GOLPH3 is expressed in both prostate and metastatic lymph nodes, with higher expression in metastatic lymph nodes. High GOLPH3 expression was associated with the occurrence of LVI, higher-risk group in the EAU classification, and lower efficacy of the RP, but there was no significant correlation with other pathological features or postoperative outcomes.

KEYWORDS

GOLPH3, prostate cancer, lymph nodes metastases, radical prostatectomy, golgi phosphoprotein 3

1 Introduction

Prostate cancer (PCa) is the second most commonly diagnosed cancer in men worldwide, and is one of the leading causes of cancer-related deaths in the male population (1). The incidence of PCa increases with the age of patients; therefore, due to increasing life expectancy, there will be more patients with PCa. This will be an even more significant health problem in society than it is today (2). Due to the dynamic development of methods for the diagnosis and treatment of PCa, the results of treatment of patients are gradually improving. Despite the presence of constantly improving therapy protocols, choosing the best treatment plan for a given patient is difficult, and the final effect is uncertain. This is due to the fact that there is still a lack of more precise tools to accurately assess the survival prognosis and the risk of progression or metastasis after primary treatment of PCa.

Differences in the treatment effects between patients with PCa may be related to the high heterogeneity of prostate tumors, which may affect the effectiveness of primary or adjuvant therapy. Numerous studies suggest that the analysis of the expression of immunohistochemical (IHC) markers in the tissues of patients with PCa, such as Golgi phosphoprotein 3 (GOLPH3), may be an important tool for improving diagnosis, assessing prognosis, risk of progression, and potential effects of primary treatment or response to adjuvant treatment (3–7). In addition, they can be a valuable supplement to the already used classic prognostic factors, such as prostate specific antigen (PSA) level, clinical stage, or histological grade, determined on the basis of prostate biopsy results. Despite the promising results of these studies, analysis of the expression of these markers is not routinely recommended by the guidelines.

The presence of lymph node metastases is an important risk factor with a decidedly negative impact on survival and the risk of recurrence after primary treatment in patients with PCa. Nodal metastases also affect the therapeutic process in patients through the selection of adequate adjuvant treatment and more rigorous follow-up after primary treatment (8, 9). Despite continuous intensive technological developments, the assessment of nodal involvement using radiological imaging techniques remains inferior to lymphadenectomy (10, 11). Currently, the gold standard for detecting dissemination to the lymph nodes is extended pelvic lymphadenectomy during radical prostatectomy (RP). However, this is an additional invasive procedure that does not bring survival benefits and significantly increases the risk of treatment side effects, such as increased blood loss, longer surgery and hospitalization time, and an increased risk of lymphocele development in the postoperative period (12). In the absence of more accurate methods to determine lymph node status, extended pelvic lymphadenectomy should be performed in intermediate- and high-risk PCa patients (13).

In this study, we investigated the GOLPH3 protein, which performs key functions in the Golgi apparatus, such as maintaining the ribbon structure and its glycosylation, as well as intracellular vesicular transport (14–16). GOLPH3 was the first Golgi oncoprotein to be described (17). Its pro-tumor effects are complex, and a thorough understanding of all these mechanisms requires further research. To date, several possible pathways using GOLPH3 in the process of carcinogenesis have been proposed, including increased transport from the Golgi apparatus to the plasma membrane, disruption of genome structure stability, disorganization of endocytosis regulation, and changes in glycosylation of proteins in the Golgi apparatus (16). The oncogenic effect of GOLPH3 and its impact on the course of the disease have been demonstrated in studies on melanoma, colon adenocarcinoma, glioblastoma, and non-small-cell lung cancer (18).

The precise mechanism of GOLPH3 oncogenic effect in PCa pathogenesis is unknown. Several modes of action have been

Abbreviations: BMI, body mass index; EAU, European Association of Urology; ECE, extracapsular extension; EGFR, epidermal growth factor receptor; GGG ISUP, International Society of Urological Pathology grade (group) system; GOLPH3, Golgi phosphoprotein 3; HE, haematoxylin and eosin; IHC, Immunohistochemistry; IRS, immunoreactive scale; LVI, lymphovascular invasion; MMP9, matrix metalloproteinases 9; mTOR, mammalian target of rapamycin; NSCLC , non-small-cell lung cancer; NVI, neurovascular invasion; PCa, prostate cancer; PSA, prostate specific antigen; pT, pathological tumor stage; RP, radical prostatectomy; TMA, tissue microarray; TURP, transurethral resection of the prostate.

proposed based on currently available studies. One of these mechanisms is the activation of the mammalian target of rapamycin (mTOR) signalling pathway, which stimulates the activity of the kinase B protein while decreasing the transcriptional activity of the forkhead box protein O gene (3, 18–20). Furthermore, the effect of mTOR activation on cell differentiation suggests a significant role in the transition from hormone-sensitive to hormone-refractory PCa (6). Another suggested mechanism is GOLPH3 stimulating effect on matrix metalloproteinases 9 (MMP9) secretion in PCa cells via epidermal growth factor receptor (EGFR) and Src kinase, which appear to be important, especially for the formation of PCa metastases (4, 21–26).

Despite the results suggesting a correlation between GOLPH3 and malignant tumor progression, to date, no studies have assessed the expression of GOLPH3 in lymph nodes. In our study, we comprehensively investigated GOLPH3 expression in PCa cells from primary tumor tissues and metastatic lymph nodes. The evaluation of GOLPH3 expression in metastatic lymph nodes has not been previously reported, making our study unique in this regard. We correlated the obtained results with clinical data of patients with lymph node metastases to assess the application of GOLPH3 as a new negative risk factor for PCa progression.

2 Materials and methods

2.1 Patients and pathological specimens

The study included 78 patients with diagnosed PCa, in whom metastases in the lymph nodes were detected in the postoperative material. All patients underwent RP with extended lymphadenectomy between January 2012 and September 2018 at the University Urology Center (Wrocław, Poland). We retrospectively analyzed the clinical data of the patients included in the study and selected their histopathological specimens obtained after prostatectomy for further analysis. The obtained material was evaluated by an experienced uropathologist. Tumor stage and grade ware assessed according to the 2017 Tumour, Node, Metastasis (TNM) classification of PCa and the Gleason system. In addition, classifications such as International Society of Urological Pathology (ISUP) 2014 grade (group) system and European Association of Urology (EAU) risk groups for biochemical recurrence of localized and locally-advanced PCa were used to better characterize patients. Efficacy of RP was defined as a PSA level <0.1 ng/ml at the first measurement after RP, usually 6 weeks after surgery.

2.2 Tissue microarrays

The tissue microarrays (TMAs) technique is widely recognized as a valid approach for preserving material in paraffin blocks, offering numerous benefits such as cost-effectiveness, consistent IHC reaction conditions, and efficient evaluation of IHC results, with only minor limitations (27, 28). In our study 8 TMAs were prepared. Prior to performing TMAs blocks the histological slides stained with hematoxylin and eosin (HE) were obtained from whole samples of prostate and lymph nodes with detected prostate adenocarcinoma cells archived in the form of paraffin blocks (donor blocks). The slides were scanned using the Pannoramic Midi II histological scanner (3DHISTECH Ltd.). After that by using the Panoramic Viewer Program (3DHISTECH Ltd.), the representative areas from the entire sections where selected by uropathologist. In addition, to increase the representativeness of each case, 3 representative cores with a size of 1.5 mm from the donor block were selected and then transferred to the TMA 'recipient' block using the TMA Grand Master (3DHISTECH Ltd.). The TMA creation process is presented in Figure 1.

2.3 Immunohistochemistry

IHC reactions were performed on 4 µm paraffin sections obtained from TMA blocks using an automated staining platform, Autostainer Link48 (Dako, Glostrup, Denmark). First, the slides were deparaffinized, rehydrated, and antigen retrieval was performed by boiling the sections in EnVision FLEX Target Retrieval Solution, High pH (97°C, 20 min; pH 9) in PTLink (Dako). Endogenous peroxidase activity was blocked by incubation for 5 min with the EnVision FLEX Peroxidase-Blocking Reagent (Dako). Monoclonal mouse anti-GOLPH3 antibody (1:2000; cat. No. LS-B5044, LS Bio, Lynnwood, DC, USA) was used as the primary antibody (20 min incubation), followed by incubation with a secondary antibody conjugated with horseradish peroxidase (EnVision FLEX/HRP, 20 min incubation). Subsequently, 3,3-diaminobenzidine was applied and the sections were incubated for 10 min at RT. All sections were counterstained with EnVision FLEX Hematoxylin (Dako) for 5 min. After dehydration in graded ethanol concentrations (70%, 96%, absolute) and xylene, all slides were closed with coverslips in SUB-X Mounting Medium. The slides were scanned using a histologic scanner, Pannoramic MIDI (3DHistech). Reactions were evaluated with the use of Quant Center software (3DHistech) under researcher supervision. In order to evaluate the expression of GOLPH3, for every case, six TMA cores (3 from prostate and 3 from metastatic lymph node) were assessed using a Pannoramic Viewer Digital image analysis. The expression assessment of GOLPH3 was performed by an experienced uropathologist unaware of detailed patient clinical information, by using immunoreactive scale (IRS) by Remmele and Stegner (29, 30), presented in Table 1.

In short, IRS score taking into account the percentage of positively stained PCa cells (A) and the intensity of staining (B) and final score is the result of multiplying these values (A X B). Material from the prostate and the metastatic lymph node were assessed separately for each patient. The final IRS score for prostate and metastatic lymph node was the average score obtained from the assessment of each of the 3 cores of a given tissue type. The figures show a comparison of the intensity of GOLPH3 expression in the evaluated prostate preparations (Figure 2) and metastatic lymph nodes (Figure 3).

2.4 Statistical analysis

The mean, standard deviation (SD), minimum (Min), maximum (Max), median (Me), lower quartile (Q1), and upper quartile (Q3) for quantitative variables were calculated. The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to confirm that quantitative variables' empirical distribution fits to a normal distribution. To evaluate the connection between monotonic relationships between variables, Spearman's rank correlation coefficient was determined. In contingency tables, qualitative (nominal and categorical) variables were presented as numbers (n) and percentages (%). The Mann-Whitney U test was applied to verify the significance of differences in quantitative parameters between the two groups, and Pearson's Chi squared test was used to confirm the independence of two qualitative factors. In all analyzed cases, the associations were considered statistically significant for $p < 0.05$. All statistical analyses were performed using Statistica v.13.3 (TIBCO Software Inc., Palo Alto, CA, USA).

3 Results

General characteristics of the patients presented in Table 2.

In all the analyzed tissue samples, we found a positive immunohistochemical reaction in PCa cells confirming the expression of GOLPH3 in the analyzed material.

The level of GOLPH3 expression, assessed using the IRS scale, was higher in the material from the metastatic lymph node than from the prostate (IRS score: 8 vs. 6 score; p=0.056). However, the statistically significant difference between prostate and metastatic lymph nodes was only in the percentage of GOLPH3 positive cancer cells found in the evaluated tissue sample (A: 4 vs. 3; p=0.046), with no significant difference in the intensity of staining (p=0.278).

Using a simplified GOLPH3 expression level classification based on the IRS score, most prostate samples were found to have low GOLPH3 expression levels, whereas metastatic lymph node material was found to have high GOLPH3 expression. However, these differences were not statistically significant (p=0.148). The results of the statistical analysis are presented in Table 3.

A significant positive correlation was found between the level of GOLHP3 expression in prostate and in the metastatic lymph node (rho=0,294, p<0.05; Figure 4A).

There were no significant correlations between the level of GOLPH3 expression (expressed by IRS score) in the prostate or metastatic lymph nodes and the patients' age, BMI, EAU risk group, postoperative GGG ISUP, or preoperative PSA level.

A significant positive correlation was found between the level of GOLPH3 expression in metastatic lymph nodes and the percentage of affected lymph nodes (p=0.036, Figure 4B). Table 4 shows the results of the statistical analysis.

No statistically significant correlation was found between the level of GOLPH3 expression in prostate and metastatic lymph nodes (assessed based on the IRS score) and the pathological features or postoperative outcomes of patients. Table 5 contains the results of the statistical analysis.

FIGURE 1
Tissue microarray (TMA) preparation and immunohistochemical (IHC) staining for GOLPH3 expression analysis. **(A)** Tissue microarrayer TMA Grand Master (3DHISTECH Ltd.), with selected donor blocks containing prostate tissue. **(B)** Finished tissue microarray. **(C)** Slides after IHC staining of prostate tissue prepared for scanning. **(D)** The result of scanning a single slide in the program is ready for evaluation of GOLPH3 expression in prostate tissue. The tissue on the left side was IHC stained. On the right side, the same material was stained with haematoxylin and eosin **(HE)** to aid the evaluation.

TABLE 1 Immunoreactive scale (IRS) by Remmele and Stegner.

Immunoreactive Scale (IRS)			
A – Percentage of positive cancer cells		B – Staining intensity	
Score		Score	
0	no cells with positive reaction	0	no colour reaction
1	< 10% cells with positive reaction	1	mild reaction
2	10–50% cells with positive reaction	2	moderate reaction
3	51–80% cells with positive reaction	3	intense reaction
4	> 80% cells with positive reaction		
IRS SCORE (A X B): 0-12 points			
Final score		Level of expression	
1-7		Low expression	
8-12		High expression	

IRS score taking into account the percentage of positively stained prostate cancer cells (A) and the intensity of staining (B) and final score is the result of multiplying these values (A X B). Based on the IRS score, patients were divided into a group of low and high GOLPH3 expression as presented.

However, when analyzing the relationship between these features and the percentage of GOLPH3 positive cancer cells ("A" score in IRS scale) in the prostate and metastatic lymph node samples, a significant correlation was found with the efficacy of the RP - the efficacy of RP was higher in patients in whom the percentage of GOLPH3 positive cancer cells in prostate was lower (75% vs. 36.7% for 51-80% GOLPH3 positive cells and >80% GOLPH3 positive cells respectively; p=0.001). In addition, a statistically significant correlation was found between the percentage of GOLPH3 positive cancer cells in metastatic lymph nodes and the occurrence of lymphovascular invasion (LVI) (p=0.02). LVI was significantly less common in patients with a percentage of GOLPH3 positive cancer cells between 11-50% than 51-80% (0% vs. 75%; p=0.026) and >80% (0% vs. 83%; p=0.004). The results of this analysis are summarized in Table 6.

We also found a statistically significant relationship related to the efficacy of RP, similar to that already described, between the intensity of staining GOLPH3 positive cancer cells ("B" score in IRS scale) in metastatic lymph node (p=0.026). The efficacy of RP was

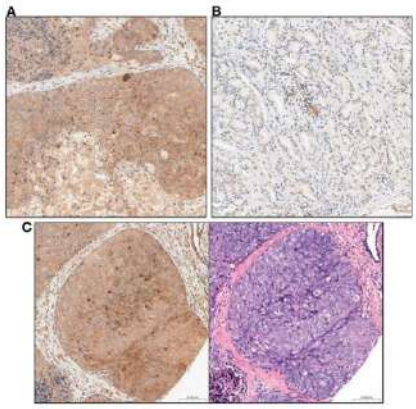

FIGURE 2
Comparison of GOLPH3 expression in prostate tissue **(A)** High GOLPH3 expression in prostate tissue. **(B)** Low GOLPH3 expression in the prostate tissue. **(C)** Comparison of the image after immunohistochemical staining with high expression of GOLPH3 in the prostate tissue and the same tissue stained only with haematoxylin and eosin (HE). Magnification, x15.

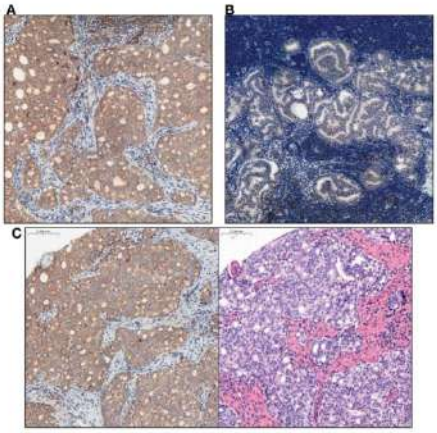

FIGURE 3
Comparison of GOLPH3 expression in lymph nodes with prostate cancer metastasis. **(A)** High GOLPH3 expression in the metastatic lymph node tissues. **(B)** Low GOLPH3 expression in the metastatic lymph node tissues. **(C)** Comparison of the image after immunohistochemical staining with high expression of GOLPH3 in metastatic lymph node tissue and the same tissue stained only with haematoxylin and eosin (HE). Magnification, x15.

