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Review

STAT3 and Its Pathways Dysregulation – Underestimated Role in Urological Tumors.

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Abstract: Nowadays molecular research is essential for the better understanding of tumor cells pathophysiology. The increasing number of neoplasms is taken under 'the molecular magnifying glass' therefore it is possible to discover complex relationships between cytophysiology and tumor cells. Signal transducer and activator of transcription 3 (STAT3) belongs to the family of latent cytoplasmic transcription factors called STATs which comprises seven members: STAT1, STAT2, STAT3, STAT5A, STAT5B, STAT6. Those proteins play important role in cytokine-activated gene expression by transducing signals from the cell membrane to the nucleus. Abnormal prolonged activation results in tumorigenesis, metastasis, cell proliferation, invasion, migration and angiogenesis. Inhibition of this transcription factor inhibits previously mentioned effects in cancer cells whereas normal cells are not affected. Hence STAT3 might be a viable target for cancer therapy.

Keywords: STAT3; prostate cancer; bladder cancer; upper tract urothelial carcinoma; renal cell carcinoma; penile cancer; testicular cancer

1. Introduction

All urological cancers made up 13% of incidence and 8% of mortality of all cancers in 2020 worldwide. Prostate cancer (PCa) is one of the most common cancer in men, being the most common cancer in urology, representing about 56% of all urological cancers in 2020 [1]. The most important risk factors are age (the majority of PCa diagnosis occurs between the age 65 and 74) and familial history of PCa [2]. Bladder cancer is the second most common and deadly cancer met in urology, diagnosed, and causing death in men approximately 4 times those among women. Known risk factors are smoking, exposure to aromatic amines, chronic urinary tract infections, pelvic radiotherapy or cyclophosphamide chemotherapy [1,3]. Renal cell carcinoma (RCC) takes the third place in both incidence and mortality among urological cancers, representing about 17% and 23% of those, respectively [1]. Smoking, hypertension or obesity predispose to its occurrence [4]. Upper tract urothelial carcinoma (UTUC) is a rare tumor with an incidence estimated at 1-2/100 000. Major risk factors are similar to those of RCC [5]. Penile cancer (PeCa) is another occasional condition, accounting for 0,2% incidence and 0,1% mortality of all cancers, described as epidemiologically significant mainly in countries of South America or Africa [1,6]. Similarly to PeCa, testicular cancer is one of the most uncommon cancers. It is

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frequently diagnosed in young men, with the highest rate between the age 25-29 and 30-43 [1,2].

Signal transducer and activator of transcription (STAT) protein family includes STAT3 [7]. In normal conditions STAT3 is latently located in cytoplasm. However, there are many pathways which may activate STAT3 and the major one is interleukin 6 (IL-6) pathway. After stimulation STAT3 phosphorylation, dimerization and nuclear translocation occur, respectively. In the nucleus the protein acts as a transcriptional factor, resulting in enhanced cell survival, proliferation, migration and angiogenesis, and inhibited apoptosis. Main downstream genes are Bcl-2, Bcl-xL, Bcl-6, survivin, MYC, cyclin D1, MMPs and VEGF [8].

STAT3 has already been described as a prooncogenic protein in many different tumors like breast, head and neck, lung, gastric or pancreatic cancer [9,10]. Moreover, the relationship between not only STAT3 and cancerous cells, but also STAT3 and tumor microenvironment (TME) was shown many times, also in case of urological cancers. This process may include changes in myeloid-derived suppressor cells (MDSCs) or tumor-associated macrophages (TAMs) phenotype or differentiation of mesenchymal stem cells (MSCs) into osteoblasts in PCa bone metastasis [11–13].

Considering epidemiological importance of urological cancers as well as multidimensional STAT3 activity in tumor progression and metastasis we strongly believe that STAT3 is underestimated in urologic oncology. Therefore we consider this issue worth describing. The aim of this work is to gather and discuss the major recently published findings in the field of urological cancers through the prism of STAT3 used as a biomarker or therapeutical target to help researchers revising their view and exploring this matter.

3. Role of STAT3 in cancers

STAT3 belongs to the family of latent cytoplasmic transcription factors called STATs which comprises seven members: STAT1, STAT2, STAT3, STAT5A, STAT5B, STAT6. Those proteins play important role in cytokine-activated gene expression by transducing signals from the cell membrane to the nucleus [14,15].

STAT3 is characterized by six main structural motifs: amino-terminal domain, coiled-coil domain, DNA binding domain, linker domain, SRC2 Homology (SH2) and transactivation domain [15]. The SH2 domain is the most specific part of the protein. It is responsible for identifying and binding of phosphotyrosine motifs. Furthermore, it provides recognition and binding by JAK protein activation. Finally, it allows dimerization of STAT3 either with another STAT3 molecule or with remaining STAT family members [16]. In regular conditions STAT3 is in a latent state in the cytoplasm, after external signals cell surface receptors oligomerize which leads to proximation of the tyrosine kinases and triggers its transphosphorylation, finally resulting in activation of kinases. Further phosphorylation of the internal domain of receptor by kinases ends up with the recruitment of STAT3 and its phosphorylation. Activation of STAT3 causes either homodimerization or heterodimerization of protein and translocation to the nucleus where it stimulates gene expression by binding to DNA [17]. The STAT3/JAK pathway is immediately quenched by suppressors of cytokine signaling (SOCS), protein inhibitors of activated STATs (PIAS), protein tyrosine phosphatases (PTPases), or through protein degradation by ubiquitin–proteosome machinery [8]. STAT3 is activated mainly by IL-6 and epidermal growth factor (EGF), however many other factors were exposed to take part in this process [18].

Upon binding to DNA STAT3 regulates the expression of many important genes such as Bcl-2, Bcl-xl, Bcl-6 and survivin responsible for cell survival and inhibition of apoptosis; MYC and cyclin D1 regulators of proliferation; MMPs (matrix metalloproteinases) promotors of metastasis and migration; VEGF (vascular endothelial growth factor) mediator of angiogenesis; IL-6 pro-inflammatory cytokine and IL-10 immunosuppressive cytokine. Interestingly, the transcriptional effect of STAT3 varies among tissue types [17]. Aberrant phosphorylation of STAT3 was reported in 70% of cancers and was associated with poor prognosis [17]. The aforementioned refers not only to solid tumors but also to hematological malignancies.

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Constitutive activation of this transcription factor was detected previously in acute myeloid leukemia, multiple myeloma, non-Hodgkin lymphoma, chronic lymphocytic leukemia (CLL), as well as solid tumors of the lung, breast, ovary, cervix, prostate, bladder, kidney, colon, liver, stomach and head and neck [17]. Aberrant activation of STAT3 might be caused by spontaneous mutation of protein, however it is mostly assigned to autocrine and paracrine cytokine stimulation [7,17]. Additionally, hyperactivation of STAT3 was observed as a result of MEK therapeutical inhibition [19]. Abnormal prolonged activation results in tumorigenesis, metastasis, cell proliferation, invasion, migration and angiogenesis. Inhibition of this transcription factor inhibits previously mentioned effects in cancer cells, whereas normal cells are not affected. Hence STAT3 might be a viable target for cancer therapy [20].

4. Role of STAT3 in prostate cancer

In 2020 prostate cancer was the second most frequent cancer in men worldwide. It was also the fifth cause of cancer death in this group. Incidence has the highest rate in Northern and Western Europe, Caribbean and Australia/New Zealand area [1]. However, a sharp reduction in prostate cancer incidence between 2010 and 2014 was reported. Five-year relative survival rate of localized and regional PCa is estimated to be >99%, whereas in distant cancer the rate dramatically decreases to 30% [21]. This may indicate why scientists put so much effort into developing new strategies of managing PCa. It was also shown that Africans are more susceptible to develop PCa during their life and Asians are less affected. Such differences are yet to be explained [22].

