REVIEW PAPER

UROLOGICAL ONCOLOGY

The relationship of cancer stem cells in urological cancers

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Numerous studies are ongoing to identify and isolate cancer stem cells from cancers of genito-urinary tracts. Better understanding of their role in prostate, urothelial and kidney cancer origin, growth and progression opens new pathways in development of more effective treatment methods. However there are still many issues before advances in this field can be introduced for clinical application. This review addresses current achievements in cancer stem cells research in uro-oncology.

Key Words: cancer stem cells ‹› stem cells ‹› urological oncology

INTRODUCTION

Cancer Stem Cells (CSC) are population of cancer cells that has self-renewing capacity and heterogenic differentiation potential. These cells may give rise to all types of tumor cells of particular tumor and thereby constantly sustain tumor growth. The cancer stem cells theory was developed based on studies, which demonstrated that only minority of cells harvested from cancers could initiate metastatic spreading or re-growth after allo-and xeno-transplantation [1]. The number of CSC in a tumor population is estimated on the range between 1- 5% of all tumor cells [2]. The small number of CSC within the tumor mass together with lack of specific molecular markers for their identification and targeting make isolation process challenging. CSC reside in cancer stem cell niches, which are characterized as a growth environment, consisting of different cell types and Extra Cellular Matrix (ECM) components [3]. The architecture of niches is variable from cancer to cancer [4]. CSC niche should be viewed as a compartment that comprises numerous signaling pathways regulating CSC fate and provides necessary support for paracrine and adhesive signaling regulating their life cycle.

The existence of CSC was proofed in cases of many tumors including urological cancers as well. CSC of Prostate Cancer (PC) were successfully isolated and characterized. Currently these findings are being translated into new therapies that may bring soon revolution into PC treatment. One the other hand, the origin of bladder cancer and renal cancer from CSC is still a hypothesis because CSC have not been so far isolated from samples of these tumors. The theories regarding prostate, urinary bladder and kidney cancer development do not fully explain many unique features of these neoplasms such as intra-tumoral heterogeneity, metastasizing, drug resistance and distant relapses after effective primary treatment. Widely postulated involvement of cancer stem cells in urological malignancies initiation and progression provides new insight into understanding their biology, clinical course and brings great opportunity for future

less empirical management. The highlighted feature of CSC behind this statement is their well-evaluated resistance to radiation and chemotherapeutic agents. Based on gathered research evidence CSCs are in fact responsible for tumor regrowth after primary therapy (Figure 1). The assumption that tumors contain population of constantly multiplying stem cells capable to survey systemic treatment and then initiating tumor regrowth should become major concept of designing new treatment approach and establishing new goals of cancer management.

Testicular germ cell tumors – The proof of concept

The origin of TGCTs is the most striking link between normal stem cells and CSCs.
Historically, teratomas have given the first proof that cancer cells are able to differentiate into tissues originated from three embryonic germ layers. Now it is obvious that pluripotent stem cells derived from normal stem cells are responsible for this phenomenon. The fate of germ stem cells that finally give rise to tumor in testis is the best proof of concept that stem cells may transform into cancer stem cells.
TGCTs arise from primordial germ stem cells, that share many morphological similarities with embryonic stem cells of blastocyst inner mass and gonocytes [5]. Early germ cells are *in vivo* equivalent of ES cells [6]. The expression of genes, such as OCT3/4, SOX2 and Lin28, which determine the pluripotency and self – renewal ability of primordial germ cells (PGCs), was found in TGCTs [7]. Cells isolated from TGCTs indicate a molecular and developmental dependence to embryonic cells from which they arise [8]. Oncogenic transformation occurs during the migration of germ stem cells to genital ridge or during early stages of gonadal organogenesis. Than transformed cells are supposed to form the intratubular germ cell neoplasia, which is compartment of pluripotent, cells able to progress during adult life [9]. During fetal life, genetic factors prepare the background for further stages of oncogenic transformation, which involves postnatal environmental factors [10]. Therefore CIS (*Carcinoma in situ*) cells arise from embryonic cells in early prenatal development before the gonadal tissue is mature. They are oriented to neoplasia progression, finally leading to invasive cancer [11].

