The present article highlights the diverse role of stem cells in normal kidney and renal cancer, with special emphasis on surface markers. Proteins such as CD105 and CD133 have been reported as being significant in clear cell renal cell carcinoma (ccRCC) cancer stem cells. The role of normal kidney progenitor cells and their surface markers is compared with the role of those surface markers in ccRCC. Subsequently, we state the current hypothesis about origin of tumour-initiating cells along with their clinical and prognostic potential in RCC. Finally, we present future perspectives with respect to recent studies.

Key words: tumour-initiating cells, cancer stem cells, renal cancer.

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Biology of renal tumour cancer stem cells applied in medicine

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Introduction

The National Cancer Institute (NCI) defines renal cell cancer (RCC) as the most common type of kidney cancer, which begins in the lining of the renal tubules in the kidney [1]. The highest incidence of RCC was observed in the Czech Republic and North America [2]. Despite progress in general cancer diagnosis techniques, around 20-30% of patients are diagnosed with metastatic RCC (mRCC) and a further 20% will have a relapse after nephrectomy and develop mRCC in the first 12 months post-surgery. Current achievements in advanced RCC treatment are mainly due to molecular therapies targeting the vascular endothelial growth factor (VEGF) and mammalian target of rapamycin (mTOR). Angiogenesis plays a pivotal role in renal tumour growth since RCC is one of the most vascularised solid cancers, probably due to dysfunctional mutation in the *von Hippel Lindau* (*VHL*) gene, which induces overexpression of hypoxia-inducible factor (HIF), and this leads to up-regulation of pro-angiogenic VEGF and platelet-derived growth factor (PDGF) [3, 4]. Receptors for VEGF and PDGF play crucial roles in angiogenesis, mainly via cell survival, invasion, and proliferation, and exhibit tyrosine kinase activity. Tyrosine-kinase inhibitors (TKIs) were developed to block the physiological function of the receptors. Currently available TKIs approved by the Food and Drug Administration (FDA) in first and second or third line therapy are: sunitinib, pazopanib, sorafenib, and axitinib [5]. Bevacizumab, which is an anti-VEGF monoclonal antibody [3], is commonly used, with clinical benefit. Other new treatment strategies target the mTOR pathway, which is responsible for angiogenesis. Everolimus and temserolimus are mTOR inhibitors also approved by the FDA [5].

According to the latest hypothesis about cancer development and progression, "cancer stem cells" (CSCs) are responsible for cancer initiation and maintenance. Based on the definition, CSCs possess several features: clonogenic ability, expression of stem cell markers, lack of differentiation markers, multipotency, growth in non-adhesive spheroids, and most importantly generation of *in vivo* serially transplantable carcinomas that contain differentiated and undifferentiated cells. The tumourigenicity is analysed by generation of neoplasms after injection of a low number of cells into severe combined immune deficient (SCID) mice [6]. In the literature, the best tumour-generating cells were called CSCs, and the xenotransplantation causes inclusion of the analysed cell population into the CSCs class. The terminology in this case is inadequate – some other names have been postulated such as tumour-initiating cells (TICs), stemloids, or tumour propagating cells (TPCs).

The CSC term suggests that its origin is from somatic stem cells, and that CSCs constitute a distinct population from other cancer cells. Stemloids [7] also exhibit similarities with somatic stem cells; however, this term suggests that stemloids and somatic stem cells are different. The operational term TIC [8] is linked to the cells that generate tumours in immunocompromised animals. Tumour-initiating cell suggests that the cells have initiated a tumour *in vivo*, which is not true – the cells have initiated a tumour after xenotransplantation. There is no clear confirmation which cancer cell population initiated the primary tumour *in vivo*, but current evidence indicates that it could only be from populations able to reconstitute a tumour in xenografts. On the basis of these facts, we support tumour-propagating cell (TPC) or tumour stem-like cell terms as better reflecting the features of cells mostly characterised by tumourigenicity potential in SCID mice in the literature. In our opinion these cells were improperly called CSCs.