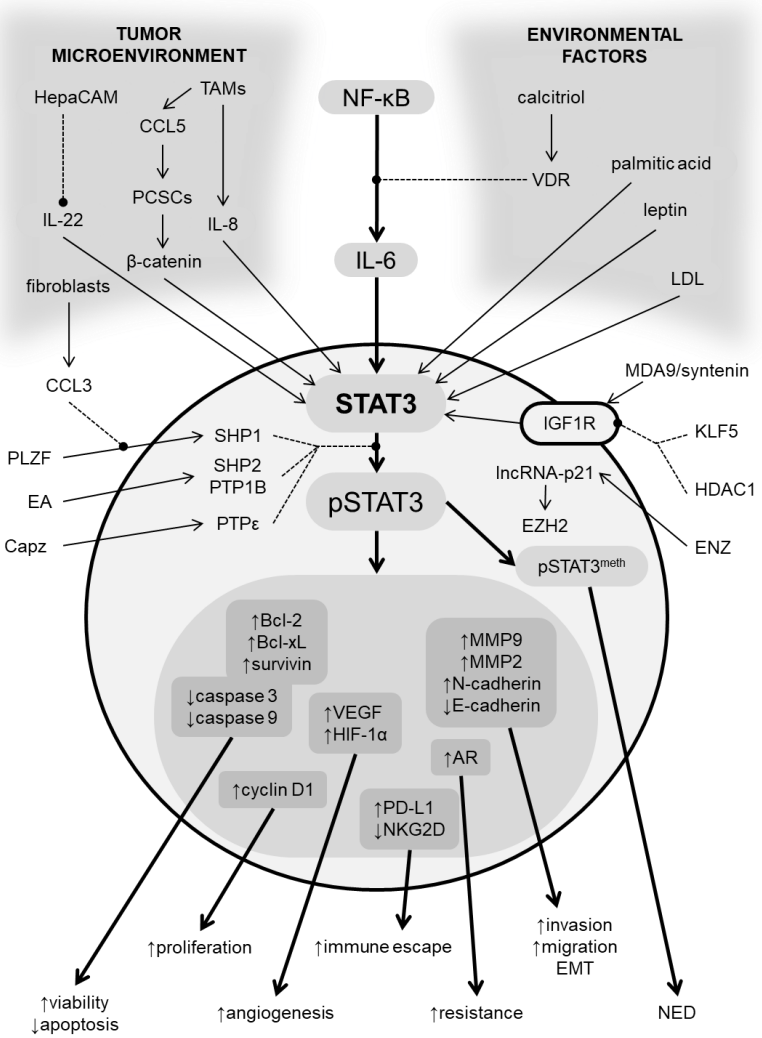

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Figure 1. Schematic and simplified depiction of selected pathways and factors regulating STAT3 expression counting its downstream target proteins with the consequences of their overexpression in prostate cancer. Dashed line ended with a dot pictures inhibition; arrow pictures stimulation.

Plenty of research papers highlighted the importance of STAT3 in PCa development. Many factors and different conditions may trigger STAT3 activation, but the core seems to be NF-κB/IL-6/STAT3 axis [23–27]. There is also lots of downstream targets being under the control of STAT3. Overexpressed STAT3 forces cyclin D1, Bcl-2, Bcl-xL and survivin expression which have proliferative and antiapoptotic properties [28–30]. PCa cells may also avoid apoptosis by inhibited expression of caspase 3 and caspase 9 through STAT3 activity [31,32]. On the other hand, elevated levels of N-cadherin, vimentin, MMP2 and MMP9 (mesenchymal factors) and decreased level of E-cadherin (epithelial marker) enhance invasive and migrative capabilities, leading to epithelial-mesenchymal transition (EMT) [25,33,34]. Overexpression of VEGF results in augmented angiogenesis [35]. Lastly, induced expression of PD-L1 helps a tumor to avoid physiological immune response [36]. Collectively, high expression and activation of STAT3 result in increased cell survival, proliferation, angiogenesis, invasion and migration, leading to distal metastasis [37–39].

Tumors might be described as complicated organs which consist of not only tumorous cells, but also benign cells, e.g. stromal cells. All of them create TME which has recently caught scientists' interest. Exemplary stromal cells are fibroblasts – critical regulators of metastatic progression in PCa. Promyelocytic leukemia zinc finger (PLZF) takes part in self-renewal or stem cells differentiation and may act as a tumor suppressor. Collected data showed that PLZF level is decreased whereas phosphorylated STAT3 (pSTAT3) level is increased with PCa progression. Exogenous overexpression of PLZF resulted in substantial inhibition in STAT3 phosphorylation by increased SHP1 expression which has an ability to deactivate JAK/STAT3 pathway. On the other hand, fibroblasts produce CCL3, which prevents PLZF expression. Scientists presented a part of complicated relationship between fibroblasts and PCa cells, describing a potentially useful CCL3/PLZF/SHP1/STAT3 cascade [40]. Zhao et al. reported that highly concentrated lactoferrin (LTF) significantly decreases STAT3 and granulocyte-macrophage colony-stimulating factor (GM-CSF) levels, which result in immune TME changes [41]. One of the most abundant immune cell population in TME is TAMs. CCL5 is a chemokine produced by TAMs. Elevated CCL5 level was noticed in PCa tissues and has been associated with migration, invasion and EMT promotion. CCL5 promotes self-renewal of prostate cancer stem cells (PCSCs) by activating their CCL5 receptor (CCR5) and thereby stimulating β-catenin/STAT3 signaling pathway, which results in STAT3 upregulation. In vivo studies revealed that CCL5 blockage leads to inhibition in PCa growth, bone metastasis and PCSCs self-renewal. Therapy focused on inhibiting TAMs or CCL5 and CCR5 might be a promising future approach [33]. TAMs may be divided into two groups – M1, which acts as anticancer cells, and M2, promoting cancerous characteristics. One phenotype can switch into the other under specific conditions. It was evidenced that the use of PC3 (human PCa cell line) supernatant results in disappearance of M1 and emergence of M2 biomarkers. Additional STAT3 inhibitor completely changes this trend and forces differentiation into M1 phenotype. Modifying TME by TAMs manipulations may contribute to develop new strategies [42]. Another study was focused on exploring the role of IL-8 secreted by M2. It was shown that IL-8 activates STAT3/MALAT1 (lncRNA upregulated in PCa tissues) pathway and therefore promotes PCa progression. MALAT1 gene knockdown results in inhibition of proliferation and invasion. It has to be explored whether STAT3 activation is pivotal for MALAT1 overexpression [43]. Another part of TME are MDSCs. MDSCs characterized as $CD33^+$ $pSTAT^+$ are more frequent in PCa TME comparing to benign prostate hyperplasia (BPH) tissues. Described phenomenon may be the basis for future studies on developing new treatment strategies [11]. MDSCs play a crucial role in suppressing antitumor immunity and their generation may be induced by PCa cells. Galiellalactone, a STAT3 inhibitor, effectively downregulates MDSCs level. Researchers conclude that galiellalactone may impair arising immunological immunity and suggest a

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potential use of STAT3 inhibitors in advanced PCa [44]. Innovative approach to change TME in favor to patients may come with combined treatment of STAT3 inhibition and Toll-like Receptor 9 (TLR9) stimulation. Developed therapy significantly changed TME by neutrophiles and CD8+ T cells recruitment and decrease in MDSCs' population at the same time. Results suggest that bifunctional and immunostimulatory combination has the potential to disrupt tumor immunological escape mediated by STAT3 [45]. McGuire et al. described a process of MSCs recruitment by PCa bone metastasis. Firstly, MSC-derived IL-28 leads to PCa cells apoptosis, but persistent exposure results in the selection of cells resistant not only to IL-28-induced apoptosis but also to chemotherapeutics like docetaxel or etoposide. Scientists reported that the use of STAT3 inhibitor, S32-201, selectively inhibited MSC-selected PCa cells. Such cells are notably susceptible to STAT3 inhibition in vivo [13]. Witt et al. analyzed how the use of STAT3 inhibitor will influence anti-CTLA-4 (antibody enhancing immune response) treatment. Cotreatment resulted in a significant enhancement in survival time in mice when compared to group treated with antibody only. However, complete tumor regression was not observed. Significant $CD45^+$ (characteristic for all leucocytes) cells tumor infiltration, as well as a substantial reduction in regulatory T cells population (which may contribute to tumor's resistance development), was noticed intratumorally, respectively. The authors point at the therapeutical potential of such an approach [46]. Apoptotic cells are supposed to produce paracrine molecules that promote a compensatory proliferation mechanism among survived cells. Conducted experiment showed that after etoposide-induced apoptosis, PC3 repopulation, EMT and chemoresistance occur through caspase-3/cPLA2/COX-2/PGE-2/EP4-/2/STAT3 axis, called Phoenix Rising. Although Phoenix Rising is a physiological process responsible for regeneration in healthy tissues, some epigenetic-induced changes may lead to pathological repopulation of cancerous cells. New insight into the repopulation mechanism may result in new therapeutical strategies [47].