TABLE 2 General characteristics and clinicopathological parameters of the patients.

Variable	Statistics
General characteristics of patients	
Age (years):	
M ± *SD*	65.0 ± 5.6
BMI (kg/m^2):	
M ± *SD*	28.1 ± 3.6
Preoperative PSA (ng/ml):	
Me [Q1; Q3]	19.7 (10.8; 36.1)
EAU risk group, n (%):	
Low-risk	1 (1.3)
Intermediate-risk	8 (10.3)
High-risk	39 (50.0)
High-risk locally advanced	30 (38.5)
Clinicopathological parameters	
pT, n (%):	
2a	1 (1.3)
2c	9 (11.5)
3a	14 (17.9)
3b	54 (69.2)
Postoperative Gleason, n (%):	
3 + 3	1 (1.3)
3 + 4	11 (14.1)
3 + 5	4 (5.1)
4 + 3	19 (24.4)
4 + 4	3 (3.8)
4 + 5	29 (37.2)
5 + 3	2 (2.6)
5 + 4	8 (10.3)
5 + 5	1 (1.3)
Postoperative GGG ISUP, n (%):	
1	1 (1.3)
2	11 (14.1)
3	19 (24.4)
4	9 (11.5)
5	38 (48.7)
Extracapsular extension of prostate, n (%):	
Yes	67 (85.9)
No	11 (14.1)
Resection margin, n (%):	
Positive	55 (70.5)
Negative	23 (29.5)
Neurovascular invasion, n (%):	
Yes	71 (91.0)
No	1 (1.3)
No data	6 (7.7)
Lymphovascular invasion, n (%):	
Yes	57 (73.1)
No	16 (20.5)
No data	5 (6.4)

(Continued)

TABLE 2 Continued

Variable	Statistics
Affected lymph nodes (%):	
Me [Q1; Q3]	12.1 (8.0; 27.3)
Efficacy of RP, n (%):	
Yes	37 (47.4)
No	41 (52.6)

M, arithmetic mean; SD, standard deviation; BMI, body mass index; PSA, prostate specific antigen; Me, median; Q1, lower quartile; Q3, upper quartile; EAU, European Association of Urology; n, number; %, percentage; pT, pathological tumor stage; GGG ISUP, International Society of Urological Pathology (ISUP) 2014 grade (group) system; efficacy of RP, defined as an PSA level <0.1 ng/ml at the first measurement after radical prostatectomy.

higher in patients with moderate staining intensity than in those with intensive staining (68.4% vs. 25%; p=0.002). The statistical analysis is shown in Table 7. Additionally, we also found a positive correlation between the intensity of GOLPH3 staining ("B" score in IRS scale) in the metastatic lymph nodes and the percentage of all metastatic lymph nodes (rho=0.298, p<0.05; Figure 4C) and also the EAU classification (rho=0.242, p<0.05; Figure 4D).

4 Discussion

PCa, the second most commonly diagnosed cancer in men, presents a serious diagnostic and therapeutic challenge for clinicians and pathologists. As life expectancy is increasing worldwide and PCa incidence is correlated with age, an increase in the number of men newly diagnosed with this type of cancer in the near future is expected (2). Nevertheless, despite significant advancements in adjuvant therapy resulting in increased cancer-specific survival, we still rely on classic factors such as PSA level, histological grade group, and clinical stage when establishing prognosis (31). Incorporating additional data such as IHC marker expression in postoperative specimens could improve patient prognosis after RP. Therefore, it is necessary to identify reliable biomarkers. The role and application of IHC biomarkers in the diagnosis and prognosis of PCa, including the formation of metastases, are the subject of many ongoing studies. Many of the results from these studies are promising but are not currently reflected in urological guidelines regarding PCa (13, 32). Although prostatic expression of GOLPH3 has been evaluated in several studies, to date, no study has examined the lymph node expression of this marker, which makes our research innovative (3, 4, 6, 7, 33).

According to our statistical analysis, GOLPH3 expression assessed using the IRS scale was higher in the material from the metastatic lymph node than from the prostate (IRS score: 8 vs. 6; p = 0,056), which may suggest that it plays a significant role not only in proliferation and cell cycle regulation, but also in the formation of distant metastases. Our study also found a positive correlation

TABLE 3 Basic descriptive statistics of the evaluation of GOLPH3 expression in prostate and metastatic lymph node tissues and the results of comparisons (N = 78).

GOLPH3 expression (IRS scale)	Prostate	Metastatic lymph node	p-value
A - Percentage of GOLPH3 positive cancer cells (score)			**0.046**
***Me* [Q1; Q3]**	3 [3; 4]	4 [3; 4]	
Min - Max	2 - 4	2 - 4	
B - Intensity of staining (score)			0.278
***Me* [Q1; Q3]**	2 [1; 2]	2 [2; 2]	
Min - Max	1 - 3	1 - 3	
IRS score (A × B)			0.056
***Me* [Q1; Q3]**	6 [4; 8]	8 [6; 8]	
Min - Max	2 - 12	2 – 12	
GOLPH3 expression level:			0.148
Low expression (1 – 7 score), n (%)	47 (60.3)	37 (47.4)	
High expression (8 – 12 score), n (%)	31 (39.7)	41 (52.6)	

IRS, immunoreactive scale; A, percentage of positive cancer cells (value from IRS scale); B, staining intensity (value from IRS scale); Me, median; Q1, lower quartile; Q3, upper quartile; Min, minimum; Max, maximum; n, number; %, percentage.

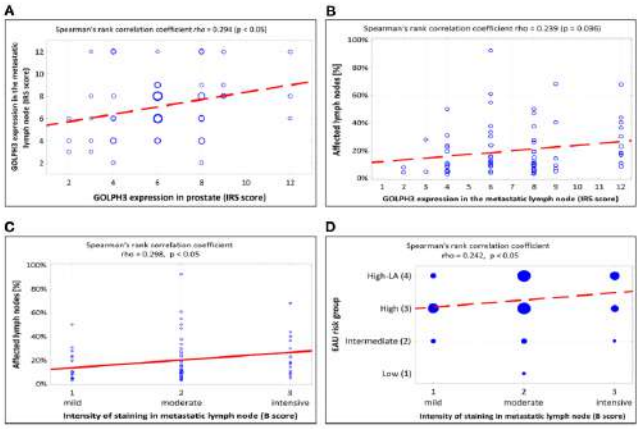

FIGURE 4
Summary of scatterplots and Spearman rank correlation coefficients. **(A)** Correlation between GOLPH3 expression in the metastatic lymph node and GOLPH3 expression in prostate (IRS score). **(B)** Correlation between the percentage of affected lymph nodes and GOLPH3 expression in the metastatic lymph node (IRS score). **(C)** Correlation between the percentage of affected lymph nodes and intensity of staining in metastatic lymph node (B-score in IRS scale). **(D)** Correlation between the EAU risk group and intensity of staining in metastatic lymph node (B-score in IRS scale). IRS - Immunoreactive scale, EAU - European Association of Urology.

between the level of GOLPH3 expression in prostate tissue and metastatic lymph node tissue (rho = 0,294). Future lymph node examination of GOLPH3 expression might be a promising direction in tissue marker diagnostics, considering its increased nodal expression compared to that in prostate specimens. GOLPH3 involvement in metastasis has already been demonstrated in a study by Song et al. (34), where GOLPH3 overexpression correlated positively with clinicopathological characteristics, such as nodal status ($p = 0.007$), in patients with non-small-cell lung cancer (NSCLC). In the same study, the authors reported that NSCLC cells expressing GOLPH3 at high level injected into the tail vein of a mouse model presented higher metastatic capabilities than GOLPH3-silenced cells. Moreover, the migratory and invasive abilities of NSCLC cells were significantly higher in GOLPH3-overexpressing cell lines. We found a positive correlation between the level of GOLPH3 expression on both the IRS scale and the intensity of staining in the metastatic lymph nodes and the percentage of total lymph nodes with metastases. These results suggest that GOLPH3 may be an important factor in pre-metastatic niche formation; however, since correlation does not necessarily constitute causation, more data and biological proof are needed to prove this hypothesis. In the future, this observation may also be helpful in improving the models used to determine the risk of nodal metastases of PCa, such as the Briganti nomogram. This issue is extremely important in the decision-making process regarding the determination of the indications for lymphadenectomy, which significantly increases the risk of surgery and potential complications (12, 35–37).

GOLPH3 is also considered a negative prognostic factor in patients with PCa. In a study by El-Maqsoud et al., patients with high GOLPH3 expression had a higher Gleason score and disease stage. Moreover, moderate or intense marker levels were the sole

TABLE 4 Correlation analysis between GOLPH3 expression in prostate and metastatic lymph node assessed in IRS score and quantitative variables.

	Prostate		Metastatic lymph node	
	rho	p	rho	p
Preoperative PSA (ng/ml)	-0.016	0.885	0.100	0.380
Affected lymph nodes (%)	0.137	0.230	0.239	0.036
Age (years)	-0.121	0.286	-0.030	0.795
BMI (kg/m^2)	0.126	0.271	-0.139	0.223
EAU risk group	0.045	0.692	0.183	0.109
Postoperative GGG ISUP	0.002	0.989	0.055	0.632

BMI, body mass index; PSA, prostate specific antigen; EAU, European Association of Urology; %, percentage; GGG ISUP, International Society of Urological Pathology (ISUP) 2014 grade (group) system.

TABLE 5 Number (percentage) of patients in groups differing in the level of GOLPH3 expression (based on IRS score) in the material from the prostate or metastatic lymph node, risk factors, and results of tests of independence.

GOLPH3 expression level (IRS score based)							
Variables		Expression of GOLPH3 in PROSTATE			Expression of GOLPH3 in METASTATIC LYMPH NODE		
		Level of expression		p-value	Level of expression		p-value
		High N = 31	Low N = 47		High N = 41	Low N = 37	
		n (%)	n (%)		n (%)	n (%)	
pT	3a and 3b	30 (96.7)	38 (80.8)	0.087	35 (85.4)	33 (89.2)	0.869
	2a and 2c	1 (3.2)	9 (15.2)		6 (14.6)	4 (10.8)	
ECE of prostate	Yes	28 (90.3)	39 (83.0)	0.511	33 (80.5)	34 (91.9)	0.199
	No	3 (9.7)	8 (17.0)		8 (19.5)	3 (8.1)	
Resection margin	Positive	22 (71.0)	33 (70.2)	0.855	28 (68.3)	27 (73.0)	0.804
	Negative	9 (29.0)	14 (29.8)		13 (31.7)	10 (27.0)	
ECE of lymph node	Yes	6 (19.4)	13 (27.7)	0.435	11 (26.8)	8 (21.6)	0.610
	No	25 (80.6)	34 (72.3)		30 (73.2)	29 (78.4)	
NVI	Yes	27 (100.0)	44 (97.8)	1.000	38 (100.0)	33 (97.1)	0.472
	No	0 (0.0)	1 (2.2)		0 (0.0)	1 (2.9)	
LVI	Yes	22 (78.6)	35 (77.8)	1.000	31 (83.8)	26 (72.2)	0.269
	No	6 (21.4)	10 (22.2)		6 (16.2)	10 (27.8)	
Efficacy of RP	Yes	12 (48.0)	25 (61.0)	0.321	20 (62.5)	17 (50.0)	0.332
	No	13 (52.0)	16 (39.0)		12 (37.5)	17 (50.0)	
Expression of GOLPH3 in metastatic lymph node	High	20 (64.5)	21 (44.7)	0.107	XX	XX	XX
	Low	11 (35.5)	26 (55.3)		XX	XX	
Expression of GOLPH3 in prostate	High	XX	XX	XX	20 (48.8)	11 (29.7)	0.107
	Low	XX	XX		21 (51.2)	26 (70.3)	

IRS, immunoreactive scale; n, number; %, percentage; pT, pathological tumor stage; ECE, extracapsular extension; NVI, neurovascular invasion; LVI, lymphovascular invasion; efficacy of RP, defined as an PSA level <0.1 ng/ml at the first measurement after radical prostatectomy.

predictors of overall survival (5). Overexpression of GOLPH3 was also associated with the transition of PCa from hormone-sensitive to hormone-resistant and shorter disease-free survival and overall survival (6). In our study, we found that LVI tended to be more common in patients with a higher percentage of GOLPH3-positive cells ($p = 0.02$). Although we failed to demonstrate a correlation between GOLPH3 expression level and oncological outcome, LVI was established as a negative prognostic factor in patients with PCa (38–40). We also found a positive association between the intensity of GOLPH3 staining in the metastatic lymph nodes and EAU classification (rho = 0.242), which was also highlighted in previous studies (3, 5). In the subject of the use of GOLPH3 expression assessment as a prognostic parameter, in our study, we found a significant correlation between GOLPH3 expression and efficacy of RP. The higher the percentage of GOLPH3 positive cancer cells ("A" score in IRS scale) in the prostate and the higher the staining intensity ("B" score in IRS scale) in the metastatic lymph node, the efficacy of RP was lower ($p<0.05$). We define efficacy of RP as a PSA level <0.1 ng/ml at the first measurement after RP, approximately 6 weeks after surgery (this value was also used to define so-called persistent PSA). Persistent PSA after RP occurs in 5–20% of patients and may result from various causes, including pre-existing metastases or residual benign prostate tissue (41, 42). Studies have shown that persistent PSA after RP is associated with more advanced disease characteristics (for example, higher pathologic stage, positive nodal status, or pathologic ISUP grade > 3) and poorer prognosis (worse 5-year biochemical recurrence-free survival and ten-years overall survival than patients without persistent PSA after RP) (43). Also, detectable PSA after RP (>0.1 ng/ml) significantly increases the risk of metastasis formation (44, 45). Although not all patients with persistent PSA experience disease recurrence (46), it remains a significant factor in predicting adverse oncological outcomes.

We are aware of the limitations of this study. Firstly, it was conducted on a relatively small group of patients, which could have resulted in a lack of statistical power to detect subtle differences and

TABLE 6 Number (percentage) of patients in groups differing in the percentage of GOLPH3 positive prostate cancer cells ("A" score in IRS scale) in the material from the prostate or metastatic lymph node, risk factors, and results of chi-square tests of independence.