The main aim of this article is to indicate inaccuracies within the terminology used for cells with stem-like features. Moreover, inconsistent data about proper identification and characterisation of renal cancer cells with stem-like features are discussed. Many techniques used to isolate the cells are compared and described within the article. At the beginning, the physiological role of the renal tumor-propagating cell markers (TPCs) is discussed to show putative connections with each marker and TPCs. The next chapter describes the role of TPC markers in clinical patient prognosis to find new therapeutic targets. Finally, perspectives for new approaches to TPC identification and characterisation and new ideas that may change the direction of TPC research are described.

Putative implications of renal tumour-propagating cell markers in cell physiology

Molecular markers help support treatment planning because of more accurate individual risk assessment. Since the introduction of targeted therapies in RCC, more individualised treatment options have become available. Most of the TPCs in RCC research are isolated based on molecular markers that may play important physiological roles in the cell (Fig. 1). Molecular markers offer potential for additional information in tumour detection and diagnosis, prognostic and predictive values, as well as determination of therapeutic targets.

CD105

CD105 is a transmembrane protein expressed in angiogenic endothelial cells associated with proliferation and hypoxia (Fig. 2). Moreover, CD105 is a receptor for transforming growth factors TGF-1 and TGF-3. The exact molecular mechanisms of CD105 angiogenesis and vascular development are not fully understood [9]. CD105 is an integral