Castration resistance prostate cancer (CRPC) often remains lethal or refractory for available therapies. Many researchers consider developing tools to break this resistance as a key to save PCa patients. Cytotoxic natural killer (NK) cells were tried to manage CRPC. It was shown that IL-6-producing tumors were more resistant to their cytotoxicity, which suggests the importance of IL-6 signaling in determining tumor cells' sensitivity. What is more, high IL-6 expression led to excessed PD-L1 expression at CRPC cells, which in turn resulted in T cells death or inactivation, and decreased NKG2D ligand expression, which disrupted NK cells with recognizing tumor cells. Collected data showed that using JAK1 inhibitor or STAT3 inhibitor resulted in decreasing PD-L1 and increasing NKG2D levels. PD-L1 antibodies increased NK cells cytotoxicity as mentioned inhibitors did. Collectively, combined therapy of JAK inhibitor or STAT3 inhibitor with the PD-L1 antibody showed much stronger effects than using these molecules in monotherapy. Combined therapy leads to increased susceptibility of CRPC cells to cytotoxicity mediated by NK cells. Inhibitory targeting of IL-6 or its downstream proteins with PD-L1 antibody might be a successful way of managing CRPC [36]. It was also presented that fructose-1,6-bisphosphatase (FBP1), a glycolysis inhibiting enzyme, acts through STAT3 pathway and its loss leads to increased expression of PD-L1. Carried experiments revealed that FBP1, independently of its enzyme activity, interferes with STAT3 and inhibits STAT3 binding to the locus of PD-L1 gene. This effect was reversed by ionizing radiation or IL-6 administration which increased STAT3 phosphorylation. Knockdown of FBP1 gene led to a significant enhancement of PD-L1 protein and mRNA expression and tumor growth. Interestingly, loss of FBP1 gene correlated with stronger resistance to anti-PD-L1 treatment. Collectively, FBP1 loss may be involved in the tumor immune escape. Further studies are needed to define new strategies [48]. Hepatocyte cell adhesion molecule (HepaCAM) is an immunoglobulin-like molecule poorly expressed or absent in malignant tumors. Recent study showed HepaCAM interferes IL-22/STAT3 axis and consequently blocks STAT3 phosphorylation and noticeably decreases proliferation, migration and invasion of CRPC cells. Results were pictured not only by reduced levels of STAT3 target genes, but also by the absence of any lung metastatic areas in mice. Restoring expression of

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HepaCAM might be a promising perspective for CRPC patients [29]. Lin et al. reported that CYP1B1, an enzyme catalyzing the synthesis of 4-hydroxy-17β-estradiol (4-OHE2) from estradiol, might contribute to the development of CRPC by promoting PCSCs characteristics. 4-OHE2 is supposed to increase IL-6 expression which in turn intensifies IL-6/STAT3 pathway and its downstream genes. Scientists found out that CYP1B1 expression positively correlates with the Gleason Score (GS) and is higher expressed in CRPC tissues comparing to androgen-dependent PCa cells. Moreover, CYP1B1 enhanced CRPC resistance to bicalutamide while its knockdown reversed this effect. CYP1B1 seems to be a new therapeutic target in CRPC patients [49].

Different therapeutical strategies have a potential to induce resistance in PCa cells which may contribute to cancerous cells survival. Hu et al. focused on autophagy phenomenon, which may lead to chemoresistance acquirement, in CRPC cells induced by docetaxel. Collected data showed that STAT3 negatively regulates this process. Activated STAT3 is supposed to decrease CRPC cells viability during chemotherapy by apoptosis promotion. Finding a molecular explanation for these outcomes may be a basis to new treatment strategies [50]. Therefore, managing acquired resistance may be a key to develop the proper treatment. It was shown that alantolactone (ALT) decreases cancer stem cells (CSCs) viability through STAT3 inhibition and sensitizes those cells to cisplatin [51]. Galiellalactone, a direct STAT3 inhibitor, was reported to significantly decrease docetaxel-resistant PCa cells viability and may be used in combined therapy [52]. Similarly, another team proved galiellalactone's efficacy in managing enzalutamide-resistant PCa both in monotherapy and in combination [53]. Furthermore, metformin is capable of reversing EMT promoted by enzalutamide (ENZ) by targeting TGF-β1/STAT3 axis. Combined therapy of metformin and ENZ was especially effective and promising [24]. Although ENZ prolongs PCa patients' lives by about 5 months, it can also induce neuroendocrine differentiation (NED) by activating lncRNA-p21/EZH2/STAT3 pathway. Developed neuroendocrine PCa cells (NEPCs) are insensitive to androgen deprivation therapy (ADT) and therefore exacerbate the course of illness. Scientists suggest that EZH2 targeting may reduce enzalutamide-induced changes [54]. Collected data implied that STAT3 activation may result in radioresistance. Zhang et al. proved that the use of STAT3 inhibitor or STAT3 knockdown increase sensitivity of PCa cells to irradiation. Complex treatment of radio- and chemotherapy (STAT3 inhibition or knockdown) demonstrated synergistic effect pictured by augmented apoptosis. These results seem to be an interesting approach to PCa managing, combining two methods of cancer treatment [55].

Recently some studies focused on targeting NF-κB/IL-6/STAT3 axis have been published. It was shown that IL-8 level is elevated in PCa cells and it considerably promotes proliferation as well as migration and invasion while inhibiting apoptosis. Mechanistically, IL-8 works by activating STAT3/AKT/NF-κB axis. This finding may contribute to develop new treatment strategies [56]. Considering the pivotal role of mentioned axis in PCa progression, scientists developed dual STAT3/NF-κB inhibitor. Iridium(III), showing anti-NF-κB properties, was conjugated with benzofuran, which acts as a STAT3 inhibitor. Collected data shows that synthesized complex not only inhibits STAT3 activation and binding of already activated STAT3 to DNA but also decreases nuclear translocation of NF-κB from the cytoplasm. Interestingly, benzofuran-iridium(III) was relatively more toxic against DU145 cells than cisplatin and doxorubicin with simultaneous lower toxicity to normal human cell lines. This conjugation seems to be an interesting and promising way of treatment [27]. N-myc downstream-regulated gene 1 (NDRG1) is an important molecular regulator, inhibiting PCa progression and metastasis. It inhibits many precancerous signaling pathways which promote CRPC development. It was proved that NDRG1 significantly decreases levels of activated STAT3, IL-6 and NF-κB. Considering that NDRG1 affects crucial steps in NF-κB/IL-6/STAT3 axis, and therefore disrupts androgen-independent AR activation pathways, it might be a promising solution for CRPC patients [57].

Some scientific teams have recently tried to use RNA molecules as a treatment targeted at STAT3. Wei et al. found out that long non-coding RNA (lncRNA) called MAGI2-

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AS3 is one of the most downregulated lncRNA in PCa tissues. MAGI2-AS3 is supposed to act as a sponge for another lncRNA appearing in PCa cells – miR-424-5p, which activates STAT3 pathway. It was shown that MAGI2-AS3 forced overexpression decreases STAT3 concentration and, in turn, inhibits cell viability and enhances apoptosis [58]. Another study presented that miRNA-583 expression in PCa tissues is significantly inhibited. miRNA-583 transfection resulted in considerable diminished proliferation and invasion of PCa cells due to JAK1 inhibition and, as a result, abolished activation of STAT3. Collected data suggest a potential use of miRNA-583 as a treatment. Described mechanism of action has remained unknown until this study [59]. A relationship between specific lncRNA and TAMs has also been described. It was proved that LINC00467 induces macrophage polarization from M1 to M2 phenotype which is responsible for STAT3 pathway activation through miR-494-3p/STAT3 cascade. Potential use of blocking this molecule might benefit patients by inhibiting proliferation and infiltration of PCa cells [60]. Similar properties to those mentioned before are represented by another lncRNA – LINC00473. It activates JAK/STAT3 pathway and therefore contributes to proliferation of PCa cells. Consequently, inhibitory targeting of LINC00473 might be a new way of treatment [61].

It is commonly known there are many environmental risk factors of developing cancers. The way we live and create our habits might also contribute to PCa emerging. Kwan et al. proved that a high-fat diet increases tumor size, STAT3 phosphorylation and palmitic acid (PA) levels in the xenograft tissues. PA upregulates STAT3 mRNA and protein. Moreover, PA strongly binds to STAT3, which changes its conformation and activity [26]. Another team noted that LDL cholesterol significantly increases pSTAT3 level by enhanced JAK1 and JAK2 phosphorylation. LDL intensifies the proliferative and invasive abilities of PCa cells. The use of statins may benefit PCa patients [28]. The impact of obesity on PCa development was investigated by exploring the role of leptin. It was shown that increased leptin concentration boosts EMT by inducing STAT3 phosphorylation. What is more, it was noticed that the level of leptin receptor is much higher in adenocarcinoma than in BPH [25]. 27-hydroxycholesterol (27HC) was found to impair lipid rafts and inhibit their signaling pathways. In vivo experiment showed that 27HC treatment resulted in a statistically significant difference in tumor size in treated group when compared to control group. Mechanistically, 27HC disrupts IL-6/JAK/STAT3 pathway and, what is more, acts synergistically with STAT3 inhibitors. Further studies are needed to apply these findings clinically [62]. Despite calcitriol's importance in regulating calcium and phosphorus metabolism, it also has anti-inflammatory or anti-tumor properties. It was revealed that calcitriol inhibits lipopolysaccharide(LPS)-induced migration and invasion of PCa cells. Calcitriol enhances physical interaction between STAT3 and vitamin D receptors (VDR), resulting in disrupted nuclear translocation of STAT3. Moreover, calcitriol leads to VDR and NF-κB binding, which in turn downregulate IL-6 and IL-8. Clinical relevance and in vitro studies have to be carried out [63]. Same pro-cancerous mechanism was analyzed through the prism of melatonin activity. This molecule not only inhibited LPS-induced invasion and migration but also affected not stimulated cells in the same way. Expression of NF-κB was noticed to be downregulated as well as IL-6 and STAT3 levels. In vivo studies might confirm therapeutical potential [64].