Percentage of GOLPH3 positive prostate cancer cells ("A" score in IRS scale)															
Variables		Percentage of GOLPH3 positive prostate cancer cells in PROSTATE							Percentage of GOLPH3 positive prostate cancer cells in METASTATIC LYMPH NODE						
		A score (% ranges of GOLPH3 positive cancer cells)						p-value	A score (% ranges of GOLPH3 positive cancer cells)						p-value
		2 (11-50%) N = 4		3 (51-80%) N = 37		4 (>80%) N = 37			2 (11-50%) N = 2		3 (51-80%) N = 26		4 (>80%) N = 50		
		n	(%)	n	(%)	n	(%)		n	(%)	n	(%)	n	(%)	
pT	3a and 3b	3	75.0	30	81.1	35	94.6	0.167	2	100.0	22	84.6	44	88.0	0.788
	2a and 2c	1	25.0	7	18.9	2	5.4		0	0.0	4	15.4	6	12.0	
ECE of prostate	Yes	3	75.0	30	81.1	34	91.9	0.333	2	100.0	23	88.5	42	84.0	0.734
	No	1	25.0	7	18.9	3	8.1		0	0.0	3	11.5	8	16.0	
Resection margin	Positive	2	50.0	24	64.9	29	78.4	0.290	2	100.0	16	61.5	37	74.0	0.344
	Negative	2	50.0	13	35.1	8	21.6		0	0.0	10	38.5	13	26.0	
ECE of lymph node	Yes	1	25.0	10	27.0	8	21.6	0.863	0	0.0	8	30.8	11	22.0	0.503
	No	3	75.0	27	73.0	29	78.4		2	100.0	18	69.2	39	78.0	
NVI	Yes	4	100.0	34	97.1	33	100.0	0.585	1	100.0	23	95.8	47	100.0	0.363
	No	0	0.0	1	2.9	0	0.0		0	0.0	1	4.2	0	0.0	
LVI	Yes	4	100.0	26	74.3	27	79.4	0.484	0	0.0	18	75.0	39	83.0	**0.020**
	No	0	0.0	9	25.7	7	20.6		2	100.0	6	25.0	8	17.0	
Efficacy of RP	Yes	2	50.0	24	75.0	11	36.7	**0.010**	2	100.0	11	44.0	24	61.5	0.172
	No	2	50.0	8	25.0	19	63.3		0	0.0	14	56.0	15	38.5	
Expression of GOLPH3 in metastatic lymph node	High	0	0.0	22	59.5	19	51.4	**0.035**	XX	XX	XX	XX	XX	XX	XX
	Low	4	100.0	15	40.5	18	48.6		XX	XX	XX	XX	XX	XX	
Expression of GOLPH3 in prostate	High	XX	XX	XX	XX	XX	XX	XX	1	50.0	7	26.9	23	46.0	0.261
	Low	XX	XX	XX	XX	XX	XX		1	50.0	19	73.1	27	54.0	

IRS, immunoreactive scale; n, number; %, percentage; pT, pathological tumor stage; ECE, extracapsular extension; NVI, neurovascular invasion; LVI, lymphovascular invasion; efficacy of RP, defined as an PSA level <0.1 ng/ml at the first measurement after radical prostatectomy.

may have introduced bias. Secondly, there was an absence of follow-up data for patients who underwent RP. The lack of long-term data hampers our ability to evaluate the impact of the expression level of GOLPH3 on patient outcomes, such as biochemical recurrence (BCR) or overall survival. Thirdly, the expression of the GOLPH3 evaluation method employed in this study, the IRS scale, has certain limitations that require further improvement. To address this issue, future studies should consider the use of the H-score method (47, 48) (requiring more experience from the uropathologist, but allowing for a more detailed assessment of the material, taking into account even the heterogeneity of staining within one sample) as a method of assessing GOLPH3 expression in preparations. The last limitation of the study was that we tested GOLPH3 expression only in PCa tissue without comparison with the control group; for example, normal prostatic tissue adjacent to tumor cells obtained via biopsies, tissues of benign prostatic hyperplasia after transurethral resection of the prostate (TURP) or lymph nodes of patients after RP without metastases. However, the limitations we identified should not detract from the strengths of our study, which originate from its innovative nature and the rigorous methodology applied. The unique feature of our study is that it was the first time that GOLPH3 expression was tested in PCa metastatic lymph nodes; however, we see a need and plan to extend our study in the future with the above-described comparison to a control group. This will further define the role of GOLPH3 in PCa and its potential clinical implications.

TABLE 7 Number (percentage) of patients in groups differing in the intensity of staining in GOLPH3 positive prostate cancer cells ("B" score in IRS scale) in the material from the prostate or metastatic lymph node, risk factors, and results of chi-square tests of independence.

Intensity of staining in GOLPH3 positive prostate cancer cells ("B" score in IRS scale)															
Variables		Intensity of staining in GOLPH3 positive prostate cancer cells in PROSTATE							Intensity of staining in GOLPH3 positive prostate cancer cells in METASTATIC LYMPH NODE						
		B score (staining intensity)						p-value	B score (staining intensity)						p-value
		1 (mild) N = 20		2 (moderate) N = 47		3 (intensive) N = 11			1 (mild) N = 19		2 (moderate) N = 41		3 (intensive) N = 18		
		n	(%)	n	(%)	n	(%)		n	(%)	n	(%)	n	(%)	
pT	3a and 3b	18	90.0	39	83.0	11	100.0	0.286	18	94.7	33	80.5	17	94.4	0.177
	2a and 2c	2	10.0	8	17.0	0	0.0		1	5.3	8	19.5	1	5.6	
ECE of prostate	Yes	18	90.0	39	83.0	10	90.9	0.658	17	89.5	34	82.9	16	88.9	0.729
	No	2	10.0	8	17.0	1	9.1		2	10.5	7	17.1	2	11.1	
Resection margin	Positive	15	75.0	32	68.1	8	72.7	0.838	16	84.2	27	65.9	12	66.7	0.321
	Negative	5	25.0	15	31.9	3	27.3		3	15.8	14	34.1	6	33.3	
ECE of lymph node	Yes	6	30.0	12	25.5	1	9.1	0.412	3	15.8	9	22.0	7	38.9	0.229
	No	14	70.0	35	74.5	10	90.9		16	84.2	32	78.0	11	61.1	
NVI	Yes	18	94.7	43	100.0	10	100.0	0.243	16	94.1	39	100.0	16	100.0	0.194
	No	1	5.3	0	0.0	0	0.0		1	5.9	0	0.0	0	0.0	
LVI	Yes	16	84.2	34	77.3	7	70.0	0.665	12	66.7	32	80.0	13	86.7	0.350
	No	3	15.8	10	22.7	3	30.0		6	33.3	8	20.0	2	13.3	
Efficacy of RP	Yes	7	41.2	25	61.0	5	62.5	0.356	8	50.0	26	68.4	3	25.0	**0.026**
	No	10	58.8	16	39.0	3	37.5		8	50.0	12	31.6	9	75.0	
Expression of GOLPH3 in metastatic lymph node	High	7	35.0	24	51.1	10	90.9	**0.011**	XX	XX	XX	XX	XX	XX	XX
	Low	13	65.0	23	48.9	1	9.1%		XX	XX	XX	XX	XX	XX	
Expression of GOLPH3 in prostate	High	XX	XX	XX	XX	XX	XX	XX	6	31.6	16	39.0	9	50.0	0.515
	Low	XX	XX	XX	XX	XX	XX		13	68.4	25	61.0	9	50.0	

IRS, immunoreactive scale; n, number; %, percentage; pT, pathological tumor stage; ECE, extracapsular extension; NVI, neurovascular invasion; LVI, lymphovascular invasion; efficacy of RP, defined as an PSA level <0.1 ng/ml at the first measurement after radical prostatectomy.

5 Conclusions

GOLPH3 is expressed in both the prostate and metastatic lymph nodes, with higher expression in the metastatic lymph nodes (however, this difference was not statistically significant, p=0.056) and a positive correlation between GOLPH3 expression levels in the prostate and metastatic lymph nodes, suggesting a potential connection between primary and metastatic tumors. High GOLPH3 expression is associated with LVI, the percentage of all metastatic lymph nodes, and the high-risk group in the EAU classification, but there was no significant correlation between GOLPH3 expression levels and the other pathological features or postoperative outcomes of patients. Further research is needed to understand the functional significance and potential clinical applications of GOLPH3 in prostate cancer.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethics Committee of Wroclaw Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from a by- product of routine care or industry. Written informed consent for participation was not

required from the participants or the participants' legal guardians/ next of kin in accordance with the national legislation and institutional requirements.

Author contributions

PK: Conceptualization, Investigation, Methodology, Writing – original draft. MK: Conceptualization, Investigation, Methodology, Validation, Writing – review & editing. KK: Resources, Writing – original draft. AP: Conceptualization, Methodology, Visualization, Writing – review & editing. ŁN: Data curation, Resources, Writing – review & editing. WK: Resources, Writing – review & editing. AG: Writing – review & editing. KD: Data curation, Writing – review & editing. PD: Methodology, Supervision, Writing – review & editing. AH: Methodology, Supervision, Writing – review & editing. TS: Project administration, Supervision, Writing – review & editing. BM: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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7.3 Role of IL-17A and IL-17RA in Prostate Cancer with Lymph Nodes Metastasis: Expression Patterns and Clinical Significance.

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Simple Summary: Prostate cancer (PCa) is the second most common type of cancer among men. The expression of IL-17A cytokine and its receptor IL-17RA may be used to predict the risk of aggressive prostate cancer. We examined the clinical data of 77 patients with PCa and lymph node metastasis (LN+) and then evaluated the levels of IL-17A and IL-17RA expression in the prostate and LN+. We found significant correlations between the investigated markers' expression levels in examined tissues and clinical data, such as body mass index (BMI), the percentage of involved lymph nodes, or the European Association of Urology (EAU) risk group. The findings of this study suggest that IL-17A and IL-17RA may be useful in predicting the risk of aggressive prostate cancer; however, further studies are needed to determine their roles and potential clinical applications.

Abstract: Prostate cancer (PCa) is the second most frequently diagnosed cancer among men. The use of IL-17A and its receptor IL-17RA as prognostic markers for PCa has shown promising results. We analyzed the clinical data of 77 patients with PCa after radical prostatectomy with lymphadenectomy and lymph node metastasis (LN+). We assessed the expression levels of IL-17A and IL-17RA in cancer cells in prostate and, for the first time, also in LN+. Prostate IL-17A expression positively correlated with BMI ($p = 0.028$). In LN+, the expression of IL-17A was positively correlated with the percentage of affected lymph nodes ($p = 0.006$) and EAU risk groups ($p = 0.001$). Additionally, in the group with high IL-17A expression in LN+, the extracapsular extension (ECE) of the prostate was significantly more frequent ($p = 0.033$). Also, significant correlations with the level of IL-17RA expression was found—expression was higher in prostate than in LN+ ($p = 0.009$); in LN+, expression positively correlated with the EAU risk group ($p = 0.045$), and in the group of high expression in LN+ ECE of lymph nodes was detected significantly more often ($p = 0.009$). Our findings support the potential role of IL-17A and IL-17RA as PCa markers; however, further studies are needed to determine their roles and potential clinical applications.

Keywords: IL-17; IL-17A; IL-17RA; prostate cancer; lymph nodes metastases; radical prostatectomy

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1. Introduction

Prostate cancer (PCa) is one of the major causes of cancer-related deaths in the male population and the second most frequently diagnosed cancer in men worldwide [1]. Owing

to longer life expectancies and the fact that PCa incidence increases with patient age, there will be an increase in the number of PCa patients. The impact on society's health will be even greater than it is now [2]. Despite the availability of therapy protocols that are constantly being improved, selecting the best course of action for a particular patient is challenging and the outcome is unpredictable. This is because more accurate tools are still lacking for determining survival prognosis and the likelihood of progression or metastasis following primary PCa treatment. Lymph node metastases are a significant risk factor for PCa patients and have a significant negative effect on survival and the risk of recurrence after primary treatment. Through the selection of appropriate adjuvant therapy and more stringent follow-up after primary therapy, nodal metastases also have an impact on the therapeutic process in patients [3,4]. Despite intensive improvements in technology, lymphadenectomy persists as a superior method in comparison to the use of radiological imaging techniques for the detection of positive (metastatic) lymph nodes (LN+) [5,6]. Radical prostatectomy (RP) with extended pelvic lymphadenectomy is the gold standard for identifying LN+. However, this is an additional challenging step added to an already complex operation, that is, RP. It is important to emphasize that lymphadenectomy does not improve survival and greatly increases the risk of side effects (e.g., longer hospital stay, increased blood loss, and a higher probability of lymphocele development) [7]. Extended pelvic lymphadenectomy should be performed in patients with intermediate- and high-risk PCa in the absence of more precise techniques to assess the lymph node status [8].

Inflammation is considered an increasingly important factor in the pathogenesis of many cancers, including PCa [9]. The inflammatory response is a complex process involving many different cells of the immune system and the chemokines and cytokines produced by them. Active oxygen and nitrogen radicals formed during inflammation are believed to be responsible for the suppression of antitumor activity and stimulation of carcinogenesis [10,11]. This inflammatory response probably promotes the survival, proliferation, and spread of tumor cells [12,13]. This is particularly important for the formation of PCa metastases. Many studies have shown a link between prostatitis and increased risk of developing PCa. This relationship has been observed in relation to chronic and acute prostatitis [14–16]. In addition, the occurrence of inflammation (mainly chronic) in patients with benign prostatic hyperplasia increases the risk of developing PCa, especially high-grade tumors [17]. Some authors also indicate that the evidence of the significant role of the inflammatory process in the development of PCa are studies that have shown that the use of antioxidants and anti-inflammatory drugs may reduce the risk of PCa [18,19].

Many different factors affect the treatment outcomes and prognosis of patients with PCa. This is due to the high heterogeneity of prostate tumors, which results in different treatment effects between patients. Currently, to determine the risk of progression, we rely on predictors such as the prostate-specific antigen (PSA) level or the stage and histological grade of PCa determined in the prostate biopsy material. In recent years, research on potentially new markers that may complement these known predictors has been gaining increasing interest. Preliminary conclusions from these analyses of the expression of immunohistochemical (IHC) markers in PCa, such as IL-17A and its receptor IL-17RA, suggest their potential usefulness in the process of improving diagnostics, determining the risk of progression (including metastasis), and response to primary and adjuvant treatment. It should be noted, however, that despite promising results, there are still too few unambiguous studies confirming the usefulness of these potential new PCa prognostic markers in clinical practice [20]. Therefore, routine assessment of their expression is currently not recommended by the urological guidelines.

One of the most important pro-inflammatory cytokines is IL-17. It is secreted by various immune cells, including helper T 17 cells and NK cells. Its precise effect on cancer pathogenesis is still not fully understood. According to the existing evidence, IL-17 has been suggested to promote angiogenesis, inhibit cancer cell apoptosis, and enhance cancer cell proliferation. Additionally, it has been hypothesized that IL-17 affects the development of a microenvironment favorable for cancer growth and potential metastasis [21]. Research

has shown that it promotes the growth of colorectal, breast, pancreatic, and PCa cancers [22]. IL-17 is a cytokine family comprising six ligands (IL-17A–IL-17F) and five receptors (IL-17RA–IL-17RE) [23]. In this study, we examined IL-17A and its receptor IL-17RA. However, it is unclear how IL-17 contributes to PCa pathogenesis. Various studies have shown an increased expression of IL-17A and IL-17RA receptor in PCa and BPH cells [24–26]. According to previous studies, IL-17 has a stimulatory effect on PCa growth and metastasis even under castration conditions [27–29]. The results of various studies on the expression of individual ligands and IL-17 receptors in PCa remain unclear. For instance, in a relatively recent study, an increased expression of IL-17 was observed in low-grade PCa and BPH, whereas no expression of the IL-17RA receptor was detected in the tested material [30].

In this study, we extensively investigated the expression of IL-17A and IL-17RA in PCa cells from primary tumor tissues and LN+. Our study is unique because it is the first to analyze the expression of IL-17A and IL-17RA in LN+. To evaluate the utilization of the investigated markers as potential new negative risk factors for PCa progression, we compared the obtained results with the clinical data of patients with LN+.

2. Materials and Methods

2.1. Patients Selection

In this study, we included 77 patients with PCa who had lymph node metastases in the postoperative material. Between January 2012 and September 2018, all patients underwent RP with extended lymphadenectomy at the University Urology Center, Wroclaw, Poland. A retrospective clinical data analysis was performed on the study participants, and histopathological specimens collected during RP were selected for additional examination. An experienced uropathologist examined the selected specimens. The 2017 PCa Tumor, Node, Metastasis (TNM) classification and the Gleason system were used to evaluate tumor stage and grade. Additionally, classifications, including the European Association of Urology (EAU) risk categories for biochemical recurrence of localized and locally advanced PCa and the International Society of Urological Pathology (ISUP) 2014 grade (group) system, were used to better categorize patients. A PSA level 0.1 ng/mL at the first measurement after RP, typically six weeks after surgery, was used to determine the radicality of the procedure.

2.2. Tissue Microarrays (TMAs) and Immunohistochemical (IHC) Staining

For this study, we prepared histopathological samples for immunohistochemical staining and its further examination using the tissue microarrays (TMAs) technique. Sixteen TMAs were created for our study. The donor blocks were paraffin blocks containing material from the prostate with PCa or LN+. Donor blocks were then used to create histopathological slides stained with hematoxylin and eosin (HE). A Pannoramic Midi II histological scanner (3DHISTECH Ltd., Budapest, Hungary) was used to scan slides. Representative areas from the entire section were selected by a uropathologist using the Panoramic Viewer Program (3DHISTECH Ltd.). In order to further increase the representativeness of each case, 3 representative cores with a size of 1.5 mm from the donor block were chosen and transferred to the TMA'recipient' block using the TMA Grand Master (3DHISTECH Ltd.).