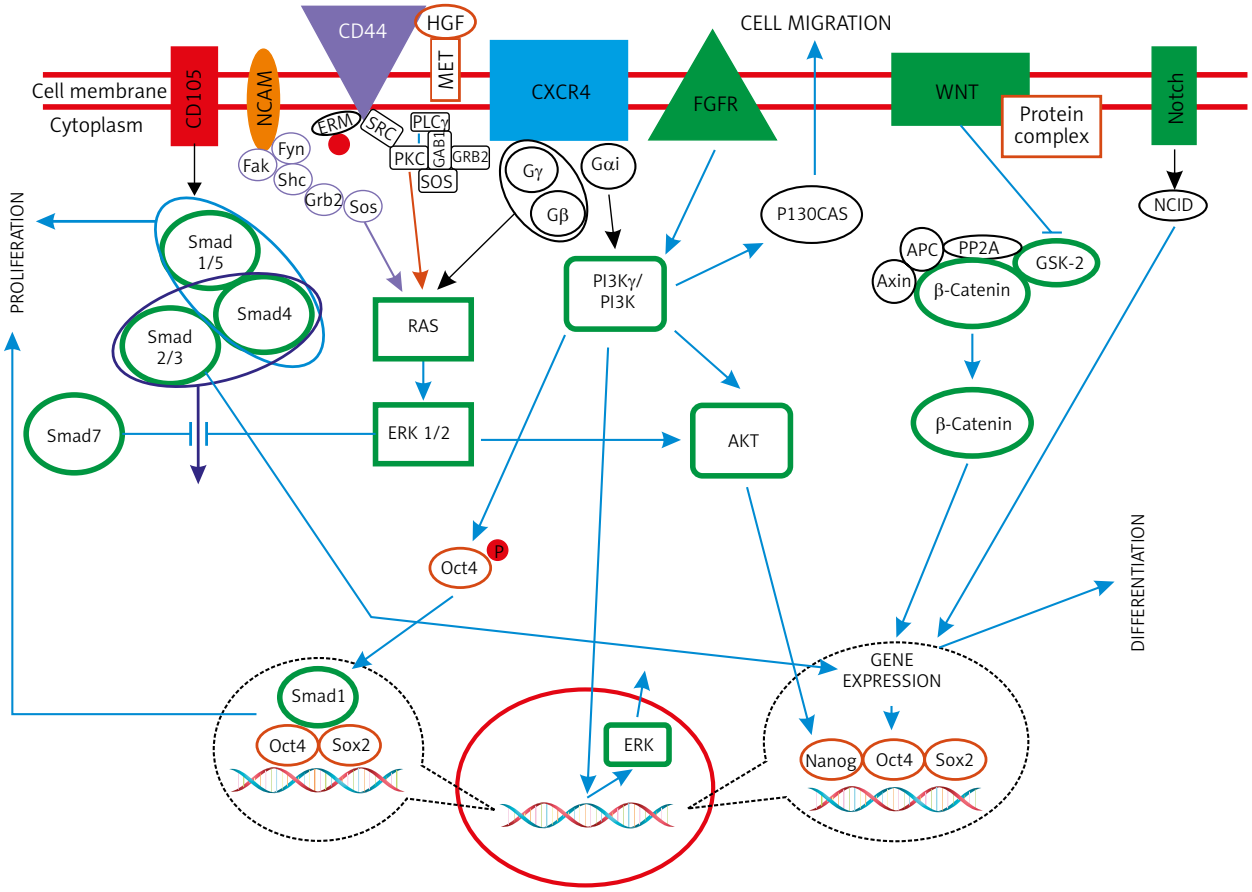

Fig. 1. The association of renal tumour-propagating cell markers in kidney development. Signalling pathways of kidney development is shown in green colour (frames and figures)