Prostate cancer stem cells

Prostate Cancer Stem Cells (PCSC) represent only a small fraction of all tumor cells. Collins et al estimated that 0.1% of prostate cancer tumor cells correspond to characteristics of stem cells [12]. Despite low numbers of stem cells, their successful identification were reported in several studies. The main strategy of CSC identification utilized the assumption that normal prostate stem cells share markers with PCSC. The following stem cell markers were used in different studies CD44, CD133, $\alpha 2\beta 1$hi integrin, FAM65B, MFI2, and LEF1 in order to detect PCSCs [13]. The results of numerous studies which tried to answer the question about the cellular origin of PCSCs are inconclusive. According to the current state of research origin of PCSC comprise: Transient Proliferating/Amplifying (TP/A) intermediate stem cells, prostate stem cells, basal cells or luminal cells (Figure 2) [14]. There is approximately equally number of research reports informing that PCSC developed either from basal layer or luminal layer of prostatic epithelium (Figure 2 A, B).
The origin of prostate cancer stem cells might be related to differences in cancer presentation and outcomes.

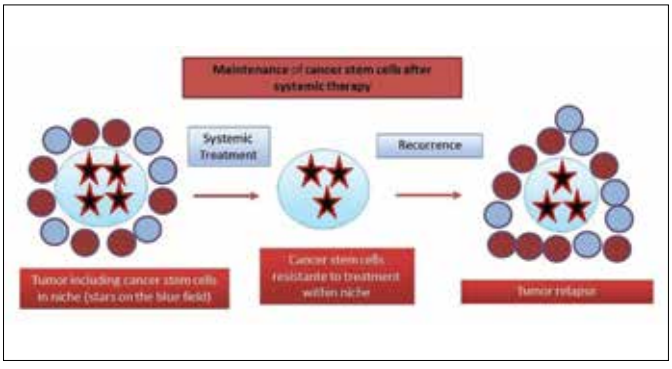

Figure 1. *Cancer stem cells are mostly resistant to systemic treatment. They survive within their niches and contribute to tumor recurrence.*

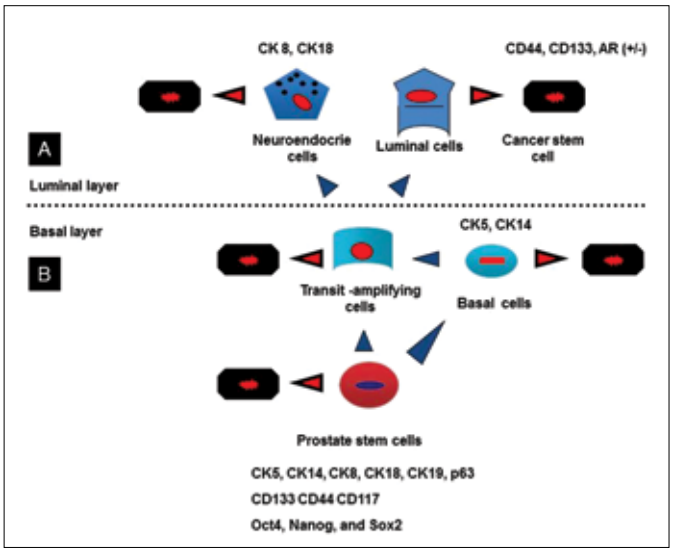

Figure 2. *The figure presents the model of cellular hierarchy of prostate including cancer origin pathway.*

This hypothesis could be also an excellent explanation why prostate carcinoma is such a molecularly, phenotypically and clinically heterogeneous disease. However, there is lack of any reports to support this concept. Generation of new progeny of cancer stem cells is very likely to be associated with diseases progression. Targeting cellular niches of CSC is challenging, due to heterogeneous structure of prostate gland and prostate cancer [15]. The most likely cancer stem cell niche is primary located on the basement membrane of the prostatic glands. During the malignant transformation of the prostatic epithelium and tumor growth, the proliferative zone is inverted and shifts to luminal side. This process is accompanied with formation of neoplastic basement membrane that might be rich in new niches of cancer stem cells [16].