IHC reactions were performed on 4 µm TMA paraffin sections using an Autostainer Link48 (Dako, Glostrup, Denmark). Deparaffinization, rehydration, and antigen retrieval were performed using EnVision FLEX Target Retrieval Solution, High pH (97 °C, 20 min; pH 9), in PTLink (Dako). Endogenous peroxidase was blocked using the EnVision FLEX Peroxidase-Blocking Reagent (Dako) for 5 min. Primary antibodies—polyclonal rabbit anti-IL-17/IL-17A antibody (1:1600, cat. no NBP1-76337, Novus Biologicals, Minneapolis, MN, USA) and monoclonal mouse anti-IL17RA/IL-17R antibody (1:200, cat. No NBP2-25258, Novus Biologicals)—were applied for 20 min. Following this, the secondary antibody, conjugated with horseradish peroxidase (EnVision FLEX/HRP—20 min incubation), was applied. 3,3′-diaminobenzidine (DAB, Dako) was used as the peroxidase substrate, and the sections were incubated for 10 min. Finally, all sections were counterstained for 5 min with

EnVision FLEX Hematoxylin (Dako). After dehydration in ethanol (70%, 96%, absolute) and xylene, all slides were closed with coverslips in SUB-X Mounting Medium in a coverslipper. The primary antibodies were diluted in the EnVision FLEX Antibody Diluent (Dako). The slides were scanned using a histologic scanner, Pannoramic MIDI (3DHistech). Reactions were evaluated with the use of Quant Center software (3DHistech) under researcher supervision. In order to evaluate the expression of IL-17A and IL-17RA, for every case, six TMA cores (3 from prostate and 3 from metastatic lymph node) were assessed using a Pannoramic Viewer Digital image analysis.

Next, an experienced uropathologist who did not have access to patient clinical data assessed IL-17A and IL-17RA expression using the immunoreactive scale (IRS) developed by Remmele and Stegner [31,32] presented in Table 1.

Table 1. Immunoreactive scale (IRS) by Remmele and Stegner. IRS score taking into account the percentage of positively stained prostate cancer cells (A) and the intensity of staining (B), and the final score is the result of multiplying these values (A × B). Based on the IRS score, patients were divided into groups of low and high IL-17A and IL-17RA expressions, respectively, as presented.

Immunoreactive Scale (IRS)			
A—Percentage of Positive Cancer Cells		B—Staining Intensity	
Score		Score	
0	no cells with positive reaction	0	no color reaction
1	<10% cells with positive reaction	1	mild reaction
2	10–50% cells with positive reaction	2	moderate reaction
3	51–80% cells with positive reaction	3	intense reaction
4	>80% cells with positive reaction		
IRS SCORE (A X B): 0–12 points			
Final score		Level of expression	
1–7		Low expression	
8–12		High expression	

The final IRS score was determined by multiplying the percentage of stained PCa cells ("A" score) with the staining intensity ("B" score). The prostate and LN+ samples from each patient were assessed independently. The final IRS score for the prostate and LN+ was calculated using the average score obtained from the assessment of each of the three cores of a specific tissue type.

2.3. Statistical Analysis

For quantitative variables, the mean, standard deviation (SD), minimum (Min), maximum (Max), median (Me), lower (Q1), and upper (Q3) quartiles were calculated. The empirical distribution of quantitative variables was examined to fit a normal distribution using the Kolmogorov–Smirnov and Shapiro–Wilk tests. Spearman's rank correlation coefficient was calculated to assess the relationship between monotonic relationships between variables. Qualitative (nominal and categorical) variables were presented in contingency tables as numbers (n) and percentages (%). The significance of differences in quantitative parameters between the two groups was assessed using the Mann–Whitney U test, and the independence of the two qualitative factors was established using Pearson's chi-squared test. In all analyzed cases, the associations were considered statistically significant at $p < 0.05$. Statistica v.13.3 (TIBCO Software Inc., Palo Alto, CA, USA) was used for all statistical analyses.

3. Results

The general characteristics of the patients are presented in Table 2.

Table 2. General characteristics and clinicopathological parameters of the patients. M—arithmetic mean, SD—standard deviation, BMI—body mass index, PSA—prostate-specific antigen, Me—median, Q1—lower quartile, Q3—upper quartile, EAU—European Association of Urology, n—number, %—percentage, pT—pathological tumor stage, GGG ISUP—International Society of Urological Pathology (ISUP) 2014 grade (group) system, radical procedure—defined as a PSA level <0.1 ng/mL at the first measurement after radical prostatectomy.

Variable	Statistics
General characteristics of patients	
Age (years):	
$M \pm SD$	64.9 ± 5.5
BMI (kg/m^2):	
$M \pm SD$	28.1 ± 3.7
Preoperative PSA (ng/mL):	
Me (Q1; Q3)	19.8 (12; 36.1)
EAU risk group, *n* (%):	
Low-risk	1 (1.3)
Intermediate-risk	8 (10.4)
High-risk	38 (49.3)
High-risk locally advanced	30 (39)
Clinicopathological parameters	
pT, n (%):	
2a	1 (1.3)
2c	9 (11.7)
3a	14 (18.2)
3b	53 (68.8)
Postoperative Gleason, n (%):	
3 + 3	1 (1.3)
3 + 4	10 (13)
3 + 5	4 (5.2)
4 + 3	19 (24.7)
4 + 4	3 (3.9)
4 + 5	29 (37.6)
5 + 3	2 (2.6)
5 + 4	8 (10.4)
5 + 5	1 (1.3)
Postoperative GGG ISUP, n (%):	
1	1 (1.3)
2	10 (13)
3	19 (24.7)
4	9 (11.7)
5	38 (48.3)
Extracapsular extension of prostate, n (%):	
Yes	66 (85.7)
No	11 (14.3)
Extracapsular extension of lymph node, n (%):	
Yes	19 (24.7)
No	58 (75.3)
Resection margin, n (%):	
Positive	54 (70.1)
Negative	23 (29.9)
Neurovascular invasion, n (%):	
Yes	70 (90.9)
No	1 (1.3)
No data	6 (7.8)

Table 2. *Cont.*

Variable	Statistics
Lymphovascular invasion, n (%):	
Yes	57 (74)
No	15 (19.5)
No data	5 (6.5)
Affected lymph nodes (%):	
Me (Q1; Q3)	12.5 (8.3; 27.3)
Radical procedure, n (%):	
Yes	36 (46.7)
No	41 (53.3)

3.1. IL-17A

IL-17A expression in the prostate and LN+ was found in 98.7% (n = 76) and 100% (n = 77) of patients, respectively. Figure 1 shows a comparison of IL-17A expression levels in the prostate and LN+.

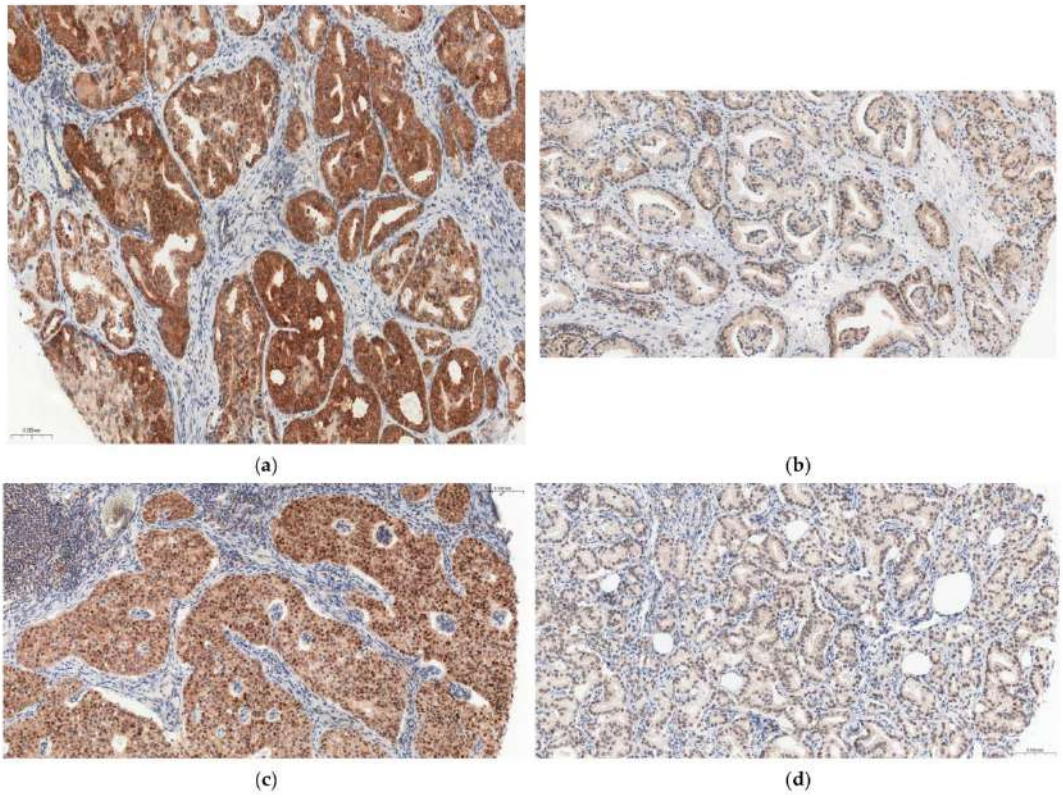


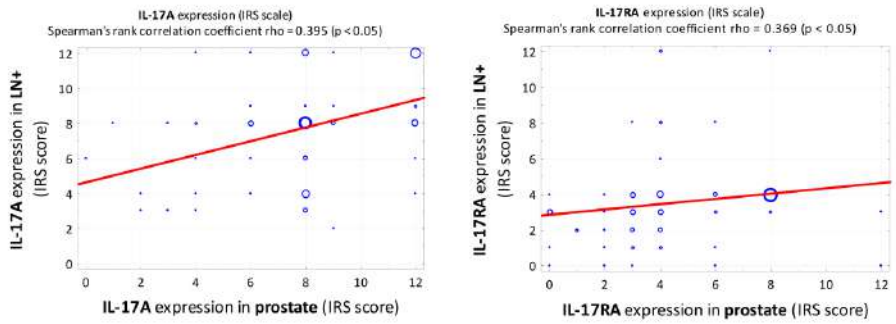
Figure 1. Comparison of IL-17A expression levels in the prostate and metastatic lymph nodes. (**a**) High IL-17A expression in prostate tissue; (**b**) Low IL-17A expression in prostate tissue; (**c**) High IL-17A expression in metastatic lymph node; (**d**) Low IL-17A expression in metastatic lymph node. Magnification, ×15.

As presented in Table 3, IL-17A expression levels were comparable in the prostate and LN+ ($p = 0.415$), with no statistically significant difference between the percentage of positively stained cancer cells ($p = 0.634$) and intensity of staining ($p = 0.446$).

Table 3. Basic descriptive statistics of the evaluation of IL-17A and IL-17RA expression in prostate and metastatic lymph node tissues and the results of comparisons. IRS—immunoreactive scale, A—percentage of positive cancer cells (value from IRS scale), B -staining intensity (value from IRS scale), Me—median, Q1—lower quartile, Q3—upper quartile, Min—minimum, Max—maximum, n—number, %—percentage.

	Expression (IRS Scale)					
	IL-17A			**IL-17RA**		
	Prostate	**Metastatic Lymph Node**	***p*-Value**	**Prostate**	**Metastatic Lymph Node**	***p*-Value**
A—Percentage of positively stained cancer cells (score)			0.634			0.271
Me (Q1; Q3)	4 [3; 4]	4 [3; 4]		3 [3; 4]	3 [2; 4]	
Min–Max	0–4	2–4		0–4	0–4	
B—Intensity of staining (score)			0.446			0.112
Me (Q1; Q3)	2 [2; 3]	2 [2; 3]		1 [1; 2]	1 [1; 1]	
Min–Max	0–3	1–3		0–3	0–3	
IRS score (A × B)			0.415			0.009
Me (Q1; Q3)	8 [6; 12]	8 [6; 9]		4 [3; 6]	3 [2; 4]	
Min–Max	0–12	2–12		0–12	0–12	
Expression level:			0.308			0.012
Low expression (1–7 score), n (%)	19 (25)	25 (32.5)		52 (74.3)	65 (90.3)	
High expression (8–12 score), n (%)	57 (75)	52 (67.5)		18 (25.7)	7 (9.7)	

A statistically significant positive correlation was observed between IL-17A expression levels in the prostate and LN+ (rho = 0.395; Figure 2a).



(**a**) (**b**)

Figure 2. Summary of scatterplots and Spearman rank correlation coefficients. (**a**) Correlation between IL-17A expression in the metastatic lymph node and IL-17A expression in prostate (IRS score). (**b**) Correlation between IL-17A expression in the metastatic lymph node and IL-17A expression in prostate (IRS score). IRS—immunoreactive scale, LN+—metastatic/positive lymph node.

IL-17A expression (expressed by the IRS score) in the prostate and LN+ was not significantly correlated with patient age, postoperative GGG ISUP, or preoperative PSA level. IL-17A expression levels in the prostate and BMI were significantly positively correlated ($p = 0.028$). Additionally, a statistically significant positive connection between the level of IL-17A expression in LN+ and the percentage of the affected lymph nodes and the EAU risk group was found ($p = 0.006$ and $p = 0.001$, respectively). Table 4 presents the results of the statistical analyses.

Table 4. Correlation analysis between IL-17A and IL-17RA expression in prostate and metastatic lymph node assessed in IRS score and quantitative variables. BMI—body mass index, PSA—prostate-specific antigen, EAU—European Association of Urology, %—percentage, GGG ISUP—International Society of Urological Pathology (ISUP) 2014 grade (group) system.

	IL-17A				IL-17RA			
	Prostate		Metastatic Lymph Node		Prostate		Metastatic Lymph Node	
	rho	*p*	rho	*p*	rho	*p*	rho	*p*
Preoperative PSA (ng/mL)	0.033	0.778	0.027	0.813	0.057	0.623	0.100	0.387
Affected lymph nodes (%)	−0.007	0.952	0.312	0.006	0.100	0.385	0.144	0.211
Age (years)	−0.037	0.752	−0.037	0.749	0.047	0.682	0.019	0.873
BMI (kg/m^2)	0.251	0.028	0.013	0.912	0.096	0.404	0.079	0.494
EAU risk group	0.159	0.168	0.376	0.001	0.051	0.660	0.229	0.045
Postoperative GGG ISUP	−0.020	0.862	0.146	0.205	−0.111	0.335	−0.023	0.841

When analyzing the differences between the groups with low and high expressions of IL-17A (assessed based on the IRS score) and the pathological features or postoperative outcomes of patients, only one statistically significant correlation was detected between the extracapsular extension (ECE) of the prostate and the level of IL-17A expression in the LN+—it was significantly more common in the high expression group ($p = 0.033$). No statistically significant correlation was observed between these variables and IL-17A expression in the prostate (Table 5).

Next, the IRS scale variables—the percentage of IL-17A-positive cancer cells ("A" score in the IRS scale) and the intensity of staining IL-17A-positive cancer cells ("B" score in the IRS scale) in the prostate and LN+—were independently examined to further the analysis of the pathological characteristics or postoperative results of the patients. There was a correlation between the ECE of the lymph node and the percentage of IL-17A-positive cancer cells in the prostate ($p = 0.009$), as well as between the intensity of staining IL-17A-positive cancer cells in LN+ ($p = 0.014$).

3.2. IL-17RA

IL-17RA expression in the prostate and LN+ was found in 90.9% (n = 70) and 93.5% (n = 72) of patients, respectively. Figure 3 shows a comparison of IL-17RA expression levels in the prostate and LN+.

A statistically significant difference was observed in the level of IL-17RA expression between the prostate and LN+. The level of IL-17RA expression according to the IRS score was higher in the prostate than in LN+ (4 vs. 3; $p = 0.009$). In addition, the level of expression was significantly more often marked as low in the material from LN+ than in the prostate (90.3% vs. 74.3%; $p = 0.012$). IL-17RA, like IL-17A, showed a statistically significant positive correlation between expression in the prostate and expression in LN+ (rho = 0.369; Figure 2b). As shown in Table 4, there was only one statistically significant positive correlation between the EAU risk group and the level of IL-17RA expression (IRS

score) in LN+. No significant correlations were observed between the level of expression in the prostate and the previously mentioned quantitative variables.

Table 5. Number (percentage) of patients in groups differing in the level of IL-17A expression (based on IRS score) in the material from the prostate or metastatic lymph node, risk factors, and results of tests of independence. IRS—immunoreactive scale, n—number, %—percentage, pT—pathological tumor stage, ECE—extracapsular extension, NVI—neurovascular invasion, LVI—lymphovascular invasion, radical procedure—defined as a PSA level <0.1 ng/mL at the first measurement after radical prostatectomy.