There are many different approaches to manage PCa. Krüpple-like transcription factor 5 (KLF5) is a zinc-finger transcription factor regulating proliferation, apoptosis or invasion. It was observed that PCa tissues are characterized by downregulation of KLF5. KLF5 expression is significantly inhibited in tissues described as GS8-GS10 and its concentration is lower in PCa metastasis rather than in localized PCa. That is why scientists assumed that loss of KLF5 might promote invasive abilities of PCa and consequently it was proved in experiments. Mechanistically KLF5 downregulation activated IGF1/STAT3 pathway which in turn led to PCa invasion. KLF5 was also reported to cooperate with HDAC1 (histone deacetylase) in binding to the promoter of IGF1 gene [23]. The role of insulin-like growth factor (IGF) and its receptor (IGF1R) was also explored in another study. MDA-9/syntenin is a protein overexpressed in many types of human cancers and its upregulation caused enhanced invasive abilities of PCa cells. Overexpression of MDA-

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9/syntenin was observed to correlate with STAT3 overactivation. MDA-9/syntenin physically interacts with IGF1R and leads to its autophosphorylation which consequently activates STAT3. All in all, MDA-9/syntenin-IGF1R interaction is yet to be explored [65]. Ethacrynic acid (EA) is a diuretic with a potential of inhibiting STAT3 by activating phosphatases SHP2 and PTP1B which consequently dephosphorylate STAT3 at Tyr705. In vivo experiments revealed EA's antiproliferative properties [66]. Similar effects were previously observed using capsazepine (Capz), a synthetic analogue of capsaicin. Capz reduced STAT3 phosphorylation and nuclear translocation by blocking phosphorylation at Tyr705. It was discovered that Capz increases the expression of protein tyrosine phosphatase ε (PTPε) which consequently deactivates STAT3. Capz dramatically reduces downstream STAT3 target genes levels. Potential involvement of NF-κB axis in this mechanism is still unknown [67]. Post-translational modifications (PTMs) of STAT3 might not only be useful as a biomarker, but also as a potential therapy. Inhibiting STAT3 phosphorylation, acetylation or glutathionylation at specific amino-acids could be a way to disturb driving intracellular signals conducting to PCa progression [68]. Commonly used antiandrogens might inhibit PCa proliferation, but excessing PCa invasive abilities at the same time. It was shown that ASC-J9® is able to induce STAT3 sumoylation which leads to decreased STAT3 phosphorylation. Adding this result to previous known ability of degrading AR, ASC-J9® seems to be a promising candidate for supporting ADT or radiotherapy [69]. Virotherapy seems to be an interesting and promising way of managing PCa. In vitro studies showed that Newcastle disease virus (NDV) induces immunogenic cell death (ICD) markers of PC cells. Addition of STAT3 inhibitor resulted not only in decreased STAT3 phosphorylation but also significant enhancement of released ICDs. The mechanism of this synergistic effect waits to be explored [70]. Metformin is a drug widely used for type 2 diabetes. Tang et al. tried to use its potential to inhibit EMT of PCa cells induced by ADT. Metformin reduced migration and invasion by about 50% which was statistically significant and it was consistent with biomarkers' levels specific for EMT. Finally, it was shown that metformin significantly decreased pSTAT3 level without influencing total STAT3 (tSTAT3) by inhibiting COX2/PGE2/STAT3 axis. Interestingly, highly concentrated metformin is capable of inhibiting STAT3 directly even with exogenous PGE2 presence [34]. S-adenosylmethionine (SAM), which acts as a biological methyl donor, seems to be another promising STAT3 inhibitor. Studies showed a significant reduction of STAT3 protein and phosphorylated form after 72h and 120h of SAM treatment [39]. It was demonstrated that cell lines without androgen receptor (AR) expression show the highest levels of fibrinogen. Knockdown fibrinogen gene, which remains under IL-6/STAT3 control, resulted in inhibited proliferation and mobility of PCa cells [71].

A large number of recently published research papers focused on exploring antitumor properties of natural compounds may suggest that many scientists pin their hopes on mother nature. Lots of molecules have a potential to become an anticancer drug indeed. Fucoidan, a polysaccharide sourced from brown algae, was found to reduce activated JAK and STAT3 levels in PCa tissues, presenting a massive antiangiogenic ability [35]. Atractylenolide II, a natural sesquiterpene lactone, inhibits JAK2/STAT3 pathway activity [72]. Proscillaridin A, a cardiac glycoside obtained from *Urginea maritima*, disrupts the same pathway and, even more importantly, substantially enhances antiapoptotic abilities of doxorubicin [73]. Liu et al. reported acetyl-11-keto-β-boswellic acid, a pentacyclic triterpenic acid collected from gum resin trees, as a strong cytotoxic agent in PC3 cells resistant to docetaxel. This compound downregulates not only pJAK2 and pSTAT3, but also IGF1R or pAKT levels [74]. *Ganoderma lucidum*, a mushroom used in Chinese medicine, contains triterpenes which are supposed to act as an anticancer factor. It was shown *G. lucidum* is also cytotoxic to PC3 cell line through JAK1/STAT3 pathway deactivation [32]. On the other hand, carvacrol, a natural flavoring approved for food use, significantly reduces IL-6 and STAT3 expressions, resulting in limited invasion, migration, proliferation and viability of PCa cells. The whole mechanistic picture is yet to be explored [75]. There are also some organic molecules or their derivatives that inhibit STAT3 activity through direct binding, preventing STAT3 dimerization and nuclear translocation [76–

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78]. Compound K (CK), a saponin obtained from ginseng, was reported to increase miR193a-5p expression in DU145 cell line, which leads to attenuated STAT3 and PD-L1 expression. Thanks to those, CK demonstrates its proapoptotic properties through the lack of T cells inactivity [30]. Similarly, PD-L1 inhibition through STAT3 pathway deactivation seems to be possible with the use of CFF-1, a traditional Chinese medicine cure. CFF-1 is likely to act alone or with docetaxel combination to present its effects. It is reported to inhibit tumor growth and lung metastasis [79]. Methyllucidone, a cyclopentenedione isolated from some Lauraceae family plants' fruit, has abilities to inhibit STAT3 activation even by 90%. Mechanistically, methyllucidone exerts MEG2 expression, a PTP known from its capability of STAT3 dephosphorylation [80]. On the other hand, Qi Ling, another medication from traditional Chinese medicine, has the potential to alter TME and force TAMs to switch from M2 to M1 phenotype through IL-6/STAT3 pathway inhibition. Moreover, Qi Ling is supposed to decrease the paclitaxel resistance in PCa tissues [12]. Furthermore, a polymethoxyflavone obtained from citrus called nobiletin was reported to decrease STAT3 expression with consecutive enhancement in bicalutamide cytotoxicity [81]. Astaxanthin, which is naturally produced by marine organisms like algae, was proved to inhibit proliferation and colony forming by PCa cells through disrupting STAT3 and related pathways, e.g. JAK2 or NF-κB [31]. The final described product of Chinese medicine called compound 154 is collected from the skin of giant toads and was evidenced to work as a STAT3 and AR inhibitor. Besides its increased cytotoxicity to normal tissue, it might be a therapeutical option after molecular modifications [82].