Prostate cancer stem cells – clinical implications

Understanding of prostate cancer biology is key factor for optimizing existing therapies, as well as development of new treatment methods. Isolation of androgen dependet and androgen independent PCSC gave new insight into the mechanisms responsible for androgen resistance on deprivation therapy in patients suffering from advanced prostate cancer [17]. The hormone dependency of PCSC is unsolved. The most current findings suggest that at least a subset of prostate cancers stem cells express AR [18]. Vinagolu et al. identified stem-like human prostate tumor-initiating cells that do not express androgen receptor and Prostate Specific Antigen (PSA). It is highly probable that these cells resist treatment and diagnostic procedures and contribute to disease recurrence [19]. The PCS are mostly resistant to current treatment. Direct targeting these cells may bring revolution to prostate cancer therapy, due to ability to eliminate cells responsible for metastasis and recurrence. Liu et al. has recently showed that such approach is possible and introduces new potential therapeutical agent the microRNA miR-34a. These molecules inhibit prostate cancer stem cells and metastasis by directly repressing CD44 expression [20]. The determination of cancer stem cell markers can provide prognostic information. Vis et al. showed that loss of CD44 on surface of prostate PCSC is independent prognostic predictor of clinical recurrence [21].

Bladder cancer (transitional cell carcinoma – TCC, urothelial cancer). Bladder cancer origin

The belief in the existence of urothelial cancer stem cells is derived mainly from clonal hypothesis of urothelial cancer origin. The clonal hypothesis asserts that all urothelial cancer cells in the urinary tracts evolve from a single transformed cell of which all progeny share several identical genetic mutations. This hypothesis explains multifocal and recurrent urinary bladder tumor nature by seeding or intraepithelial spread of transformed cell clones [22]. The path from oncogenic transformation to multifocal tumors seems to be the consequence of gradual acquisition of genetic alternations and several populations of cancer stem cells could be generated during it.

The oligoclonal theory assumes an overall change in the urothelium, with many transformed cells evolving independently into tumors and therefore being genetically unrelated. According to this concept, urothelial cells undergo field malignant transformation that affects the entire urothelium within urinary tracts and causes a myriad of independent mutations responsible for further simultaneous tumors growth [23]. Experience gained during clinical practice reveled that multifocal advanced urothelial carcinomas are frequently monoclonal, whereas others ,especially low grade superficial papillomas, show oligoclonal origin and expansion [24]. With regards to the inexact nature of bladder cancer, low grade and high-grade carcinomas may originate from different cancer stem cells or from the same one depending on carcinogenic pathway.

High-grade tumors may arise *de novo* or share common carcinogenesis pathway with low-grade urinary bladder tumors. In this case, mutation of gene encoding structure of FGF receptor is first hit, which initiate urothelial hyperplasia [25]. Subsequent mutations in two major tumor suppressors p53 and retinoblastoma (Rb), which occurred in 15% of low-grade cases, lead to genomic instability and tumor progression to high-grade [26, 27].