IL-17A Expression Level (IRS Score-Based)							
Variables		Expression of IL-17A in PROSTATE			Expression of IL-17A in METASTATIC LYMPH NODE		
		Level of Expression		*p*-Value	Level of Expression		*p*-Value
		Low (N = 19)	High (N = 57)		Low (N = 25)	High (N = 52)	
		n (%)	*n* (%)		*n* (%)	*n* (%)	
pT	3a and 3b	15 (79.0%)	51 (89.5%)	0.257	20 (80.0%)	47 (90.4%)	0.279
	2a and 2c	4 (21.0%)	6 (10.5%)		5 (20.0%)	5 (9.6%)	
ECE of prostate	Yes	16 (84.2%)	49 (86.0%)	1.000	18 (72.0%)	48 (92.3%)	0.033
	No	3 (15.8%)	8 (14.0%)		7 (28.0%)	4 (7.7%)	
Resection margin	Positive	11 (57.9%)	42 (73.7%)	0.251	18 (72.0%)	36 (69.2%)	0.986
	Negative	8 (42.1%)	15 (26.3%)		7 (28.0%)	16 (30.8%)	
ECE of lymph node	Yes	6 (31.6%)	13 (22.8%)	0.543	3 (12.0%)	16 (30.8%)	0.094
	No	13 (68.4%)	44 (77.2%)		22 (88.0%)	36 (69.2%)	
NVI	Yes	16 (100.0%)	53 (98.2%)	1.000	20 (95.2%)	50 (100.0%)	0.296
	No	0 (0.0%)	1 (1.8%)		1 (4.8%)	0 (0.0%)	
LVI	Yes	12 (80.0%)	44 (78.6%)	1.000	16 (69.6%)	41 (83.7%)	0.288
	No	3 (20.0%)	12 (21.4%)		7 (30.4%)	8 (16.3%)	
Radical procedure	Yes	7 (43.8%)	28 (58.3%)	0.389	12 (57.1%)	24 (54.5%)	0.944
	No	9 (56.3%)	20 (41.7%)		9 (42.9%)	20 (45.5%)	
Expression of IL-17A in metastatic lymph node	Low	8 (42.1%)	16 (28.1%)	0.287	XX	XX	XX
	High	11 (57.9%)	41 (71.9%)		XX	XX	
Expression of IL-17A in prostate	Low	XX	XX	XX	8 (33.3%)	11 (21.2%)	0.178
	High	XX	XX		16 (66.7%)	41 (78.8%)	

After analyzing the results to identify potential differences between the groups with low and high expression of IL-17RA (based on the IRS scale) and the pathological features or postoperative outcomes of patients were found only in the case of the frequency of ECE of the lymph node. This phenomenon was more common in the group with high expression of IL-17RA (71.4% vs. 20.0%, $p = 0.009$). Similar to IL-17A, no statistically significant correlation was observed between these variables and IL-17RA expression in the prostate (Table 6).

Furthermore, as with IL-17A, an in-depth analysis of the IRS variables ("A" score and "B" score) used to assess IL-17RA expression was performed. There were no statistically significant differences in this regard in either the prostate or LN+ samples.

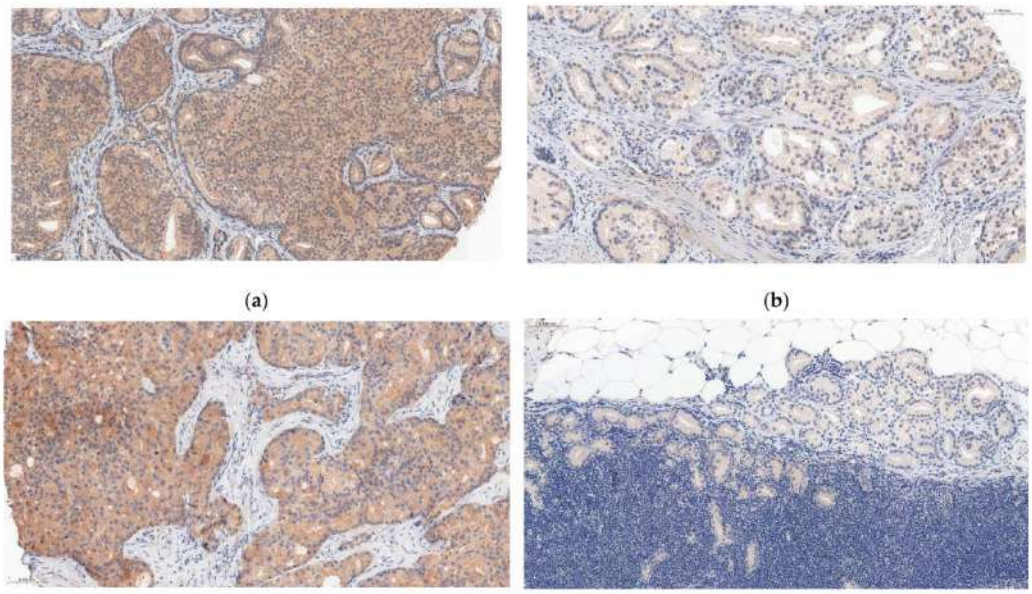


Figure 3. Comparison of IL-17RA expression levels in the prostate and metastatic lymph nodes. (**a**) High IL-17RA expression in prostate tissue; (**b**) Low IL-17RA expression in prostate tissue; (**c**) High IL-17RA expression in metastatic lymph node; (**d**) Low IL-17RA expression in metastatic lymph node. Magnification, ×15.

Table 6. Number (percentage) of patients in groups differing in the level of IL-17RA expression (based on IRS score) in the material from the prostate or metastatic lymph node, risk factors, and results of tests of independence. IRS—immunoreactive scale, n—number, %—percentage, pT—pathological tumor stage, ECE—extracapsular extension, NVI—neurovascular invasion, LVI—lymphovascular invasion, radical procedure—defined as a PSA level <0.1 ng/mL at the first measurement after radical prostatectomy.

IL-17RA Expression Level (IRS Score-Based)							
Variables		**Expression of IL-17RA in PROSTATE**			**Expression of IL-17RA in METASTATIC LYMPH NODE**		
		Level of Expression		***p*-Value**	**Level of Expression**		***p*-Value**
		Low (N = 52)	**High (N = 18)**		**Low (N = 65)**	**High (N = 7)**	
		***n* (%)**	***n* (%)**		***n* (%)**	***n* (%)**	
pT	3a and 3b	45 (86.5%)	16 (88.9%)	1.000	57 (87.7%)	6 (85.7%)	1.000
	2a and 2c	7 (13.5%)	2 (11.1%)		8 (12.3%)	1 (14.3%)	
ECE of prostate	Yes	47 (90.4%)	14 (77.8%)	0.222	58 (89.2%)	5 (71.4%)	0.209
	No	5 (9.6%)	4 (22.2%)		7 (10.8%)	2 (28.6%)	
Resection margin	Positive	37 (71.2%)	13 (72.2%)	1.000	45 (69.2%)	6 (85.7%)	0.665
	Negative	15 (28.8%)	5 (27.8%)		20 (30.8%)	1 (14.3%)	
ECE of lymph node	Yes	13 (25.0%)	3 (16.7%)	0.745	13 (20.0%)	5 (71.4%)	0.009
	No	39 (75.0%)	15 (83.3%)		52 (80.0%)	2 (28.6%)	
NVI	Yes	45 (97.8%)	18 (100.0%)	1.000	59 (100.0%)	7 (100.0%)	1.000
	No	1 (2.2%)	0 (0.0%)		0 (0.0%)	0 (0.0%)	

Table 6. *Cont.*

IL-17RA Expression Level (IRS Score-Based)							
Variables		**Expression of IL-17RA in PROSTATE**			**Expression of IL-17RA in METASTATIC LYMPH NODE**		
		Level of Expression		***p*-Value**	**Level of Expression**		***p*-Value**
		Low (N = 52)	**High (N = 18)**		**Low (N = 65)**	**High (N = 7)**	
		***n* (%)**	***n* (%)**		***n* (%)**	***n* (%)**	
LVI	Yes	40 (81.6%)	12 (70.6%)	0.491	48 (80.0%)	6 (85.7%)	1.000
	No	9 (18.4%)	5 (29.4%)		12 (20.0%)	1 (14.3%)	
Radical procedure	Yes	25 (58.1%)	8 (50.0%)	0.769	31 (57.4%)	2 (33.3%)	0.394
	No	18 (41.9%)	8 (50.0%)		23 (42.6%)	4 (66.7%)	
Expression of IL-17RA in metastatic lymph node	Low	43 (87.8%)	16 (94.1%)	0.667	XX	XX	XX
	High	6 (12.2%)	1 (5.9%)		XX	XX	
Expression of IL-17RA in prostate	Low	XX	XX	XX	43 (72.9%)	6 (85.7%)	0.667
	High	XX	XX		16 (27.1%)	1 (14.3%)	

4. Discussion

For clinicians and pathologists, PCa, the second most frequently diagnosed cancer in men, presents a significant diagnostic and therapeutic challenge. A rise in the number of new PCa diagnoses in men is anticipated in the near future. It is due to the correlation between PCa incidence and age and the rising life expectancy [2]. But, despite substantial progress in adjuvant therapy that have increased cancer-specific survival, we continue to base prognosis on conventional variables like PSA level, histological grade group, and clinical stage [33].

Numerous ongoing studies are investigating the function and use of IHC biomarkers in the diagnosis and prognosis of PCa, including the development of metastases. Although many of the findings from these studies are encouraging, urological guidelines for PCa currently do not take these findings into account [8,20].

IL-17A and IL-17RA are members of a large family of IL-17 cytokines that have been demonstrated to have both pro-cancer (in most cases) and cancer-inhibiting effects [20,34–37]. Zang suggested a mechanism of action for IL-17 in the development of PCa in a mouse model. According to the author, IL-17 promotes PCa carcinogenesis via matrix metalloproteinase 7 (MMP7), which is also increased in PCa and triggers epithelial-to-mesenchymal transition (EMT), resulting in the development of PCa [22].

Our research is novel because, despite the fact that IL-17A and IL-17RA expressions in the prostate have been assessed in a number of studies, no study has investigated this marker's expression in LN+ [24–30].

The expressions of IL-17A and IL-17RA in the examined tissues can be regarded as an indicator of local inflammation, which is significant in the context of neoplastic processes. Studies have shown that a chronic inflammatory process can promote the occurrence and progression of neoplasms. The exact mechanism of action is not known, but one proposed explanation is that an inflammatory process can alter the tumor microenvironment, leading to the production of cytokines that promote tumor growth and metastasis. This is a component of the pre-metastatic niche theory, which proposes that the conditions for the formation of metastases are present when the microenvironment is favorable to cancer cells at the potential site of metastasis [38].

Studying the expression of inflammatory mediators in cancer cells is especially important because recent research has shown that the process of immune escape is one of the most important factors in the development of cancer. Cancer cells develop resistance to immune system neutralization as well as resistance to anticancer drugs during this process. Current research is focused on identifying factors that influence the development of immune escape cancer cells, as well as the development of effective anticancer immunotherapies [39,40].

Cytokines, including IL-17A, are an important group of factors that are involved in this process [41]. In a recent study, the authors showed that melittin, an anti-inflammatory drug, inhibits the proliferation and migration of castration-resistant prostate cancer cells by downregulating the IL-17 signaling pathway [42].

Available studies confirm that a chronic inflammatory process promotes the development of BPH and PCa by stimulating angiogenesis or stimulating cell growth, but so far, there is no clearly described mechanism of this action [43–45]. It has been shown that the infiltration of inflammatory cells selectively promotes the proliferation of prostate epithelial cells, which may be the source of PCa development [43]. In a study by De Marzo et al., the stimulating effect of the inflammatory process on the development of proliferative inflammatory atrophy (PIA) was found, which may be a precursor to the transformation into prostatic intraepithelial neoplasia (PIN) or PCa [44]. Despite these data, the effect of prostatitis on PCa progression has not been unequivocally demonstrated [46].

According to Liu et al., IL-17 stimulation increased the expression of proinflammatory genes, including IL-17RA, in mice, resulting in the development of a more aggressive form of PCa [47]. Another study by the same author found an increased expression of IL-17A and IL-17RA in PCa and BPH, concluding that IL-17A action through IL-17RA contributes to PCa development [26].

We found IL-17A and IL17RA expression in a very high percentage of prostate and LN+ samples (over 90% of all analyzed samples). In contrast to the findings of Janiczek et al., who did not find IL-17RA expression in either the prostate or BPH, our findings regarding IL-17RA expression in the prostate support the hypothesis made by Liu et al.

We discovered no significant differences in IL-17A expression between the prostate and LN+, either in terms of the percentage of positively stained cancer cells or the intensity of staining, which was high in both types of tissues in most cases. However, the level of expression of Il-17RA was significantly higher in the prostate than in LN+ (IRS score 4 vs. 3; $p = 0.009$). In general, the expression level of IL-17RA (assessed by IRS scale) was lower in both types of tissues examined than that of IL-17A, and the majority of samples showed a low level of expression (especially in the case of expression in LN +). Also, we observed that prostate expression of IL-17A and IL-17RA correlated positively with their expression in LN+. This suggests that IL-17A and IL-17RA are involved in metastasis formation and are a component of pre-metastatic niche formation in lymph nodes. Further research on PCa nodal metastases is needed to draw clear conclusions from these observations. Our findings need to be confirmed in future research. This could be a promising research direction for developing new systemic therapies for PCa, or it could be an additional factor influencing the estimation of the risk of nodal metastases.

We found no significant correlation between the level of expression of Il-17A and Il-17RA in the prostate and classic factors used to assess the risk of disease progression, such as PSA or EAU risk group. The only significant positive correlation found was between the level of IL-17A expression in the prostate and BMI ($p = 0.028$). This association could be related to the chronic inflammation seen in obese and overweight people, with increased expression being the result [48–50]. This is consistent with the findings of Liu's studies on obese mice, in which he investigated the impact of hyperinsulinemia on the expression of IL-17 and its receptors as well as the progression of PCa [47].

When we evaluated the expression in the LN+, we observed a correlation between the expression levels of IL-17A ($p = 0.001$) and IL-17RA ($p = 0.045$) and the EAU risk group. This is the only significant association found between the classical model of PCa progression risk assessment and the expression levels of the markers investigated in this study. In addition, the level of IL-17A expression in LN+ was correlated with the percentage of affected lymph nodes ($p = 0.006$). These findings imply that IL-17A and IL-17RA may play a significant role in the development of pre-metastatic niches, although further evidence is required to support this theory. These results could eventually assist in improving the accuracy of models such as Memorial Sloan Kettering Cancer Center (MSKCC), Partin, and Briganti nomograms, which are used to assess the

likelihood of PCa nodal metastases [51–54]. The continuous improvement of methods to assess the risk of the presence of nodal metastases is very important because, despite the currently used tools, approximately 70% of patients undergo unnecessary extended lymphadenectomy, showing the absence of nodal metastases [55]. It should be emphasized that lymphadenectomy is an additional element that increases the risk of complications and extends the duration of RP [7]. Research on new markers to increase the accuracy of risk assessment of lymph node involvement is extremely important.

We found no statistically significant differences between groups with high and low IL-17A or IL-17RA expression in the prostate and clinicopathological characteristics of patients. In contrast, we observed these differences in LN+ expression. In the case of IL-17A, there was a significant difference in the frequency of prostate ECE; it occurred more frequently in the high-expression group than in the low-expression group. This finding is significant because the ECE of prostate is regarded as an independent risk factor for biochemical recurrence [56]. However, in the case of IL-17RA expression in LN+, a similar but not identical significant difference was noticed; the ECE of the lymph node was identified more often in the high expression group than in the low expression group. Furthermore, a statistically significant association was observed between the frequency of ECE in the lymph nodes and the percentage of IL-17A-positive cells in the prostate as well as the intensity of IL-17A staining in LN+.

This study has some limitations. Firstly, there were no follow-up data for patients who underwent RP. The ability of this study to assess the correlation between IL-17A or IL-17RA expression and patient outcomes, such as biochemical recurrence or overall survival, is hampered by the absence of long-term data. Secondly, there are certain limitations to the IRS scale, which is the evaluation method used in this study to assess the expression of IL-17A and IL-17RA. A more detailed assessment can be performed using the H-score method [57,58], which requires more experience and time from a uropathologist. A simplified classification into groups of high and low expression may turn out to be too inaccurate when it comes to detecting subtle correlations. The method we used was a compromise between the accuracy of the analysis and available resources and research needs. Thirdly, we examined the expression of IL-17A and IL-17RA exclusively in PCa tissue without comparing them to the control group, such as lymph nodes from patients who had undergone RP and lymphadenectomy, and no LN+ was detected or tissues from benign prostatic hyperplasia obtained after the transurethral resection of the prostate (TURP). The last limitation of the study was that it involved a relatively small group of patients, which would have reduced its statistical ability to identify subtle differences and may have created bias.