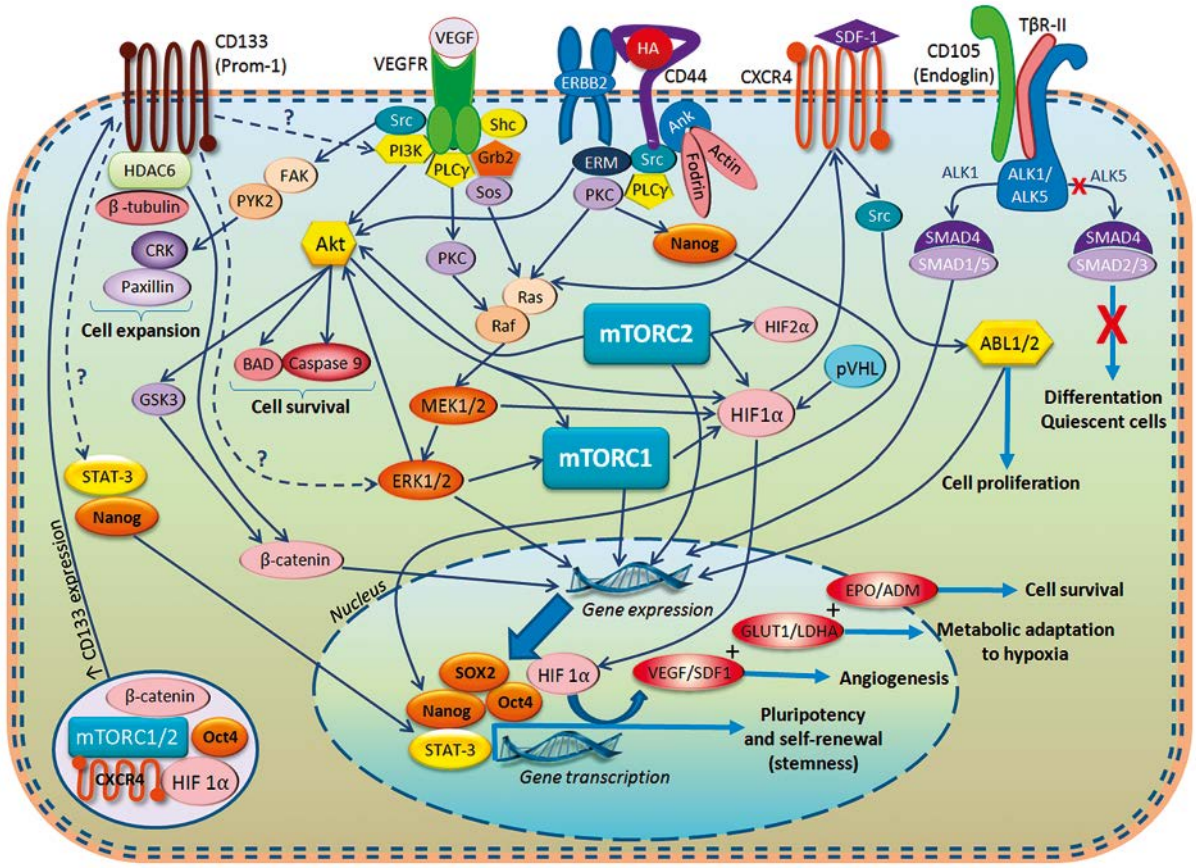

Fig. 2. Selected molecular pathways in renal tumour-propagating cells (TPCs). A view shows relations between a number of important regulators and markers that have been implicated in biology of renal cell cancer (RCC). The functions of some of the described receptors such us CD105 and CD133 still remain not well understood but are believed to play an essential role in many physiological and pathological processes. Specific for RCC proteins HIF1/2&alpha and mTOR1/2 complex integrate crucial molecular routes taking part in tumorigenesis. Demonstrated pathways finally lead to gene expression and activation of transcription factors Oct4, SOX2, and Nanog, which are responsible for pluripotency maintenance and self-renewal of TPCs

membrane glycoprotein that is expressed primarily in the vascular endothelial cells of capillaries, arterioles, and venules, as well as in activated monocytes, some leukaemic cells, and the syncytiotrophoblast, the multinucleated placental layer that constitutes the interface with maternal blood [9–11]. Using gene knockout mice, CD105 was shown to play a major role in angiogenesis and vascular and cardiovascular development in early mouse embryos [12].

CD133

Prominin-1 or CD133 is a pentaspan membrane protein, whose specific functions are still unclear; however, it is believed that CD133 is an organiser of plasma membrane topology [13]. Interactions between cholesterol and CD133 suggest that CD133 plays a role in maintaining appropriate lipid concentrations within the plasma membrane [14, 15]. Moreover, studies have confirmed CD133 as a marker of haematopoietic stem cells [16], but it is of importance that AC133 is expressed only on stem and progenitor cells, while CD133 is also expressed on differentiated cells [15]. CD133 in healthy cells is associated with tissue repair [17]. By a using a multiple tissue expression array, it was concluded that CD133 mRNA was strongly expressed in tissues such as adult kidney, mammary gland, trachea, salivary gland, placenta, pancreas, digestive tract, and testes [18]. Finally, recent findings show a relationship between CD133 and anaerobic cell metabolism; CD133 was shown to inhibit the endocytosis of the transferrin receptor, which resulted in the blockade of iron uptake and therefore of mitochondrial activity [19].

Studies have identified CD133 as a marker of CSC in various human tumours [20]; however, those cells did not show tumorigenic properties in xenotransplants in renal carcinomas, but significantly enhanced tumour growth and vascularisation [21, 22]. It has been theorised that in renal tumours, the microenvironment could recruit normal CD133 cells and promote their endothelial commitment [22].

ALDH1

Aldehyde dehydrogenase (ALDH1, ALDH1A1) is a cytosolic enzyme responsible for oxidation of intracellular aldehydes to carboxylic acids [23]. Aldehyde dehydrogenase in the cell is present, although in different isoforms, in mitochondria, cytoplasm, and the endoplasmic reticulum. Different isoforms of ALDH1 are distributed in various tissues, but the highest levels are observed in the liver and the kidney. Recently it has been stated that one of the main functions of ALDH1 is catalysis of retinol to retinoic acid

conversion, and it has important role in embryonic development. High activity of ALDH has been shown in human and murine neural and haematopoietic stem and progenitor cells [24–27]. Multiple myeloma and acute myeloid leukaemia have been characterised with upregulated activity of ALDH in the stem cell population [28].

CXCR4

Chemokine receptor type 4 (CXCR4), the receptor for α-chemokine stromal-derived factor (SDF-1), is very important in tumour biology, especially in tumour metastasis. Stromal-derived factor 1 is believed to be the mediator for many physiological and pathological processes via CXCR4. Chemokine receptor type 4 chemokine receptor belongs to the group of seven transmembrane G protein-coupled receptors (GPCRs) that are ubiquitously expressed in various normal cells and tissues, including neurons, lymphatic tissues, microglia, and haematopoietic cells [29, 30]. Chemokine receptor type 4 plays an important role in stem cell trafficking during development, tissue injury, and regeneration. Previous studies have shown that the chemokine receptor CXCR4 plays a role in breast and prostate cancer bone metastases via interactions with its ligand SDF-1 because of the role of SDF-1 in tumorigenesis and metastasis [31, 32]. As has been described in the case of other solid tumours, chemokine receptor CXCR4 is responsible for Src-mediated activation of ABL kinases. It promotes cancer cell invasion by regulation of activity and localisation of matrix metalloproteinases (MMPs) as well as by modulation of gene expression of several MMPs and factors involved in EGFR-associated pathways [33, 34]. Downregulation of CXCR4 may prove to be a good therapeutic strategy for fighting tumour metastasis in patients with RCC.