Diagnostics is the first step of a long journey of treatment. Well-developed biomarkers characterized by high sensitivity and specificity might act as a powerful weapon in clinicians' hands. Early and accurate diagnosis yields appropriate treatment, which may provide better outcomes. It seems to be crucial to diagnose benign conditions like BPH before their progression to PCa. Sanaei et al. tried to use pSTAT3 as a biomarker of MDSCs – immature cells which accumulate in pathological condition of inflammation and exist in TME. These cells were described as $CD33^{+}$ $pSTAT3^{+}$ and pSTAT3 marking was used to differentiate MDSCs from other myeloid cells, which might act as anticancer factors. The research showed that $CD33^{+}$ $pSTAT3^{+}$ cells were significantly frequent in the patients with PCa in comparison to the control group with BPH. However, there were no relevance between MDSCs level and GS. Researchers conclude that elevated MDSCs level might indicate progression from BPH to PCa [11]. STAT3 molecule undergoes some specific PTMs. Researchers found out that these alterations are characteristic for different cellular conditions. STAT3 acetylation at Lys685 was observed in overall inflammation, whereas glutathionylation or phosphorylation at Ser727 was more specific for conditions of oxidative stress. What is more, mentioned PTMs were correlated with GS. Lys685 acetylation was detected in tissues described as GS6. On the other hand, Ser727 glutathionylation or phosphorylation were noticed in GS9. In turn, phosphorylation at Tyr705 was common for all STAT3 signaling pathways. Results suggest that detecting specific STAT3 PTMs might be a biomarker for PCa prevention or differentiation [68]. Marginean at al. assessed the nuclear expression of STAT3 phosphorylated at Tyr705 and Ser727 in the prostate stromal compartment of cancer and non-cancer areas in hormone-naïve patients after radical prostatectomy due to localized PCa. Lower nuclear expression of $STAT3^{Tyr705}$ and $STAT3^{Ser727}$ in the stromal compartment was observed in cancer tissues comparing with non-cancer tissues. This decreased expression was correlated with shorter time to biochemical recurrence (BCR). Although non-cancer tissues were collected from distant areas of the tumor from patients with PCa, the data has a potential to be the foundation of developing useful biomarkers. Presented evidence reveals similar prognostic power to GS, staging or surgical margin status, which are widely used in early PCa [83]. Similar studies were carried out earlier by another Swedish team which focused on investigating the expression of tSTAT3 and two phosphorylated forms mentioned before – $STAT3^{Tyr705}$ and $STAT3^{Ser727}$ – in prostate epithelial cells and their impact on disease outcome. Surprisingly, all forms of STAT3 were lower expressed in the cancer cores than in the benign cores and the lowest expression was detected in the tissues with higher GS. Collected data

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suggests a correlation between nuclear and cytoplasmic $STAT3^{Ser727}$ and nuclear $STAT3^{Tyr705}$ expression in cancerous tissues and shorter time to BCR. The lower expression of STAT3, the poorer prognosis of the disease is. However, using gathered outcomes resulted in impairing prognostic values of GS and pT staging. Scientists conclude it might not be a good way to diagnose early stages of PCa [84].

5. Role of STAT3 in bladder cancer

Bladder cancer is the most common malignancy of the urinary tract [85]. It is also the tenth most prevalent cancer in the world with roughly 573 000 new cases and 213 000 deaths [1]. The incidence varies between geographical regions and the highest rates are observed in Europe and North America, additionally among the male population in Egypt, Syria, Israel and Turkey. The lowest incidence rates are reported in Sub-Saharan Africa, Latin America and some Middle Eastern and Central Asian countries [86]. The disorder can occur as non-muscle-invasive bladder cancer (NMIBC), muscle-invasive bladder cancer (MIBC) and metastatic form of the disease [87]. NMIBC comprises 80% of diagnosed bladder cancer cases and is often associated with FGFR3 mutation [87]. It is estimated that 15% to 20 % of NMIBCs progress to MIBCs in which the neoplasm has advanced beyond epithelial cell lining and into the muscles [87,88].

Recent investigations exposed that STAT3 plays a significant role in the progression of the disease [89]. STAT3 was not only found to be upregulated in 10 types of bladder cancer cell lines but also in invasive bladder cancer tissue samples [90]. The higher values of pSTAT3 were associated with basal bladder cancer whereas lower values were detected in luminal bladder cancer. Furthermore, the dependence on STAT3 was differentiated following the cancer cell line. 5637 cell line had the most significant response to STAT3 inhibition. The values of pSTAT3 were increasing concurrently with the progression of the disease. In addition, MIBC has shown enhanced expression of nuclear STAT3 in comparison with NMIBC [91]. The differences in STAT3 expression were also observed within urothelial cancers. Specimens with papillary patterns demonstrated significantly lower parameters of STAT3 expression than non-papillary variants [92].

Numerous research teams inspected the role of STAT3 in bladder cancer. The inhibition of the STAT3 resulted in a decrease in cell amount, which was assigned to the process of apoptosis. The anti-apoptotic genes Bcl-xL, Bcl-2 and survivin levels were downregulated in treated cell lines. The effect was observable in WH and UMUC-3 cell lines whereas no change was found in bladder smooth muscle cells. Interestingly, inhibition of the STAT3 not only led to expression reduction of apoptotic genes but also induced the cleavage of caspases 3, 8 and 9 [89]. Another study also reported that the apoptosis phenomenon was accompanied by expression attenuation of Bcl-xL and Bcl-2 in the T24 cell line [93]. Treatment with Stattic, a STAT3 inhibitor, significantly reduced the weight of the tumor xenografts. The reduction of tumour growth was up to 50%. During Stattic treatment a reduction of Ki-67 positive cells also was observed [90].

STAT3 is also implicated in process of EMT in bladder cancer. Blockage of the STAT3 pathway decreased the motility and invasiveness by inhibition of MMP2 and MMP9 expression [93]. Treatment of bladder cancer cell lines with Tanshinone IIa, extract derived from *Salvia miltiorrhiza*, contributed to an increase of epithelial marker E-cadherin level and reduction of mesenchymal markers like N-cadherin and vimentin. Additionally, transcription regulators of EMT like SLUG and SNAIL were also downregulated after therapy. This data implicated that Tanshinone IIa can suppress the process of EMT. It was established that extract from Tanshinone IIa exerts its action through inhibition of the STAT3 phosphorylation, which ends in the downregulation of CCL2 [94]. The correlation between STAT3 and EMT was spotted during examinations of IDO1 enzyme. IDO1 was found to be overexpressed in bladder cancer cell lines and tissues. The enzyme is responsible for the breakdown of tryptophan to kynurenine. It was demonstrated that IDO1 may promote EMT through the IL-6/STAT3/PD-L1 pathway. Suppressed expression of IDO1 resulted in decreased proliferation, migration and invasiveness of bladder cancer cells [95]. Cancer-associated fibroblasts were reported to be involved in process of bladder

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cancer progression through stimulation of STAT3 phosphorylation. Cancer-associated fibroblasts secreted IL-6, which activated its receptor on bladder cancer cells subsequently leading to increased phosphorylation of STAT3 and providing IL-6-activated EMT programming [96]. Certain RNA molecules affect EMT by influencing STAT3 level as well. For instance, miR-4500 was determined to be downregulated in bladder cancer cells. Ectopic expression of miR-4500 stimulated apoptosis, impaired proliferation and retarded EMT. The study demonstrated that RNA suppressed STAT3 through base pairing with the 3'-untranslated region of STAT3. Interestingly the phosphorylated STAT3 levels correlated with CCR7, increased expression of miR-4500 and repressed STAT3 which led to a decrease in CCR7 [97]. Conversely, lncRNA CARLo-7, which was established as the only bladder cancer specific lncRNA in the CARLo cluster, is dramatically overexpressed in bladder cancer cells. Silencing of CARLo-7 repressed activation of JAK/STAT and Wnt/β-catenin pathways and therefore migration, invasiveness and EMT were diminished [98].

STAT3 besides being involved in EMT participates in bladder cancer angiogenesis. Occludin is a protein which is a part of the tight junction proteins family. This protein was overexpressed in bladder cancer tissue and was linked to the progression of cancer. Importantly, occludin facilitated angiogenesis by stimulating IL-8 secretion mediated by STAT4. High levels of IL-8 triggered phosphorylation of STAT3 and led to angiogenesis [99].

STAT3 plays a significant role in bladder cancer metabolism. Phospholipase C epsilon (PLCε) was found to be upregulated in bladder cancer and caused stimulation of STAT3 phosphorylation which regulates the transcription of LDHA and, as a result, affects glucose consumption and lactate production. Knockdown of PLCε in T24 cells attenuated STAT3 phosphorylation and resulted in LDHA expression, cells proliferation, glucose consumption and lactate production downregulation [100]. The Gasdermin B protein, which is responsible for the regulation of pyroptosis in cells, similarly to PLCε was demonstrated to upregulate glucose metabolism via STAT3 activation. This interaction resulted in increased expression of LDHA, ENO2, HK2 and IGFBP3 which enhanced glycolysis [101]. What is worth mentioning is that the deprivation of glutamine decreased the values of phosphorylated STAT3. What is more, the study implied that GLN is responsible for cell growth stimulation mediated by STAT3. GLN not only regulate STAT3 by glutaminolysis and ATP supplementation but also through reactive oxygen species (ROS) level modulation in bladder cancer cell lines [102]. In addition, it was revealed that an elevated amount of RORC, which expression is considered to be downregulated in bladder cancer, has probably led to inhibition of cell proliferation and glucose metabolism by suppressing the binding of STAT3 to the promoter of STAT3-mediated genes.