The strengths of our work, derived from its novelty and the rigorous method we used, should not be diminished by the limitations we observed. Our work is unique in that it is the first to examine the expression of IL-17A and IL-17RA in PCa LN+; nevertheless, we see a need and plan to broaden our research in the future with a comparison to a control group, as mentioned above. This will further define the role of IL-17A and IL-17RA in PCa, as well as their potential clinical implications.

5. Conclusions

The results presented above show that IL-17A and IL17-RA have a statistically significant positive correlation between expression in the prostate and expression in metastatic lymph nodes. The prevalence of their expression suggests their role in local inflammation, which is associated with neoplastic processes. Our study is the first to assess IL-17A and IL17-RA expression not only in prostate tissue, but also in LN+. The findings of this study highlight the potential significance of IL-17A and IL-17RA in PCa metastasis and premetastatic niche formation. The correlations observed between marker expression and clinical parameters such as BMI and EAU risk point to possible links between chronic inflammation and disease progression. Although more evidence is needed, these markers

could contribute to improved risk assessment models for nodal metastases, helping to avoid unnecessary lymphadenectomies.

In summary, this study sheds light on the potential of IL-17A and IL-17RA as markers in PCa, and further studies, ideally with a control group and long-term outcomes, are required to determine the role and possible application of both markers in PCa.

Author Contributions: Conceptualization, P.K. and B.M.; methodology, P.K., M.K. and A.P.; validation, P.K., M.K. and K.K.; formal analysis, B.M.; investigation, P.K. and M.K.; resources, B.M.; data curation, P.K., K.D. and W.K.; writing—original draft preparation, P.K. and K.K.; writing—review and editing, B.M., Ł.N. and J.C.; visualization, J.C., K.D. and Ł.N.; supervision, T.S., P.D., A.H. and B.M.; project administration, B.M.; funding acquisition, P.K. and B.M. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Wroclaw Medical University (protocol code KB - 755/2022, approved on 27 October 2022).

Informed Consent Statement: Informed consent was waived due to the retrospective nature of this research.

Data Availability Statement: The data are available from the authors upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

BMI	body mass index
EAU	European Association of Urology
ECE	extracapsular extension
EMT	epithelial-to-mesenchymal transition
GGG ISUP	International Society of Urological Pathology grade (group) system
HE	hematoxylin and eosin
IHC	immunohistochemistry
IL	interleukin
IRS	immunoreactive scale
LN+	metastatic/positive lymph node
LVI	lymphovascular invasion
MMP7	matrix metalloproteinase 7
MSKCC	Memorial Sloan Kettering Cancer Center
NVI	neurovascular invasion
PCa	prostate cancer
PIN	prostatic intraepithelial neoplasia
PSA	prostate-specific antigen
pT	pathological tumor stage
RP	radical prostatectomy
TMA	tissue microarray
TURP	transurethral resection of the prostate

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8. Nota biograficzna doktoranta

Data i miejsce urodzenia: 15.05.1992 w Przeworsku

Wykształcenie i przebieg pracy zawodowej:

2012-2018 – Studia na Wydziale Lekarskim Uniwersytetu Medycznego im. Piastów Śląskich we Wrocławiu ukończone z wyróżnieniem Rektora

2018-2019 – Staż podyplomowy, Uniwersytecki Szpital Kliniczny we Wrocławiu

10.2019 – Rozpoczęcie Szkoły Doktorskiej Uniwersytetu Medycznego im. Piastów Śląskich we Wrocławiu

11.2019– Rozpoczęcie specjalizacji z urologii w Katedrze i Klinice Urologii i Onkologii Urologicznej Uniwersytetu Medycznego we Wrocławiu (aktualnie Klinika Urologii Małoinwazyjnej i Robotycznej Uniwersyteckiego Centrum Urologii)

Członkostwa:

• Członek Polskiego Towarzystwa Urologicznego (PTU)

• Członek Europejskiego Towarzystwa Urologicznego (EAU)

9. Wykaz osiągnięć doktoranta

9.1 Publikacje

Autor **21** publikacji zarówno w Polskich, jak i międzynarodowych czasopismach.

Sumaryczny IF = 65,589 punktów. Suma punktów MEiN = 1941 punktów.

IF jako 1 autor IF = 14,6 punktów.

Lista publikacji w czasopismach z IF:

1. Kaliszewski Krzysztof, Zubkiewicz-Kucharska Agnieszka, **Kiełb Paweł**, Maksymowicz Jerzy, Krawczyk Aleksander, Krawiec Otto: Comparison of the prevalence of incidental and non-incidental papillary thyroid microcarcinoma during 2008-2016: a single-center experience, World Journal of Surgical Oncology, 2018, vol. 16, art.202 [6 s.], DOI:10.1186/s12957-018-1501-8
   IF=1,966; Punkty MEiN = 20
2. Krajewski Wojciech, Nowak Łukasz, Poletajew Sławomir, Tukiendorf Andrzej, Moschini Marco, Mari Andrea, Di Trapani Ettore, Xylinas Evanguelos, **Kiełb Paweł**, Wełna Marek, Zdrojowy Romuald: The impact of restaging transurethral resection of bladder tumor on survival parameters in T1 nonmuscle-invasive bladder cancer: systematic review and meta-analysis, Journal of Endourology, 2020, vol. 34, nr 8, s. 795-804, DOI:10.1089/end.2020.0301
   IF=2,942; Punkty MEiN = 100
3. Nowak Łukasz, Krajewski Wojciech, Chorbińska Joanna, **Kiełb Paweł**, Sut Michał, Moschini Marco, Teoh Jeremy Yuen-Chun, Mori Keiichiro, Del Giudice Francesco, Laukhtina Ekaterina, Lonati Chiara, Kaliszewski Krzysztof, Małkiewicz Bartosz, Szydełko Tomasz: The impact of diagnostic ureteroscopy prior to radical nephroureterectomy on oncological outcomes in patients with upper tract urothelial carcinoma: a comprehensive systematic review and meta-analysis, Journal of Clinical Medicine, 2021, vol. 10, nr 18, art.4197 [17 s.], DOI:10.3390/jcm10184197
   IF=4,964; Punkty MEiN = 140
4. Łuczak Mateusz, Nowak Łukasz, Chorbińska Joanna, Galik Katarzyna, **Kiełb Paweł**, Łaszkiewicz Jan, Tukiendorf Andrzej, Kościelska-Kasprzak Katarzyna, Małkiewicz Bartosz, Zdrojowy Romuald, Szydełko Tomasz, Krajewski Wojciech: Influence of virtual reality devices on pain and anxiety in patients undergoing cystoscopy performed under local anaesthesia, Journal of Personalized Medicine, 2021, vol. 11, nr 11, art.1214 [7 s.], DOI:10.3390/jpm11111214
   IF=3,508; Punkty MEiN = 70
5. Krajewski Wojciech, Nowak Łukasz, Małkiewicz Bartosz, Chorbińska Joanna, **Kiełb Paweł**, Poterek Adrian, Sporniak Bartłomiej, Sut Michał, Moschini Marco, Lonati

Chiara, Carando Roberto, Teoh Jeremy Yuen-Chun, Mori Keiichiro, Kaliszewski Krzysztof, Szydełko Tomasz: The impact of primary tumor location on long-term oncological outcomes in patients with upper tract urothelial carcinoma treated with radical nephroureterectomy: a systematic review and meta-analysis, Journal of Personalized Medicine, 2021, vol. 11, nr 12, art.1363 [15 s.], DOI:10.3390/jpm11121363
IF=3,508; Punkty MEiN = 70

6. Małkiewicz Bartosz, Ptaszkowski Kuba, Knecht Klaudia, Gurwin Adam, Wilk Karol, **Kiełb Paweł**, Dudek Krzysztof, Zdrojowy Romuald: External validation of the Briganti nomogram to predict lymph node invasion in prostate cancer - setting a new threshold value, Life, 2021, vol. 11, nr 6, art.479 [10 s.], DOI:10.3390/life11060479
IF=3,253; Punkty MEiN = 70
7. Małkiewicz Bartosz, **Kiełb Paweł**, Gurwin Adam, Knecht Klaudia, Wilk Karol, Dobruch Jakub, Zdrojowy Romuald: The usefulness of lymphadenectomy in bladder cancer - current status, Medicina, 2021, vol. 57, nr 5, art.415 [16 s.], DOI:10.3390/medicina57050415
IF=2,948; Punkty MEiN = 40
8. Kaczorowski Maciej, Matysiak Joanna, **Kiełb Paweł**, Małkiewicz Bartosz, Hałoń Agnieszka: Nuclear factor IA is down-regulated in muscle-invasive and high-grade bladder cancers, Anticancer Research, 2022, vol. 42, nr 1, s. 493-500, DOI:10.21873/anticanres.15507
IF=2; Punkty MEiN = 70
9. Małkiewicz Bartosz, Bugla Błażej, Czarnecki Maciej, Karwacki Jakub, Długosz Paulina, Gurwin Adam, **Kiełb Paweł**, Lemiński Artur, Krajewski Wojciech, Jędrzejuk Diana, Bolanowski Marek, Hałoń Agnieszka, Szydełko Tomasz: Diagnostic value of radio-guided sentinel node detection in patients with prostate cancer undergoing radical prostatectomy with modified-extended lymphadenectomy, Cancers, 2022, vol. 14, nr 20, art.5012 [12 s.], DOI:10.3390/cancers14205012
IF=5,2; Punkty MEiN = 140
10. Małkiewicz Bartosz, **Kiełb Paweł**, Karwacki Jakub, Czerwińska Róża, Długosz Paulina, Lemiński Artur, Nowak Łukasz, Krajewski Wojciech, Szydełko Tomasz: Utility of lymphadenectomy in prostate cancer: where do we stand?, Journal of Clinical Medicine, 2022, vol. 11, nr 9, art.2343 [16 s.], DOI:10.3390/jcm11092343
IF=3,9; Punkty MEiN = 140
11. Jarocki Michał, Karska Julia, Kowalski Szymon, **Kiełb Paweł**, Nowak Łukasz , Krajewski Wojciech, Saczko Jolanta, Kulbacka Julita, Szydełko Tomasz, Małkiewicz Bartosz: Interleukin 17 and its involvement in renal cell carcinoma, Journal of Clinical Medicine, 2022, vol. 11, nr 17, art.4973 [13 s.], DOI:10.3390/jcm11174973
IF=3,9; Punkty MEiN = 140

12. Zapała Piotr, Ślusarczyk Aleksander, Rajwa Paweł, Przydacz Mikołaj, Krajewski Wojciech, Dybowski Bartosz, Kubik Przemysław, Kuffel Błażej, Przudzik Maciej, Osiecki Rafał, Stamirowski Remigiusz, Zapała Łukasz, Kozikowski Mieszko, Chorągwicki Dominik, Szymańska Magdalena, **Kiełb Paweł**, Małkiewicz Bartosz, Zostawa Jacek, Roslan Marek, Zajączkowska Joanna, Jarzemski Marcin, Brzoszczyk Bartosz, Petrasz Piotr, Jarzemski Piotr, Zdrojowy Romuald, Dobruch Jakub, Paradysz Andrzej, Drewa Tomasz, Chłosta Piotr, Radziszewski Piotr: Not as black as it is painted? The impact of the first wave of COVID-19 pandemic on surgical treatment of urological cancer patients in Poland – a cross-country experience, Archives of Medical Science, 2023, vol. 19, nr 1, s. 107-115, DOI:10.5114/aoms/130927
IF=3,8; Punkty MEiN = 100
13. **Kiełb Paweł**, Kowalczyk Kamil, Gurwin Adam, Nowak Łukasz , Krajewski Wojciech, Sosnowski Roman, Szydełko Tomasz, Małkiewicz Bartosz: Novel histopathological biomarkers in prostate cancer: implications and perspectives, Biomedicines, 2023, vol. 11, nr 6, art.1552 [28 s.], DOI:10.3390/biomedicines11061552
IF=4,7; Punkty MEiN = 140
14. Małkiewicz Bartosz, **Kiełb Paweł**, Kobylański Maximilian, Karwacki Jakub, Poterek Adrian, Krajewski Wojciech Piotr, Zdrojowy Romuald, Szydełko Tomasz: Sentinel lymph node techniques in urologic oncology: current knowledge and application, Cancers, 2023, vol. 15, nr 9, art.2495 [19 s.], DOI:10.3390/cancers15092495
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15. **Kiełb Paweł**, Kaczorowski Maciej, Kowalczyk Kamil, Piotrowska Aleksandra, Nowak Łukasz, Krajewski Wojciech, Chorbińska Joanna, Dudek Krzysztof, Dzięgiel Piotr, Hałoń Agnieszka, Szydełko Tomasz, Małkiewicz Bartosz: Role of IL-17A and IL-17RA in prostate cancer with lymph nodes metastasis: expression patterns and clinical significance, Cancers, 2023, vol. 15, nr 18, art.4578 [17 s.], DOI:10.3390/cancers15184578
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Tomasz: Diagnostic value of the sentinel lymph node technique in patients with muscle-invasive bladder cancer, Journal of Clinical Medicine, 2023, vol. 12, nr 9, art.3092 [12 s.], DOI:10.3390/jcm12093092
IF=3,9; Punkty MEiN = 140

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1. Nowak Łukasz, Kaliszewski Krzysztof, Santorowska Hanna, **Kiełb Paweł**: Opis dwóch przypadków przepukliny Spiegla u pacjentek w starszym wieku - porównanie i przegląd literatury, Geriatria, 2018, vol. 12, nr 4, s. 256-261
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2. Kaliszewski Krzysztof, Nowak Łukasz, Santorowska Hanna, **Kiełb Paweł**: Szpiczak plazmocytowy tarczycy - opis przypadku, Polski Merkuriusz Lekarski, 2019, vol. 46, nr 271, s. 42-44
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3. Nowak Łukasz, Krajewski Wojciech, **Kiełb Paweł**, Śliwa Anna, Zdrojowy-Wełna Aleksandra, Zdrojowy Romuald: COVID-19 and the urological practice: changes and future perspectives, Central European Journal of Urology, 2020, vol. 73, nr 3, s. 269-272, DOI:10.5173/ceju.2020.0087
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4. Nowak Łukasz, Krajewski Wojciech, Krajewska Joanna, Chorbińska Joanna, **Kiełb Paweł**, Małkiewicz Bartosz, Szydełko Tomasz: Urological manifestations of the systemic vasculitides - a scoping review, Uro, 2021, vol. 1, nr 4, s. 209-221, [Publikacja w czasopiśmie spoza listy MNiSW], DOI:10.3390/uro1040023

9.2 Doniesienia zjazdowe

1. Kaliszewski Krzysztof, Wojtczak Beata, Balcerzak Waldemar, Forkasiewicz Zdzisław, Sutkowski Krzysztof, Aporowicz Michał, **Kiełb Paweł**, Maksymowicz Jerzy, Krawczyk Aleksander, Krawiec Otto, Domosławski Paweł: Rak rdzeniasty tarczycy - doświadczenia jednego ośrodka, W: 68 Kongres Towarzystwa Chirurgów Polskich. Kraków, 27-30 września 2017 r. Streszczenia 2017, 161 poz.P.01 1170
2. Kaliszewski Krzysztof, Sutkowski Krzysztof, Wojtczak Beata, Balcerzak Waldemar, Forkasiewicz Zdzisław, **Kiełb Paweł**, Maksymowicz Jerzy, Krawczyk Aleksander, Krawiec Otto, Domosławski Paweł: Tracheostomia u chorych z nowotworem tarczycy, W: 68 Kongres Towarzystwa Chirurgów Polskich. Kraków, 27-30 września 2017 r. Streszczenia 2017, 173 poz.P.01 1168