CD44

CD44 is a cell adhesion molecule that seems to have a role in tumour cell invasion and tumour dissemination, by mediating interactions between tumour cells and their environment [35]. Altered expression of CD44 on tumour cells suggests a pathogenic mechanism for tumour metastasis and may provide prognostic information for particular tumours. CD44 is a surface transmembrane glycoprotein that was initially identified on lymphocytes. The extracellular domain of CD44 is the principal receptor of an extracellular matrix molecule, hyaluronic acid. Hyaluronic acid is a non-sulphated glycosaminoglycan that regulates cellular processes of adhesion, migration, and proliferation, and maintains extracellular matrix osmotic balance through its receptors, including CD44. There is a correlation between overexpression of hyaluronic acid and the metastatic potential of certain human tumours. Cell-matrix interactions of CD44 are related to tumour invasion and metastasis [36, 37].

NCAM

Neural cell adhesion molecule (NCAM) is a member of the immunoglobin superfamily (IgSF) of cell surface glycoproteins. It was detected for the first time in the neural system, where it plays a role in Ca^{2+}-dependent cell adhesion. Neural cell adhesion molecule is an indicator of neuroendocrine differentiation of cells. Non-induced as well as induced foetal renal mesenchymal cells express NCAM as a specific cell surface antigen until they reach the perinatal period during kidney development in humans and rats. Induced mesenchymal cells rapidly loose expression of NCAM after the mesenchymal-epithelial transition on immature tubular cells during further differentiation of the nephron [38]. Re-expression of NCAM is detected during the regeneration process in some tubules [39] as well as in renal cell carcinoma (RCC) [40] and in RCC metastases in the central nervous system (CNS) and adrenal glands [41].

Other markers

Not all known molecular markers in RCC are described above. Other markers include, but are not limited to, vascular endothelial growth factor (VEGF), mammalian target of rapamycin (mTOR), survivin, B7-H1, p53, matrix metalloproteinases (MMP), insulin-like growth factor II mRNA-binding protein 3 (IMP3), Ki-67, FSP-1, C-reactive protein (CRP), vimentin, and fascin [42].

The role of renal tumour-propagating markers in clinical prognosis

Clear cell renal cell carcinoma is the most prevalent adult kidney tumour. Nowadays, prognoses are constructed using Fuhrman grade and stage. Those prognoses are not reliable and ccRCC is still a tumour of unpredictable presentation and clinical outcome [43]. Because of the need for reliable prognostic markers, promising targets such as CD133, CD105, and CXCR4 have been investigated (Fig. 3).

CD105

Endoglin (CD105) is a new marker of angiogenesis found to have prognostic value in various tumours, Dubinski *et al*. provided the first automated digital assessment of intratumoural microvascular density in ccRCC using CD105. According to their results CD105 is an unfavourable prognostic marker because patients with higher endoglin expression had significantly shorter PFS [44]. CD105 appears to be an interesting molecule, both as a predictive marker and therapeutic target; however, further investigation of its potential is needed.