Furthermore, RORC via blocking STAT3 might have sensitized bladder cancer cells' response to cisplatin [103]. Another study revealed that STAT3 can be a part of a process that facilitated doxorubicin resistance in bladder cancer. Phosphorylated protein was responsible for the recruiting of DNMTB3 to the promoter region of ESR1 which ended up with its hypermethylation and downregulation of ESR1. Subsequently, it led to the downregulation of miR-4324. The microRNA decreased RACGAP1 protein levels which is supposed to be a tumor promoter and doxorubicin resistance factor. Bladder cancer was proved to be resistant to radiotherapy [104]. Fractional irradiation enhanced the invasiveness, motility and stem-like characteristics of bladder cancer cells. Responsibility for this effect was assigned to STAT3 which was previously reported to be significantly phosphorylated in irradiated cells [105]. Knockdown of STAT3 brought a loss of tumor formation in immunodeficient mice. It was suggested that excessive secretory of cytokines resulted in activation of the JAK/STAT pathway. Notably, it was demonstrated that IL-6/STAT3 pathway is important to maintain stem like properties [106]. Interesting findings were made during concomitant utilization of Stattic and chemotherapeutic agent approved for bladder cancer. The combined therapy of Stattic with one of chemotherapeutics – gemcitabine, docetaxel, paclitaxel or cisplatin – showed an additive effect. This effect may find an application in the future within a group of patients with chemoresistant tumors [90].

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Besides, the combination of Stattic and Palbociclib (CDK4/6) inhibitor showed an additive effect in T24 and UMUC-3 cell lines [90].

STAT3 may be considered a new potential biomarker. It was determined that its level correlates with a poor prognosis but due to conflicting reports, it is still disputed if the utilization of STAT3 as a new survival biomarker would be relevant [90,91]. However, it may prove to be useful in the differentiation of bladder cancer types. As an aforementioned urothelial type of bladder cancer was enriched in STAT3 [92]. Moreover, it is suggested that the high expression of pSTAT3 is a strong predictor of the basal type of urothelial bladder cancer [91]. Interestingly, the value of pSTAT3 is elevated in high grade NMBIC in comparison to low grade, which indicates a possibility to detect more aggressive cells in NMBIC [91].

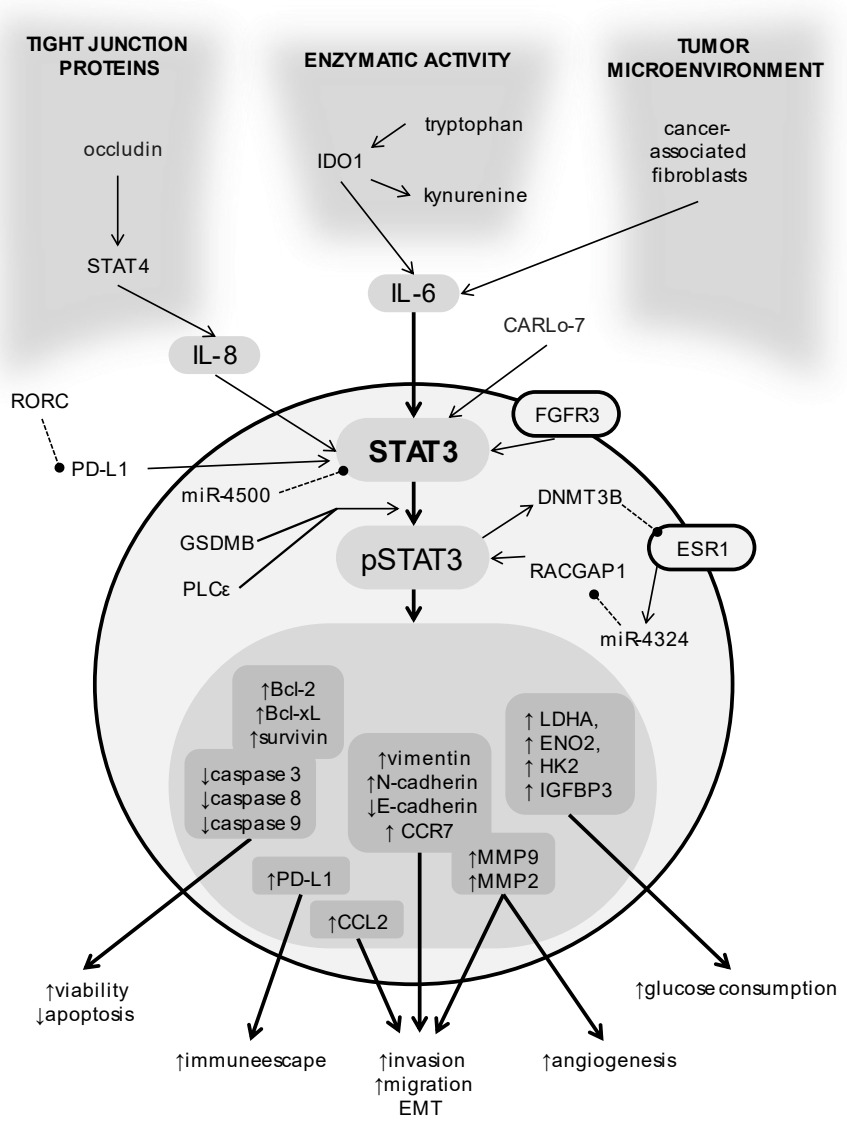

Figure 2. Schematic and simplified depiction of selected pathways and factors regulating STAT3 expression counting its downstream target proteins with the consequences of their overexpression in bladder cancer. Dashed line ended with a dot pictures inhibition; arrow pictures stimulation.

6. Role of STAT3 in upper tract urothelial carcinoma

Upper tract urothelial carcinoma (UTUC) is a rare type of neoplasm. It is defined as urothelial lining cells' malignancy within renal calyces, renal pelvis, ureter down and ureter orifice. It comprises 5% of urothelial cancers and 10% of renal cancers. Two times as frequent urothelial pelvicalyceal cancer is diagnosed as urothelial ureter cancer [107].

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UTUC is associated with FGFR3 signaling, papillary-luminal phenotype and T-cell depleted environment [108]. It is challenging to treat multifocal disease and relapses are often reported after initial therapy. 25% of patients present with metastasis when diagnosed [107]. Standard treatment in case of high-risk UTUC remains radical nephroureterectomy (RCU). Surgery is combined with platinum-based chemotherapy, which improved the prognosis in comparison with RCU solely. Furthermore, platinum agents are also utilized in metastatic cancer at first line [108].

STAT3 was proved to be a cancer-promoting factor and valuable prognostic marker in UTUC. Higher values of STAT3 were reported in ureteral UTUC than in renal UTUC. Surprisingly, no differences in levels of protein were found in muscle-invasive and non-muscle invasive cancer [109]. The expression of $pSTAT3^{Tyr705}$ in the upper urinary tract was similar to the lower urinary tract despite the diversity of these two cancer types in clinical features. Increased phosphorylation of STAT3 may be connected to invasiveness and degree of histological differentiation [110].

Investigations demonstrated that elevated amounts of $pSTAT3^{Ser727}$ were marked in 52% of patients. Higher values were identified with lower recurrence survival and cancer specific survival. Patients with increased $pSTAT3^{Ser727}$ were linked to significantly poorer prognosis, higher cancer recurrence rate and lower cancer specific survival. Elevated expression of $pSTAT3^{Ser727}$ in UTUC tissues within the advanced cancer stage group of patients was significantly associated with advanced cancer stage, and poor prognosis [111]. Another study demonstrated that STAT3 expression in the nucleus was connected to disease progression and lower cancer specific survival. The results were similar to the aforementioned investigation in that high STAT3 levels indicate UTUC progression and the risk of exacerbation of the disease is considerably higher in the advanced stage group [109].

STAT3 is demonstrated to be a viable biomarker for invasiveness of cancer and metastasis, it may provide a prediction of the need for more aggressive treatment. Interestingly, it was indicated that STAT3 could be a possible therapeutic target in UTUC [111].

7. Role of STAT3 in renal cell carcinoma

RCC is the deadliest urological cancer. Five-year survival rate prognosis is esteemed at 76%, although it decreases to 12% in late stages [112]. Only in the year 2020, 432,288 new incidence cases and 179,368 death cases were reported. Hence it was ranked as the sixteenth most common neoplasm in the world [1]. RCC can be distinguished according to the histological subtypes. Ninety percent of RCCs are of the clear cell carcinoma, papillary and chromophobe histological subtype. The ccRCC (clear cell carcinoma) appears to be the most common and aggressive histological subtype whereas the decreasing incidence of remaining subtypes is reported, respectively [112].