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5. Małkiewicz Bartosz, **Kiełb Paweł**, Jędrzejuk Diana, Krajewski Wojciech, Nowak Łukasz, Bolanowski Marek, Zdrojowy Romuald: Diagnostic value of sentinel lymph node technique in patient with invasive bladder cancer - results from a prospective study, European Urology, 2021, vol. 79, nr suppl.1, S1117 poz.P0808, [EAU21 Virtual Congress. [Online], 8-12 July 2021], DOI:10.1016/S0302-2838(21)01183-0
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7. Małkiewicz Bartosz, Kobylański Maximilian, Łątkowska Małgorzata, Handzlik Wojciech, Dębiński Paweł, Kamińska Dorota, Banasik Mirosław, Krajewski Wojciech, **Kiełb Paweł**, Nowak Łukasz, Janczak Dariusz, Krajewska Magdalena, Zdrojowy Romuald, Szydełko Tomasz: Laparoscopic donor nephrectomy - single center experience, W: The 51st Scientific Congress of the Polish Urological Association - PTU 2021. Warsaw, 21st - 23rd October 2021. A book of abstracts 2021, s. 21
8. Małkiewicz Bartosz, Wilk Karol, Gurwin Adam, **Kiełb Paweł**, Krajewski Wojciech, Nowak Łukasz, Chorbińska Joanna, Galik Katarzyna, Dudek Krzysztof, Szydełko Tomasz: External validation of the Briganti nomogram to predict lymph node invasion in prostate cancer patients undergoing extended lymph node dissection, W: The 51st Scientific Congress of the Polish Urological Association - PTU 2021. Warsaw, 21st - 23rd October 2021. A book of abstracts 2021, s. 25-26
9. **Kiełb Paweł**, Małkiewicz Bartosz, Nowak Łukasz, Krajewski Wojciech, Poterek Adrian, Janczak Dawid, Chorbińska Joanna, Kowalczyk Kamil, Dembowski Janusz, Szydełko

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10. Małkiewicz Bartosz, Karwacki Jakub, **Kiełb Paweł**, Długosz Paulina, Nowak Łukasz, Chorbińska Joanna, Krajewski Wojciech, Kowalczyk Kamil, Zdrojowy Romuald, Szydełko Tomasz: Mapping of primary lymphatic landing sites of the prostate cancer using isotope imaging and radio-guided surgery. Preliminary results, W: The 51st Scientific Congress of the Polish Urological Association - PTU 2021. Warsaw, 21st - 23rd October 2021. A book of abstracts 2021, s. 29
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12. Małkiewicz Bartosz, Kowalczyk Kamil, **Kiełb Paweł**, Froń Anita, Bińczyk Wiktoria, Krajewski Wojciech, Nowak Łukasz, Chorbińska Joanna, Kołodziej Anna, Zdrojowy Romuald, Szydełko Tomasz: Extended versus standard lymph node dissection in bladder cancer patients undergoing radical cystectomy: results from a prospective trial, W: The 51st Scientific Congress of the Polish Urological Association - PTU 2021. Warsaw, 21st - 23rd October 2021. A book of abstracts 2021, s. 33-34
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18. Nowak Łukasz , Krajewski Wojciech, Chorbińska Joanna, Małkiewicz Bartosz, **Kiełb Paweł**, Laukhtina E., Moschini M., Pichler R., Pradere B., D'Andrea D., Mori K., Albisinni S., Soria F., Szydełko Tomasz: Perioperative and oncological outcomes of simultaneous transurethral resection of bladder tumour and prostate: Systematic review and meta-analysis, European Urology, 2023, vol. 83, nr suppl.1, S1012-S1014 poz.A0715, [EAU23 - 38th Annual EAU Congress. Milan, Italy, 10-13 March 2023. Abstracts], DOI:10.1016/s0302-2838(23)00759-5
19. Tyszkowski Bartłomiej, **Kiełb Paweł**, Hackemer Paweł, Krajewski Wojciech, Nowak Łukasz , Dzięgała Mateusz: Clinical assessment of the value of the sentinel node technique in patients with bladder cancer who underwent radical cystectomy with extended lymphadenectomy, W: IX International Students' Conference of Young Medical Researchers. Wroclaw, 30.03-01.04.2023. Book of abstracts 2023, 55 poz.74, ISBN 978-83-942024-5-3
20. Karwacki Jakub, Gurwin Adam, **Kiełb Paweł**: Diagnostic value of radio-guided sentinel node detection in patients with prostate cancer undergoing radical prostatectomy with modified-extended lymphadenectomy, W: IX International Students' Conference of Young Medical Researchers. Wroclaw, 30.03-01.04.2023. Book of abstracts 2023, 56 poz.76, ISBN 978-83-942024-5-3

9.3 Granty

•Grant dla Młodych Naukowców nr STM. C090.20.081- temat :”Determinacja immunologicznych czynników ryzyka przerzutów węzłowych w raku pęcherza moczowego i gruczołu krokowego.”

10. Załączniki

10.1 Zgody komisji bioetycznej

- Zgoda Komisji Bioetycznej Nr KB – 545/2020
- Zgoda Komisji Bioetycznej Nr KB – 766/2021
- Zgoda Komisji Bioetycznej Nr KB – 755/2022

KOMISJA BIOETYCZNA
przy
Uniwersytecie Medycznym
we Wrocławiu
ul. Pasteura 1; 50-367 WROCŁAW

OPINIA KOMISJI BIOETYCZNEJ Nr KB – 545/2020

Komisja Bioetyczna przy Uniwersytecie Medycznym we Wrocławiu, powołana zarządzeniem Rektora Uniwersytetu Medycznego we Wrocławiu nr 133/XV R/2017 z dnia 21 grudnia 2017 r. oraz działająca w trybie przewidzianym rozporządzeniem Ministra Zdrowia i Opieki Społecznej z dnia 11 maja 1999 r. (Dz.U. nr 47, poz. 480) na podstawie ustawy o zawodzie lekarza z dnia 5 grudnia 1996 r. (Dz.U. nr 28 z 1997 r. poz. 152 z późniejszymi zmianami) w składzie:

prof. dr hab. Jacek Daroszewski (choroby wewnętrzne, endokrynologia, diabetologia)
prof. dr hab. Krzysztof Grabowski (chirurgia)
dr Henryk Kaczkowski (chirurgia szczękowa, chirurgia stomatologiczna)
mgr Irena Knabel-Krzyszowska (farmacja)
prof. dr hab. Jerzy Liebhart (choroby wewnętrzne, alergologia)
ks. dr hab. Piotr Mrzygłód, prof. nadzw. (duchowny)
mgr Luiza Müller (prawo)
dr hab. Sławomir Sidorowicz (psychiatria)
prof. dr hab. Leszek Szenborn, (pediatria, choroby zakaźne)
Danuta Tarkowska (pielęgniarstwo)
prof. dr hab. Anna Wiela-Hojeńska (farmakologia kliniczna)
dr hab. Andrzej Wojnar, prof. nadzw. (histopatologia, dermatologia) przedstawiciel Dolnośląskiej Izby Lekarskiej)
dr hab. Jacek Zieliński (filozofia)

pod przewodnictwem
prof. dr hab. Jana Kornafela (ginekologia i położnictwo, onkologia)

Przestrzegając w działalności zasad Good Clinical Practice oraz zasad Deklaracji Helsińskiej, po zapoznaniu się z projektem badawczym pt.

„Poszukiwane i ocena przydatności klinicznej nowych czynników genetyczno-molekularnych i markerów immunohistochemicznych zaangażowanych w etiopatogenezę nowotworów układu moczowo-płciowego"

zgłoszonym przez **dr. Bartosza Małkiewicza** zatrudnionego w Katedrze i Klinice Urologii i Onkologii Urologicznej Uniwersytetu Medycznego we Wrocławiu oraz złożonymi wraz z wnioskiem dokumentami, w tajnym głosowaniu postanowiła wyrazić zgodę na przeprowadzenie badania w: Klinice Urologii i Onkologii Urologicznej; Klinice Angiologii, Nadciśnienia Tętniczego i Diabetologii oraz w Zakładzie Patomorfologii i Cytologii Klinicznej Uniwersyteckiego Szpitala Klinicznego im. Jana Mikulicza-Radeckiego we Wrocławiu i w Zakładzie Histologii i Embriologii Katedry Morfologii i Embriologii Człowieka Uniwersytetu Medycznego we Wrocławiu **pod warunkiem zachowania anonimowości uzyskanych danych**.

Uwaga: Badanie to zostało objęte ubezpieczeniem odpowiedzialności cywilnej Uniwersytetu Medycznego we Wrocławiu z tytułu prowadzonej działalności:

Pouczenie: W ciągu 14 dni od otrzymania decyzji wnioskodawcy przysługuje prawo odwołania do Komisji Odwoławczej za pośrednictwem Komisji Bioetycznej UM we Wrocławiu

Opinia powyższa dotyczy: projektów badawczych finansowanych z subwencji
Numer rejestrowy CWN UMW: SUB.C090.19.046

Wrocław, dnia 28 września 2020 r.

BW

Uniwersytet Medyczny we Wrocławiu
KOMISJA BIOETYCZNA
Przewodniczący
prof. dr hab. Jan Kornafel

KOMISJA BIOETYCZNA
przy
Uniwersytecie Medycznym
we Wrocławiu
ul. Pasteura 1; 50-367 WROCŁAW

OPINIA KOMISJI BIOETYCZNEJ Nr KB – 766/2021

Komisja Bioetyczna przy Uniwersytecie Medycznym we Wrocławiu, powołana zarządzeniem Rektora Uniwersytetu Medycznego we Wrocławiu nr 278/XVI R/2020 z dnia 21 grudnia 2020 r. oraz działająca w trybie przewidzianym rozporządzeniem Ministra Zdrowia i Opieki Społecznej z dnia 11 maja 1999 r. (Dz.U. nr 47, poz. 480) na podstawie ustawy o zawodzie lekarza z dnia 5 grudnia 1996 r. (Dz.U. nr 514 z 2020 r.) w składzie:

dr Joanna Birecka (psychiatria)
dr Beata Freier (onkologia)
dr hab. Tomasz Fuchs (ginekologia, położnictwo)
prof. dr hab. Dariusz Janczak (chirurgia naczyniowa, transplantologia)
dr hab. Krzysztof Kaliszewski (chirurgia endokrynologiczna)
dr prawa Andrzej Malicki (prawo)
prof. dr hab. Marcin Mączyński (farmacja)
Urszula Olechowska (pielęgniarstwo)
prof. dr hab. Leszek Szenborn (pediatria, choroby zakaźne)
prof. dr hab. Andrzej Szuba (choroby wewnętrzne, angiologia
ks. prof. Andrzej Tomko (duchowny)
prof. dr hab. Mieszko Więckiewicz (stomatologia)
dr hab. Andrzej Wojnar, prof. nadzw. (histopatologia, dermatologia) przedstawiciel Dolnośląskiej Izby Lekarskiej)
dr hab. Jacek Zieliński (filozofia)

pod przewodnictwem
prof. dr hab. Jerzego Rudnickiego (chirurgia, proktologia)

Przestrzegając w działalności zasad Good Clinical Practice oraz zasad Deklaracji Helsińskiej, po zapoznaniu się z projektem badawczym pt.

„Poszukiwanie i ocena przydatności klinicznej nowych czynników genetyczno-molekularnych i markerów immunohistochemicznych zaangażowanych w etiopatogenezę nowotworów układu moczowo-płciowego"

zgłoszonym przez **dr hab. Bartosza Małkiewicza,** zatrudnionego w Katerze i Klinice Urologii i Onkologii Urologicznej Uniwersytetu Medycznego we Wrocławiu oraz złożonymi wraz z wnioskiem dokumentami, w tajnym głosowaniu postanowiła **wyrazić zgodę** na:
- rozszerzenie zespołu badawczego o nowych członków- zgodnie z wnioskiem
- dodatkowe miejsce wykonywania badań- Katedrę i Zakład Biologii Molekularnej o Komórkowej Uniwersytetu Medycznego we Wrocławiu
pod warunkiem zachowania anonimowości uzyskanych danych.

Projekt otrzymał opinię Komisji nr KB – 545/2020

Uwaga: Badanie to zostało objęte ubezpieczeniem odpowiedzialności cywilnej Uniwersytetu Medycznego we Wrocławiu z tytułu prowadzonej działalności.

Pouczenie: W ciągu 14 dni od otrzymania decyzji wnioskodawcy przysługuje prawo odwołania do Komisji Odwoławczej za pośrednictwem Komisji Bioetycznej UM we Wrocławiu.

Opinia powyższa dotyczy projektu badawczego finansowanego z subwencji nr SUB.C090.19.046

Przewodniczący Komisji Bioetycznej
przy Uniwersytecie Medycznym

prof. dr hab. Jerzy Rudnicki

Wrocław, dnia 8 października 2021r.

KOMISJA BIOETYCZNA
przy
Uniwersytecie Medycznym
we Wrocławiu

OPINIA KOMISJI BIOETYCZNEJ Nr KB – 755/2022

Komisja Bioetyczna przy Uniwersytecie Medycznym we Wrocławiu, powołana zarządzeniem Rektora Uniwersytetu Medycznego we Wrocławiu nr 278/XVI R/2020 z dnia 21 grudnia 2020 r. oraz działająca w trybie przewidzianym rozporządzeniem Ministra Zdrowia i Opieki Społecznej z dnia 11 maja 1999 r. (Dz.U. nr 47, poz. 480) na podstawie ustawy o zawodzie lekarza z dnia 5 grudnia 1996 r. (Dz.U. nr 514 z 2020 r.) w składzie:

dr Joanna Birecka (psychiatria)
dr Beata Freier (onkologia)
dr hab. Tomasz Fuchs (ginekologia, położnictwo)
prof. dr hab. Dariusz Janczak (chirurgia naczyniowa, transplantologia)
dr hab. Krzysztof Kaliszewski, prof. UMW (chirurgia endokrynologiczna)
dr prawa Andrzej Malicki (prawo)
prof. dr hab. Marcin Mączyński, prof. UMW (farmacja)
Urszula Olechowska (pielęgniarstwo)
prof. dr hab. Leszek Szenborn (pediatria, choroby zakaźne)
prof. dr hab. Andrzej Szuba (choroby wewnętrzne, angiologia
ks. prof. Andrzej Tomko (duchowny)
prof. dr hab. Mieszko Więckiewicz (stomatologia)
dr hab. Andrzej Wojnar, prof. nadzw. (histopatologia, dermatologia) przedstawiciel Dolnośląskiej Izby Lekarskiej)
dr hab. Jacek Zieliński (filozofia)

pod przewodnictwem
prof. dr hab. Jerzego Rudnickiego (chirurgia, proktologia)

Przestrzegając w działalności zasad Good Clinical Practice oraz zasad Deklaracji Helsińskiej, po zapoznaniu się z wnioskiem:

„Poszukiwane i ocena przydatności klinicznej nowych czynników genetyczno-molekularnych i markerów immunohistochemicznych zaangażowanych w etiopatogenezę nowotworów układu moczowo-płciowego"

zgłoszonym przez **dr hab. Bartosza Małkiewicza** zatrudnionego w Uniwersyteckim Centrum Urologii Uniwersytetu Medycznego we Wrocławiu

oraz złożonymi wraz z wnioskiem dokumentami, w tajnym głosowaniu postanowiła **wyrazić zgodę** na rozszerzenie badania o realizowanie zadań także w Laboratorium Genetyki i Epigenetyki Chorób Człowieka, Zakład Terapii Doświadczalnej Instytut Immunologii i Terapii Doświadczalnej PAN z udziałem zespołu w/w jednostki (wymienionym we wniosku), **pod warunkiem zachowania anonimowości uzyskanych danych**.

UWAGA: Jeśli projekt/badanie wymaga ubezpieczenia na podstawie Rozporządzenia Ministra Finansów, Funduszy i Polityki Regionalnej z dnia 2.12.2020r. w sprawie obowiązkowego ubezpieczenia odpowiedzialności cywilnej podmiotu przeprowadzającego eksperyment medyczny , Wnioskodawca zobowiązany jest do zawarcia umowy ubezpieczenia odpowiedzialności cywilnej. W takim przypadku pozytywna opinia Komisji Bioetycznej ma charakter warunkowy i będzie uprawniała do prowadzenia Badania pod warunkiem zawarcia przez Wnioskodawcę umowy ubezpieczenia OC zgodnie z Rozporządzeniem wskazanym w zdaniu poprzednim

Pouczenie: W ciągu 14 dni od otrzymania decyzji wnioskodawcy przysługuje prawo odwołania do Komisji Odwoławczej za pośrednictwem Komisji Bioetycznej UM we Wrocławiu.