CD133

D'Alterio *et al*. evaluated the role of two putative stem cell markers, CXCR4 and CD133, in 240 renal cancers. By using RT-PCR they concluded that CXCR4 was expressed in ten renal cancer cell lines, while CD133 was barely detectable. Moreover, expression of CD133 and CXCR4 was evaluated in 41 fresh RCC tumour samples. According to their results, in 31/41 samples CXCR4 mRNA was significantly upregulated, and in 7/41 samples CXCR4 expression was downregulated. CD133 mRNA was upregulated in 8/41 and downregulated in 28/41. After further evaluation of patient outcomes and expression of CXCR4 and CD133, D'Alterio *et al*. concluded that prognosis for patients

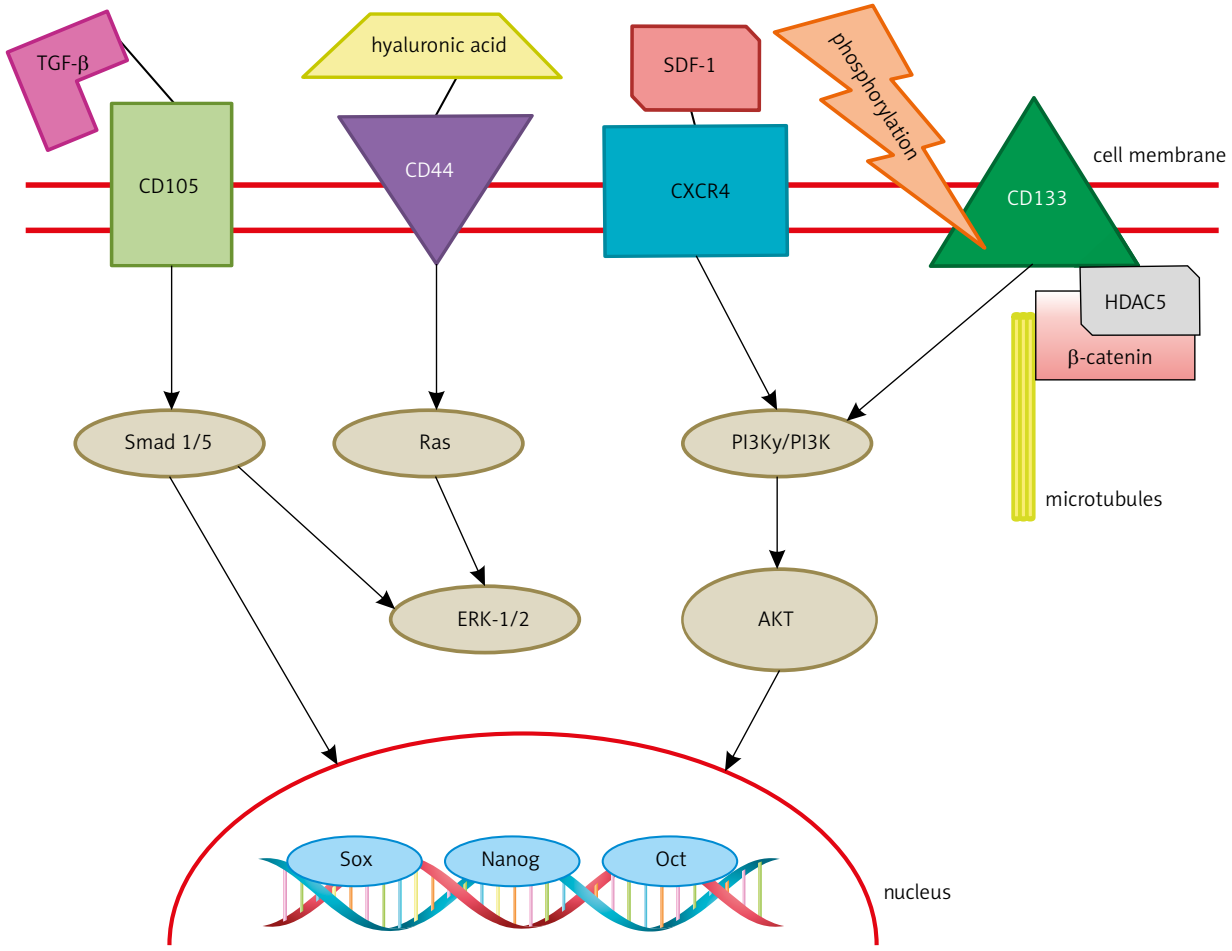

Fig. 3. The signalling pathway of renal tumour-propagating cell membrane markers and "cancer stem cell" markers