IL-6 was supposed to be an important autocrine growth factor of RCC. This cytokine proved to be detectable in renal carcinoma cell lines and genuinely stimulated the proliferation of the cancer cells [113]. However, it is still hard to say whether IL-6 is an autocrine growth factor due to contradictory data [114]. Further investigations demonstrated that IL-6 stimulate proliferation via activation of STAT3 [113]. Aberrantly phosphorylated STAT3 was observed in renal carcinoma with notably elevated expression in the metastatic stage. Moreover, STAT3 was an indicator of poor prognosis and metastasis [115]. Interestingly, the number of tumors with activated STAT3 was similar in clear cell and papillary subtypes (57%-59%), while a decreased amount of cases were found within the chromophobe subtype (33%). Furthermore, STAT3 is reported to be a key player in clear cell carcinoma as it upregulates 16 out of 32 genes which expression was evaluated. A minor quantity of genes was upregulated in papillary and chromophobe subtypes (10 and

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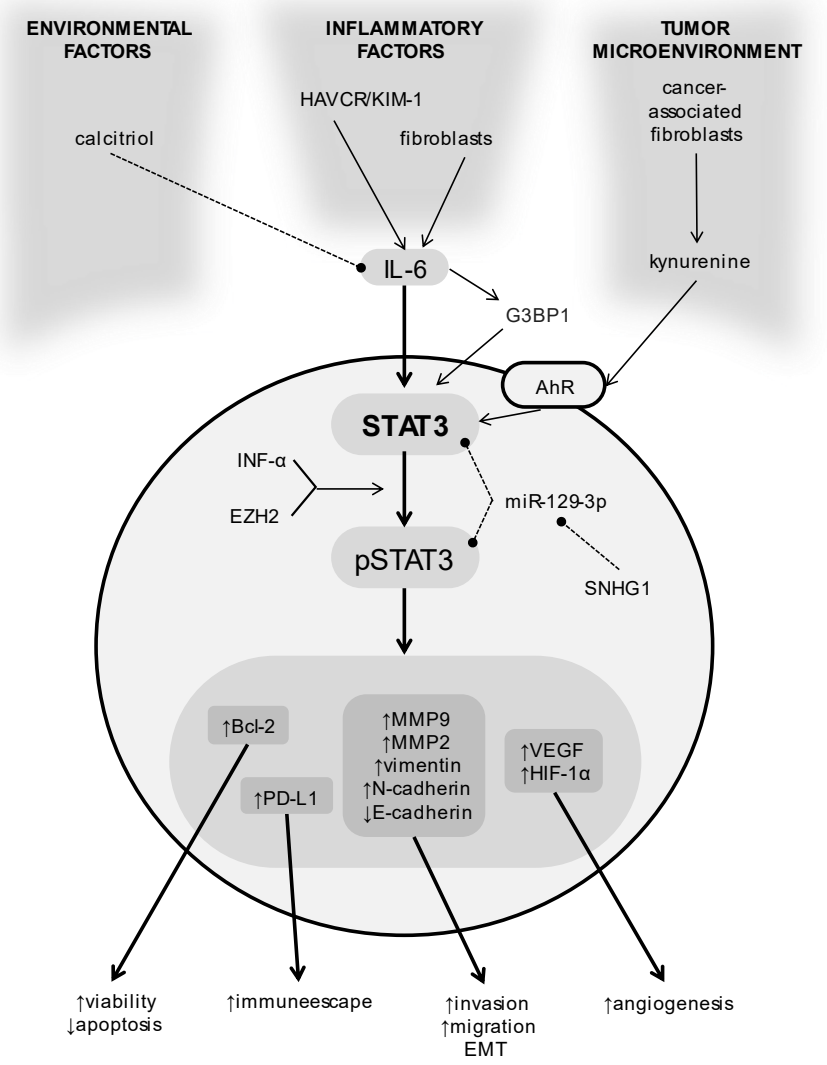
7 genes, respectively). MMP9, BIRC5 and BCL2 genes were notably upregulated, whereas FOS gene was downregulated [114].

Figure 3. Schematic and simplified depiction of selected pathways and factors regulating



STAT3 expression counting its downstream target proteins with the consequences of their overexpression in renal cell carcinoma. Dashed line ended with a dot pictures inhibition; arrow pictures stimulation.

Inhibition of STAT3 leads to induction of apoptosis, reduction of cell viability and proliferation in renal cancer cell lines. Suppression of transcription factor also downregulated Bcl-2 levels, although levels of Bcl-xL and Mcl-1 remained unchanged. Additionally, angiogenesis was suppressed concomitantly with STAT3 inhibition [93]. Investigations demonstrated increased phosphorylation and nuclear localization of STAT3 during hypoxic conditions in Caki I cell line. pSTAT3 was observed to increase the stability of hypoxia-inducible factor 1α (HIF-1α) by delaying protein degradation and accelerating its synthesis. The interaction of two proteins enhanced the expression of VEGF [116]. Notably, HIF-1α and VEGF are often upregulated in ccRCC due to VHL gene mutation and are involved in process of angiogenesis [117]. Inhibition of STAT3 suppressed VEGF expression regardless of VHL gene mutation eventually leading to reduced tumor angiogenesis. This fact might find an application in the future [116].

A well-known marker of acute kidney injury HAVCR/KIM1 is also associated with elevated levels of pSTAT3. Overexpression of this marker was spotted on 60% of ccRCC. HAVCR/KIM1 after cleavage by metalloproteinases triggers IL-6 secretion. This results in

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increased STAT3 phosphorylation and HIF-1α levels. Increased level of activated STAT3 also leads to enhanced expression of glucose transporter 1 (GLUT-1) and VEGF genes. Given that renal cell carcinoma is an angiogenic-rich neoplasm, it might be another potential pathway of cancer progression. RCC is considered as highly invasive cancer as it is speculated that 33% of cases become metastatic [118], especially within the lungs, bones and brain [119]. Investigation on G3BP1 revealed that IL-6 stimulates invasiveness and migration of RCC cells through STAT3 activation. G3BP1 was reported to be upregulated and mediate the process of STAT3 activation. Moreover, inhibition of G3BP1 decreased the STAT3 activation and, as a result, alleviated tumor growth and metastasis were observed not only *in vitro* but also in orthotopic xenografts [120].

Additionally, the change in EMT markers was spotted, indicating that STAT3 promotes the metastatic process. EZH2 was discovered to be overexpressed in RCC cells and to regulate the proliferation and invasive potential of RCC cells. Subsequently, it was determined that this effect is exerted by STAT3 activation. In addition, EZH2 increased MMP2 expression, which is responsible for extracellular matrix degradation, related to tumour invasion and metastasis [119]. Low levels of vitamin D3 were associated with increased stimulation of the IL-6/STAT3 pathway. Treatment of cell lines with calcitriol decreased almost completely STAT3 phosphorylation. This data suggested that calcitriol can block the EMT process through STAT3 inhibition, at least partially [121]. Studies conducted on cancer-associated fibroblasts exposed the stimulatory effect on the migration of RCC cells. Increased amount of kynurenine due to tryptophan 2,3-dioxygenase (TDO) overexpression contributed to the activation of aromatic hydrocarbon receptors on renal cancer cells and activation of STAT3 [122]. Surprisingly, normal fibroblasts were also associated with increased cell migration in the ccRCC. Fibroblasts were reported to promote renal cancer cells to secrete IL-6 and phosphorylate STAT3. However, they did not demonstrate to facilitate the EMT. Nevertheless, the elevated expression of MMP2 was marked [123].

Cancer-associated fibroblasts were also revealed to have an impact on drug resistance to sunitinib and sorafenib in renal cancer cells [122]. IL-6 was linked with doxorubicin resistance in RCC cells which was triggered by STAT3 phosphorylation. Attenuated STAT3 activity by si-IL6 sensitized renal cancer cells to doxorubicin [124]. Interferon-α is utilized as an immunotherapy agent in the treatment of metastatic or recurrent RCC. Nevertheless, resistance to this drug is reported. It was observed that phosphorylation of STAT3 was increased by IL-6 but also by INF-α. IL-6 is regarded as a negative regulator of the antiproliferative effect of INF-α. Further studies have shown that IL-6 inhibition induced the efficacy of INF-α, concluding addition of tocilizumab may overcome the resistance of renal cancer to INF-α [125].

An interesting fact is that STAT3 is suggested to be involved in the immune escape of RCC cells. Overexpression of miR-129-3p, which is usually downregulated, reduced STAT3 and PD-L1 values and inhibited proliferation, invasion and immune escape of RCC cells. Additionally, overexpressed microRNA led to enhancement of cytotoxicity, cytokine secretion and proliferation of CD8+ T cells. This process was regulated by lncRNA SNHG1 which inhibited the activity of miR-129-3p. This makes SNHG1 a potential treatment target due to the ability of immune escape regulation [126].

STAT3 is suggested to present diagnostic value as the phosphorylated protein at S727 residue correlated with prognosis and recurrence free survival. This investigation implies that $pSTAT3^{S727}$ would be a valuable biomarker for improved stratification and follow up of patients during the same stage of cancer and clinical score [127]. High expression of STAT3 mRNA was also associated with shorter overall survival contrary to low expression. However, due to the lack of statistical difference in the study, mRNA expression might not be useful as a biomarker [128]. Treatment with multiple tyrosine kinases inhibitors (mTKI) is a standard method in metastasis, although this therapy often results in many adverse effects such as stomatitis or hands and foot reactions [129,130]. In addition, the differentiated response efficacy to mTKI is observed. Adverse effects and treatment success were associated with the distribution of STAT3 polymorphisms. Therefore,

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assessment of STAT3 polymorphism might be a significant predictor of therapy efficacy and susceptibility to adverse effects [131].