Opinia powyższa dotyczy: projektu badawczego realizowanego w ramach SUB.C090.19.046

Projekt otrzymał opinię komisji nr: KB – 545/2020

Przewodniczący Komisji Bioetycznej
przy Uniwersytecie Medycznym

prof. dr hab. Jerzy Rudnicki

Wrocław, dnia 27.11.2020

10.2 Oświadczenia współautorów

Lek. Paweł Kiełb
Klinika Urologii Małoinwazyjnej i Robotycznej
Uniwersyteckie Centrum Urologii
Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

Wrocław, 22.09.2023

OŚWIADCZENIE

Oświadczam, że w niżej wymienionych pracach:

1. **"Novel Histopathological Biomarkers in Prostate Cancer: Implications and Perspectives."**
   Kiełb P, Kowalczyk K, Gurwin A, Nowak Ł, Krajewski W, Sosnowski R, Szydełko T, Małkiewicz B
   Biomedicines. 2023; 11(6):1552. https://doi.org/10.3390/biomedicines11061552

mój udział polegał na opracowaniu ogólnej koncepcji artykułu, doborze metodologii, ocenie uzyskanych wyników, ocenie spełnienia warunków formalnych publikacji, udziale w analizie wybranych artykułów, przygotowaniu pierwotnej wersji manuskryptu oraz jego późniejszej korekcie oraz przygotowaniu rycin.

2. **"Role of IL-17A and IL-17RA in Prostate Cancer with Lymph Nodes Metastasis: Expression Patterns and Clinical Significance."**
   Kiełb P, Kaczorowski M, Kowalczyk K, Piotrowska A, Nowak Ł, Krajewski W, Chorbińska J, Dudek K, Dzięgiel P, Hałoń A, et al.
   Cancers. 2023; 15(18):4578. https://doi.org/10.3390/cancers15184578

mój udział polegał na opracowaniu ogólnych założeń badania oraz metodologii badania, ocenie ekspresji oraz wyników analizy statystycznej, selekcji analizowanych danych pacjentów, przygotowaniu pierwotnej wersji manuskryptu oraz jego późniejszej ocenie i korekcie oraz pomocy w pozyskaniu funduszy niezbędnych do publikacji.

3. **„Comparative analysis of GOLPH3 expression in lymph node-positive prostate cancer: immunohistochemistry staining patterns and clinical significance."**
   Kiełb P, Kaczorowski M, Kowalczyk K, Piotrowska A, Nowak Ł, Krajewski W, Gurwin A, Dudek K, Dzięgiel P, Hałoń A, Szydełko T and Małkiewicz B
   Frontiers in Oncology. 2023; 13:1265788. doi: 10.3389/fonc.2023.1265788

mój udział polegał na opracowaniu ogólnych założeń badania oraz metodologii, ocenie ekspresji oraz wyników analizy statystycznej selekcji analizowanych danych pacjentów, przygotowaniu pierwotnej wersji manuskryptu oraz jego późniejszej ocenie i korekcie.

Paweł Kiełb

Podpis

Wyrażam zgodę na włączenie wyżej wymienionych prac do cyklu publikacji, na podstawie których będzie oparta rozprawa doktorska lek. Pawła Kiełba.

Uniwersytet Medyczny we Wrocławiu
Uniwersyteckie Centrum Urologii
KLINIKA UROLOGII MAŁOINWAZYJNEJ I ROBOTYCZNEJ
kierownik

Podpis promotora

dr hab. n. med. Bartosz Małkiewicz

Prof. dr hab. n. med. Agnieszka Hałoń
Zakład Patologii Klinicznej
Katedra Patologii Klinicznej i Doświadczalnej
Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

Wrocław, 19.09.2023

OŚWIADCZENIE

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   Kiełb P, Kaczorowski M, Kowalczyk K, Piotrowska A, Nowak Ł, Krajewski W, Chorbińska J, Dudek K, Dzięgiel P, Hałoń A, et al. Cancers. 2023; 15(18):4578. https://doi.org/10.3390/cancers15184578

mój udział polegał na nadzorze merytorycznym nad procesem przeprowadzania oceny preparatów oraz opracowania uzyskanych wyników.

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Wyrażam zgodę na włączenie wyżej wymienionych prac, których jestem współautorem, do cyklu publikacji, na podstawie których będzie oparta rozprawa doktorska lek. Pawła Kiełba.

Podpis

Dr n. med. Aleksandra Piotrowska
Zakład Histologii i Embriologii
Katedra Morfologii i Embriologii Człowieka
Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

Wrocław, 22.09.2023

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mój udział polegał na przygotowaniu materiału badawczego, wykonaniu mikromacierzy tkankowych oraz przeprowadzeniu reakcji immunohistochemicznych oraz udział w ocenie i korekcie treści manuskryptu.

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Podpis

Lek. Adam Gurwin
Klinika Urologii Małoinwazyjnej i Robotycznej
Uniwersyteckie Centrum Urologii
Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

Wrocław, 19.09.2023

OŚWIADCZENIE

Oświadczam, że w niżej wymienionych pracach:

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   Biomedicines. 2023; 11(6):1552. https://doi.org/10.3390/biomedicines11061552

mój udział polegał na selekcji artykułów na podstawie których dokonano przeglądu literatury i przygotowano publikację oraz współtworzeniu pierwotnej wersji manuskryptu.

2. **„Comparative analysis of GOLPH3 expression in lymph node-positive prostate cancer: immunohistochemistry staining patterns and clinical significance."**
   Kiełb P, Kaczorowski M, Kowalczyk K, Piotrowska A, Nowak Ł, Krajewski W, Gurwin A, Dudek K, Dzięgiel P, Hałoń A, Szydełko T and Małkiewicz B (2023) Frontiers in Oncology, 13:1265788. doi: 10.3389/fonc.2023.1265788

mój udział polegał na ocenie i korekcie treści manuskryptu.

Wyrażam zgodę na włączenie wyżej wymienionych prac, których jestem współautorem, do cyklu publikacji, na podstawie których będzie oparta rozprawa doktorska lek. Pawła Kiełba.

Podpis

Adam Gurwin

Dr hab. n. med. Bartosz Małkiewicz
Klinika Urologii Małoinwazyjnej i Robotycznej
Uniwersyteckie Centrum Urologii
Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

Wrocław, 19.09.2023

OŚWIADCZENIE

Oświadczam, że w niżej wymienionych pracach:

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mój udział polegał na opracowaniu ogólnej koncepcji artykułu, ocenie uzyskanych wyników, ocenie spełnienia warunków formalnych publikacji, udziale w analizie wybranych artykułów, przygotowaniu pierwotnej wersji manuskryptu oraz jego późniejszej korekcie, nadzorze merytorycznym nad procesem tworzenia pracy oraz pomocy w pozyskaniu funduszy niezbędnych do publikacji.

2. **"Role of IL-17A and IL-17RA in Prostate Cancer with Lymph Nodes Metastasis: Expression Patterns and Clinical Significance."**
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   Cancers. 2023; 15(18):4578. https://doi.org/10.3390/cancers15184578

mój udział polegał na opracowaniu ogólnych założeń badania, ocenie spełnienia warunków formalnych publikacji, selekcji analizowanych danych pacjentów, ocenie i korekcie manuskryptu, nadzorze merytorycznym nad procesem tworzenia pracy oraz pomocy w pozyskaniu funduszy niezbędnych do publikacji.

3. **„Comparative analysis of GOLPH3 expression in lymph node-positive prostate cancer: immunohistochemistry staining patterns and clinical significance."**
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Wyrażam zgodę na włączenie wyżej wymienionych prac, których jestem współautorem, do cyklu publikacji, na podstawie których będzie oparta rozprawa doktorska lek. Pawła Kiełba.

Uniwersytet Medyczny we Wrocławiu
Uniwersyteckie Centrum Urologii
KLINIKA UROLOGII MAŁOINWAZYJNEJ I ROBOTYCZNEJ
Kierownik
dr hab. n. med. Bartosz Małkiewicz

Lek. Joanna Chorbińska
Klinika Urologii Małoinwazyjnej i Robotycznej
Uniwersyteckie Centrum Urologii
Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

Wrocław, 25.09.2023

OŚWIADCZENIE

Oświadczam, że w pracy:

"Role of IL-17A and IL-17RA in Prostate Cancer with Lymph Nodes Metastasis: Expression Patterns and Clinical Significance."
Kiełb P, Kaczorowski M, Kowalczyk K, Piotrowska A, Nowak Ł, Krajewski W, Chorbińska J, Dudek K, Dzięgiel P, Hałoń A, et al. Cancers. 2023; 15(18):4578. https://doi.org/10.3390/cancers15184578

mój udział polegał na ocenie i korekcie treści manuskryptu oraz przygotowaniu rycin wykorzystanych w artykule.

Wyrażam zgodę na włączenie wyżej wymienionej pracy, której jestem współautorem, do cyklu publikacji, na podstawie których będzie oparta rozprawa doktorska lek. Pawła Kiełba.

Joanna Chorbińska
Podpis

Dr Inż. Krzysztof Dudek
Centrum Analiz Statystycznych
Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

Wrocław, 19.09.2023

OŚWIADCZENIE

Oświadczam, że w niżej wymienionych pracach:

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   Kiełb P, Kaczorowski M, Kowalczyk K, Piotrowska A, Nowak Ł, Krajewski W, Chorbińska J, Dudek K, Dzięgiel P, Hałoń A, et al. Cancers. 2023; 15(18):4578. https://doi.org/10.3390/cancers15184578

mój udział polegał na ocenie wyników badan, ich analizie statystycznej, przygotowaniu wykresów oraz tabel.

2. **„Comparative analysis of GOLPH3 expression in lymph node-positive prostate cancer: immunohistochemistry staining patterns and clinical significance."**
   Kiełb P, Kaczorowski M, Kowalczyk K, Piotrowska A, Nowak Ł, Krajewski W, Gurwin A, Dudek K, Dzięgiel P, Hałoń A, Szydełko T and Małkiewicz B (2023) Frontiers in Oncology, 13:1265788. doi: 10.3389/fonc.2023.1265788

mój udział polegał na ocenie wyników badan, ich analizie statystycznej, przygotowaniu wykresów i tabel oraz korekcie manuskryptu.

Wyrażam zgodę na włączenie wyżej wymienionych prac, których jestem współautorem, do cyklu publikacji, na podstawie których będzie oparta rozprawa doktorska lek. Pawła Kiełba.

Podpis

Lek. Kamil Kowalczyk
Klinika Urologii Małoinwazyjnej i Robotycznej
Uniwersyteckie Centrum Urologii
Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

Wrocław, 19.09.2023

OŚWIADCZENIE

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1. **"Novel Histopathological Biomarkers in Prostate Cancer: Implications and Perspectives."**
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mój udział polegał na opracowaniu ogólnych założeń pracy, opracowaniu metodologii badania, selekcji i ocenie wybranych artykułów wykorzystanych przy tworzeniu artykułu oraz przygotowaniu pierwotnej wersji manuskryptu.

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mój udział polegał na ocenie uzyskanych wyników i przygotowaniu pierwotnej wersji manuskryptu.

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   Kiełb P, Kaczorowski M, Kowalczyk K, Piotrowska A, Nowak Ł, Krajewski W, Gurwin A, Dudek K, Dzięgiel P, Hałoń A, Szydełko T and Małkiewicz B (2023) Frontiers in Oncology, 13:1265788. doi: 10.3389/fonc.2023.1265788

mój udział polegał na selekcji analizowanych danych pacjentów i przygotowaniu pierwotnej wersji manuskryptu.

Wyrażam zgodę na włączenie wyżej wymienionych prac, których jestem współautorem, do cyklu publikacji, na podstawie których będzie oparta rozprawa doktorska lek. Pawła Kiełba.

Podpis

Kamil Kowalczyk

Lek. Łukasz Nowak
Klinika Urologii Małoinwazyjnej i Robotycznej
Uniwersyteckie Centrum Urologii
Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

Wrocław, 19.09.2023

OŚWIADCZENIE

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mój udział polegał na opracowaniu metodologii badania i ocenie wybranych artykułów wykorzystanych przy tworzeniu artykułu.

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mój udział polegał na ocenie treści manuskryptu oraz przygotowaniu rycin.

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mój udział polegał na ocenie treści manuskryptu i selekcji danych poddanych analizie.

Wyrażam zgodę na włączenie wyżej wymienionych prac, których jestem współautorem, do cyklu publikacji, na podstawie których będzie oparta rozprawa doktorska lek. Pawła Kiełba.

Łukasz Nowak
Podpis

Dr n. med. Maciej Kaczorowski
Zakład Patologii Klinicznej
Katedra Patologii Klinicznej i Doświadczalnej
Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

Wrocław, 21.09.2023

OŚWIADCZENIE

Oświadczam, że w niżej wymienionych pracach:

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mój udział polegał na opracowaniu metody oceny oraz ocenie ekspresji GOLPH3 w preparatach histopatologicznych, udziale w procesie recenzji i korekty manuskryptu.

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mój udział polegał na opracowaniu ogólnych założeń badania, opracowaniu metody oceny oraz ocenie ekspresji GOLPH3 w preparatach histopatologicznych, udziale w procesie recenzji i korekty manuskryptu.

Wyrażam zgodę na włączenie wyżej wymienionych prac, których jestem współautorem, do cyklu publikacji, na podstawie których będzie oparta rozprawa doktorska lek. Pawła Kiełba.

Podpis

Prof. dr hab. n. med. Piotr Dzięgiel
Zakład Histologii i Embriologii
Katedra Morfologii i Embriologii Człowieka
Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

Wrocław, 22.09.2023

OŚWIADCZENIE

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Podpis

Prof. dr hab. n. med. Tomasz Szydełko
Klinika Urologii Małoinwazyjnej i Robotycznej
Uniwersyteckie Centrum Urologii
Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

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mój udział polegał na nadzorze merytorycznym nad przygotowaną pracą.

2. **"Role of IL-17A and IL-17RA in Prostate Cancer with Lymph Nodes Metastasis: Expression Patterns and Clinical Significance."**
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mój udział polegał na nadzorze merytorycznym nad przeprowadzonym badaniem

3. **„Comparative analysis of GOLPH3 expression in lymph node-positive prostate cancer: immunohistochemistry staining patterns and clinical significance."**
   Kiełb P, Kaczorowski M, Kowalczyk K, Piotrowska A, Nowak Ł, Krajewski W, Gurwin A, Dudek K, Dzięgiel P, Hałoń A, Szydełko T and Małkiewicz B (2023) Frontiers in Oncology, 13:1265788. doi: 10.3389/fonc.2023.1265788

mój udział polegał na nadzorze merytorycznym nad przeprowadzonym badaniem, zarządzaniu projektem badania oraz ocenie i korekcie manuskryptu.

Wyrażam zgodę na włączenie wyżej wymienionych prac, których jestem współautorem, do cyklu publikacji, na podstawie których będzie oparta rozprawa doktorska lek. Pawła Kiełba.

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Wrocław, 19.09.2023

OŚWIADCZENIE

Oświadczam, że w niżej wymienionych pracach:

1. **"Novel Histopathological Biomarkers in Prostate Cancer: Implications and Perspectives."**
   Kiełb P, Kowalczyk K, Gurwin A, Nowak Ł, Krajewski W, Sosnowski R, Szydełko T, Małkiewicz B
   Biomedicines. 2023; 11(6):1552. https://doi.org/10.3390/biomedicines11061552

mój udział polegał na selekcji artykułów na podstawie których dokonano przeglądu literatury i przygotowano publikację.

2. **"Role of IL-17A and IL-17RA in Prostate Cancer with Lymph Nodes Metastasis: Expression Patterns and Clinical Significance."**
   Kiełb P, Kaczorowski M, Kowalczyk K, Piotrowska A, Nowak Ł, Krajewski W, Chorbińska J, Dudek K, Dzięgiel P, Hałoń A, et al. Cancers. 2023; 15(18):4578. https://doi.org/10.3390/cancers15184578

mój udział polegał na selekcji i ocenie analizowanych danych.

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mój udział polegał na selekcji i ocenie analizowanych danych oraz udziale w procesie korekty manuskrytpu.

Wyrażam zgodę na włączenie wyżej wymienionych prac, których jestem współautorem, do cyklu publikacji, na podstawie których będzie oparta rozprawa doktorska lek. Pawła Kiełba.

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Wrocław, 19.09.2023

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