whose primary tumours express CXCR4 is unfavourable, while CD133 has no prognostic value [45]. Interestingly, Kim *et al*. examined the expression of CD133 in 140 cases of ccRCC using immunohistochemistry and found a correlation between expression of CD133 in ccRCC and favourable clinicopathological characteristics [46]. Moreover, Costa *et al*. evaluated the prognostic impact of the histological expression of CD133 in 142 cases of ccRCC. According to their results, 5-year disease-specific survival (DSS) was 90% for patients with high expression of CD133, while DSS for patients with low expression of CD133 was 71%. Progression-free survival (PFS) after 5 years was also higher for CD133-high (83%) than for CD133-low patients (66%). They concluded that low expression of CD133 is an independent predictor of poor DSS and PFS [47]. Unfortunately, information about CD133 from different sources is not consistent, and therefore further studies are in order.

ALDH1

ALDH1 has been recognised as a general marker of both normal stem cells and CSCs [26, 48]. Since, ALDH1-positive cells have stem cell-like characteristics it has been used as a CSC marker in many different types of cancer, including lung malignant melanoma, breast, bladder, prostate, and pancreas [49–53]. Moreover, it has been reported that high ALDH1 expression in breast cancer is associated with poor clinical prognosis because of positive correlation between ALDH1 and drug resistance. Also, it was reported that ALDH1 expression is correlated with tumour grade in RCC [54]. Ueda *et al*. showed in their experiments that ADLH1-positive cells have greater sphere forming ability and tumourigenicity than ALDH1-negative cells. Moreover, experiments performed by Ueda *et al*. on ALDH1-positive cells were done under hypoxic conditions and exposure to drugs resulting in 2- to 3-fold increased numbers of ALDH1 positive cells. They concluded that ALDH1-positive cells are resistant to conventional therapies for RCC [55]. Abourbih *et al*. performed a microarray experiment on 244 RCC specimens. They concluded that ALDH1 expression has no correlation to tumour or stage, but in the metastatic setting the expression of ALDH1 is reduced. The expression pattern of ALDH1 in ccRCC might be useful for pathological classification of renal tumours, but in RCC ALDH1 cannot be used as a prognostic marker [56]. Further studies are needed to determine the relationship between ALDH1 and clinical prognosis in RCC.

CXCR4

CXCR4 is significantly related to the biological features of the tumour (stage, Fuhrman grade, clinical presentation) and DFS [57]. Zagzag *et al*. showed that CXCR4 mRNA is upregulated in kidney cancer samples when compar-

ed to adjacent normal tissue [58]. Wang *et al*. examined 97 patients with RCC; 60 of them expressed CXCR4 at a high level, and 37 expressed CXCR4 at a low level. Expression of CXCR4 was correlated with poor prognosis [59]. Moreover, Li *et al*. examined 104 tumour samples; CXCR4 expression was found in 68 of them. In this experiment high expression of CXCR4 was associated with reduced overall survival [60]. There is growing evidence that the SDF-1/CXCR4 axis is important for tumour proliferation, survival, vascularisation, and metastasis. All the experiments listed above prove that CXCR4 may be a useful prognostic marker and therapeutic target and its expression is correlated with poor outcome for patients with RCC.

CD44

CD44 is overexpressed in many human malignancies, including breast, stomach, colorectal, lung, bladder, non-Hodgkin lymphoma, certain squamous cell carcinomas, melanoma, and RCC [37]. Noroozinia *et al*., in their studies on 64 tissue samples, showed that 30 specimens were CD44-positive, and there was no connection between CD44 and tumour size and grade [61]. There is, however, an association between CD44 and tumour progression, recurrence, and aggressiveness [36, 37]. What is more, Moskvina *et al*. concluded, after evaluation of specimens of 105 RCC, that CD44 is an independent unfavourable factor to predict RCC: hyperexpression of CD44 resulted in a 52% decrease in 5-year estimated survival rate [62]. In contrast to Noroozinia *et al*., Zhang *et al*. found a significant connection between CD44 and tumour size, grade, stage, and histological type. Moreover, they stated that CD44 might serve as a predictor of the number of metastatic sites [63].