8. Role of STAT3 in penile cancer

Penile cancer is one of the most uncommon cancers in the world. In 2020 there were 36 068 new cases and 13 211 deaths due to PeCa worldwide [1]. Although the incidence in Europe remains at a relatively low level of 1/100 000, PeCa seems to be a more serious problem in central or southern parts of America like Brazil, where the incidence is 6-8 times higher [6]. Risk factors of PeCa include phimosis, HPV infection or smoking. What is more, scientists reported that socioeconomic factors like educational level or marital status may help predicting the occurrence of this disease [132].

Much attention has been recently paid to the role of specific chemokines which are capable of regulating STAT3 expression in penile cancer. It was shown that CCL20, CXCL13 and CXCL5 are highly expressed in PeCa tissues. Elevated levels of those were also noticed in patients' serum preoperatively. Knockdown of CCL20, CXCL13 or CXCL5 gave the same results pictured by a significant suppression of pSTAT3 level and inhibition of MMP2 and MMP9 expression. Consequently, diminished proliferation, migration and invasion of PeCa cells were observed. High preoperative serum levels of mentioned chemokines were associated with tumor progression and poor outcomes. That is why scientists suggest using CCL20, CXCL13 and CXCL5 as diagnostic and prognostic biomarkers [133–135].

SHCBP1 is a gene which physiologically takes part in T cell proliferation or signaling in neural progenitor cells. Scientists reported its association with development of some cancers where it acts through STAT3 pathway. Mo et al. explored its role in PeCa. SHCBP1 was significantly expressed in PeCa tissues compared to the control group. The correlation between HPV infection and SHCBP1 expression was denied. On the other hand, it was highly correlated with grading, staging and lymph nodes status. Researchers conclude SHCBP1 may be used as a prognostic biomarker. In vitro and in vivo experiments clearly showed that SHCBP1 knockdown results in decreased proliferation, migration and invasion of PeCa cells and forced activation of STAT3 reverses this process. All in all, SHCBP1 has the potential to be used not only as a biomarker but also as a target for future treatment strategies [136].

9. Role of STAT3 in testicular cancer

Testicular cancer is one of the rarest cancers worldwide with 74 458 new cases and 9 334 new deaths in 2020 [1]. Nevertheless, it is the most frequent cancer among young men between 15 and 35 years. The incidence varies geographically – the highest rates occur in Europe (8,0-9,0/100 000), the lowest rates are reported in Asia and Africa (<1,0/100 000). The greatest risk factor is a prior history of testicle cancer contralaterally [2].

A high survival rate as well as relatively low mortality rate may explain the lack of recently published research papers concentrated on the potential role of STAT3 in testicular cancer. Cardoso et al. underlined the importance of describing how JAK/STAT signaling pathway affects testicular cancer development [137].

HOXA10 is a transcriptional factor regulating testicles' development. Scientists explored its role and mechanism of action in testicular cancer. It was shown that HOXA10 is expressed and localized in the nuclei of spermatocytes in normal tissues, whereas it is often dislocated in testicular germ cell tumor (TGCT) cells, both seminoma and non-seminoma. Although its antiproliferative properties through STAT3 pathway inhibition were proved, the exact mechanistic explanation remains unknown. Researchers admitted that detecting HOXA10 levels might not be useful in diagnostics due to its unchanged expression in TGCT. Nevertheless, this study might be the basis for further experiments [138].

10. Discussion and future perspectives

The total amount of urological cancer cases is quickly increasing due to prolonged life expectancy and population growth. Prevalent number of cases is observed in

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developed countries where widespread is an energy-dense diet, obesity and large tobacco use. Besides, not without significance is the advance in medicine and the common access to medical services in high-income countries which translates into greater diagnosed cases [21]. Urological cancers remain a challenging problem in modern society what triggers the need of novel intervention strategies and medical tools which can really tackle this issue [2].

STAT3 is believed to be a promising molecular target in terms of dealing with urological cancers. STAT3 act as a transcription factor transmitting signals from multiple receptors. STATs proteins activation is a physiological process. However, abnormal STAT3 protein activity was spotted in several cancer types. It was proved that its atypical activation is associated with tumorigenesis and invasive abilities [3].

STAT3 was revealed to be dysregulated in urological cancers. This results in stimulated proliferation and survival of cancer cells due to elevated expression of antiapoptotic genes like cyclin D1, Bcl-2, Bcl-xL or surviving [4–6]. Furthermore, cancer cells avoid apoptosis through downregulated expression of caspases 3, 8 and 9 caused by STAT3 activation [6,132]. STAT3 was noticed to induce invasiveness capabilities, EMT and promote metastasis in prostate, bladder, renal and penile cancer through enhancement of MMP2 and MMP9. The upregulation of EMT markers like vimentin and N-cadherin was observed and additional downregulation of E-cadherin was spotted [7,8,14–18]. STAT3 also promotes angiogenesis by increasing transcription of VEGF, especially in RCC. It was found that STAT3 inhibition causes loss of ability to form new blood vessels in such cases [1,19,20].

Undoubtedly, TME is significant for disease progression. Investigations exposed that cancer-associated fibroblasts are involved in enhancement of migratory properties of prostate, kidney and renal cancer by secretion several stimulating factors like IL-6 or CCL3 that activate STAT3. TAMs were also implicated in migration, invasion and EMT in PCa by CCL5 secretion [15,22–25].

Interestingly, STAT3 was found to bind to the PD-L1 promoter in cancerous cells. It is considered to be crucial in the regulation of the immune response in TME. Cancers rich in IL-6, a STAT3 activator, were more resistant to cytotoxic NK cells, and the inhibition of JAK1 or STAT3 resulted in decreased PD-L1 level in PCa cells [26]. IL-6/STAT3/PD-L1 pathway was shown to take part in EMT promotion in bladder cancer [27]. Moreover, reduced STAT3 and PD-L1 levels were linked to inhibited proliferation, invasion and immune escape of RCC cells [29].

Chemoresistance is considered as a substantial problem during cancer therapy. STAT3 is capable of inducing chemoresistance in cancer cells. Resistance to doxorubicin due to atypical STAT3 activation was observed in renal and bladder cancer [28,30]. Interestingly, STAT3 inhibition led to sensitization of PCSCs to cisplatin and docetaxel [31,32]. Additionally, increased cells' invasiveness. Motility and stem-like characteristics after fractional radiation was observed and assigned to the overactivation of STAT3. According to this, STAT3 protein inhibition in PCa cells was examined and resulted in boosted efficacy of radiotherapy [33,34].

Risk factors such as high-fat diet or obesity turned out to have significant impact on STAT3 activation. PA upregulates STAT3 mRNA and protein. Similarly, LDL cholesterol increases pSTAT3 levels by enhanced JAK1 and JAK2 phosphorylation [4,25]. Leptin was proved to promote EMT by STAT3 activation [14]. Furthermore, low level of vitamin D3 was associated with IL-6/STAT3 pathway stimulation in prostate and renal cancer. Calcitriol used as a treatment decreased STAT3 phosphorylation [35,36]. STAT3 was proven to affect cell metabolism by upregulated glucose consumption and lactate production in bladder cancer by enhanced transcription of LDHA, ENO2, HK2 and IGFBP3 [37,39].

It is worth mentioning that STAT3 might be utilized as a valuable biomarker of tumorigenesis. The level of STAT3 was increased in high-grade NMIBC tumors. It was also found useful to distinguish cancer type [38,40]. Elevated $pSTAT3^{Ser727}$ level was associated with significantly poorer prognosis, higher cancer recurrence rate and lower cancer-specific survival in UTUC. This investigation implies that $pSTAT3^{Ser727}$ may also be a valuable

biomarker for improved stratification and follow-up patients during the same cancer stage and clinical score in RCC [33,41,42]. Based on STAT3 polymorphism it seems to be possible to predict response to mTKI therapy and probability of adverse effects. Moreover, detecting specific STAT3 post-translational modifications might be a biomarker for PCa prevention or differentiation [43].

To sum up, STAT3 seems to be an up-and-coming molecular target in the context of diagnosing and treatment urological cancers. It is a convergent point of many signaling pathways induced by cytokines, growth factors or oncoproteins [11]. Either direct or indirect (via blockage of inflammatory factors) STAT3 inhibition might result in apoptosis initiation, angiogenesis inhibition, EMT attenuation or reduced metastasis risk. Combined therapy of STAT3 inhibitors and chemotherapeutics may overcome tumor cells' chemoresistance and reduce the chance of relapse. Moreover, it might be a significant biomarker in the assessment of the patient prognosis and cancer recurrence. It has capabilities of finding application in cancer type differentiation or helping evaluating either response to therapy or the probability of adverse effects.

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