NCAM

Cirović *et al*. concluded that NCAM seems to be present on RCC tissue regardless of histological type; however, NCAM expression is associated with tumour nuclear grade in ccRCC, so NCAM is not a useful marker for differential diagnosis of RCC, but its expression is correlated with aggressive behaviour and metastatic potential [64]. In a study performed by Daniel *et al*., in which they performed a retrospective immunohistochemical analysis of NCAM expression both in 338 primary renal tumours (including 249 conventional RCCs) and 31 metastases of conventional RCCs, they found association between NCAM expression and tumour size, Fuhrman grading, and lower survival rate. Finally, expression correlated with a higher risk of adrenal and CNS metastases [41].

CAIX

Carbonic anhydrase IX (CAIX) is a transmembrane member of the carbonic anhydrase family that catalyses the reversible hydration of carbon dioxide into bicarbonate and a proton. This reaction enables tumour cells to survive in an acidic microenvironment by maintaining a neutral pH. Carbonic anhydrase IX is expressed in most ccRCCs through HIF-1α accumulation driven by hypoxia and inactivation of the VHL gene; however, it is not expressed in healthy renal tissue [65].

It is widely believed that decreased CAIX levels are associated with poor survival in RCC. Many researchers support this idea [65–67]; however, Zghan *et al*. examined 730 specimens of ccRCC in which 708 were CAIX positive. One hundred and sixty-three tumours had low (≤ 85%) expression and 567 had high (> 85%) expression of CAIX. There were 265 RCC-specific deaths. The median, follow-up for the 247 patients still under observation was 13.8 years. They concluded that low CAIX expression is not statistically significantly associated with RCC death or distant metastases; therefore, CAIX is not an independent marker for ccRCC [68].

Even though some of the molecular markers offer to be promising prognostic or isolation markers, there is still much to learn about them. Moreover, there is a need for further research of known molecular markers, as well as for searching for novel ones. In order to obtain more specific information and more reliable clinical prognosis from molecular markers there might be an imminent need for designing a broad spectrum of experiments focusing on combinations of different markers.

Future perspectives

The majority of renal TPC studies concern their identification by cell surface markers, which have been used to isolate TPCs from other tumours. We already know from pathology that kidney is an organ with a completely different surface marker expression pattern, and attempts of direct translation of methodology from other tumours often give contradictory results. Cancer stem cell populations should be evaluated using functional assays, cell membrane marker analysis, and finally genetic and epigenetic signatures in combination. More large-scale research is needed. Identification of renal TPCs using a specific set of markers remains a major challenge. Moreover, It is not clear whether TPCs exist in a relatively stable or a dynamic state – this highlights the role of the dedifferentiation process and epigenetic alterations in the sought cell population.

Another limitation in the search for TPCs in patient samples is intratumoural heterogeneity. In the Gerlinger *et al*. study [69] exome sequencing, chromosome aberration analysis, and ploidy profiling were performed on multiple samples obtained from primary and metastatic RCC. From 63% to 69% of all somatic mutations were not detectable within each tumour region. Various heterogeneities have been observed in mTOR kinase, and many tumour suppressor genes which induce loss of function: *PTEN*, *SETD2*, and *KDM5C*. The characteristics of the found mutations suggest convergent phenotypic evolution because of their distinct and spatially separated features. In a search for good and poor prognostic factors the signatures of gene expression were different within various regions of the same tumour. Further allelic composition and ploidy profiling analysis confirmed intratumoural heterogeneity – an allelic imbalance profile was detected as well as ploidy heterogeneity [69]. More specific analysis of clear cell renal cell carcinoma (ccRCC) showed that intratumoural heterogeneity increased with the number of analysed biopsies [70]. The phenomenon of intratumoural hetero-

geneity and cancer drug resistance was also described by Yap *et al*. [71] as the model of a Darwinian tree with a trunk. To conclude the data about intratumoural heterogeneity, this phenomenon casts doubt on results obtained from single-tumour biopsies. Developed targeted therapy approaches should be validated once again in large-scale and multi-sample studies. The presence of various tumour genetic profiles (in other words different tumour varieties) within the tumour induces therapeutic failure through Darwinian selection.

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