Bladder cancer stem cells

Urinary bladder cancer is molecularly and phenotypically heterogeneous. Multiple genetic changes were identified to play role in urothelial cell carcinoma (UCC) development and progression [28]. Bladder cancer stem cells have not been isolated and described until now [29]. Evidence is accumulating that epithelial tumors, such as breast cancer, colon cancer contain a subpopulation of CSCs, so the search for bladder cancer stem cells is still continued. The difficulties in identification of CSCs in urinary bladder carcinoma arise from lack of any proven method allowing to separate true CSCs from other cancer cells capable of tumorigenesis [30]. Moreover, CSCs may not have tumorigenic potential at the onset. In this case, progression of disease is determined by

collection of particular genetic changes responsible for gradual acquisition of self-renewal capacity and other malignancy features [31].

The identification of urothelial stem cells

Urothelium layer consists of three hierarchically related cell types: basal cells, intermediate cells and umbrella cells (Figure 3 A). Urothelial cells form clonal units, which mean a group of cell derived from stem cell localized in basal layer [32]. Each clonal unit actively replenishes the urothelium during ageing. Castillo – Martin et al. recently postulated that two distinct progenitor cells generate intermediate cells and "umbrella" cells [33]. However, these are unconfirmed data.

Low molecular weight cytokeratins (19, 8, 7) are detected within all layers of urothelium covering inner surface of urinary bladder [34]. CD44, pancytokeratin, p63, and high molecular weight cytokeratins CK5, CK10, CK17 are exclusively expressed in the basal and intermediate cellular layers [35, 36]. Expression of CK18 and CK20 is limited only to umbrella cells. Oncogenic transformation can be recognized by monitoring changes in biomarkers expression patterns. Malfroe et al. noticed that upregulation of CK20 was associated with increased expression of p53 and Ki-67 which are well known molecular markers of malignant changes within urothelial mucosa [37].

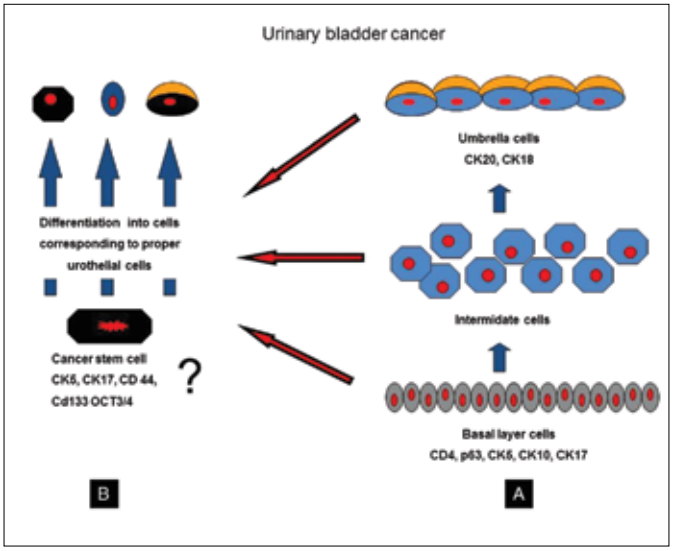

Figure 3. *The figure presents the model of cellular hierarchy of urothelium. The concept of existence of urothelial cancer stem cells is still unconfirmed. According to available data cancer stem cells might be derived from all urothelial cell types.*

On the trail of bladder cancer stem cells

That bladder cancer is hierarchically organized and may imitate the cytoarchitecture of proper urothelium (Figure 3 B). Three major types of cancer cells were identified whose morphology and cytokeratin expression patters are assigned to basal, intermediate and umbrella cells [38]. Recently Volkmer et al. also indicated that bladder cancer cells can be classified into three subtypes, on the basis of their differentiation states: basal, intermediate, and differentiated [39]. Authors emphasized in their work that each subtype contains primitive tumor initiating cells capable of generating xenograft tumors.

Similarly to linear urothelium hierarchy different subtypes of urothelial cancer cells appeared to be also derived from one cancer stem cell, but existence of this model is still unconfirmed.

High expression of human primordial stem cell marker OCT 3/4 was found in human bladder cancer cells providing evidence that undifferentiated cells must be present in population of tumor cells. The increased expression of this marker was correlated with enhanced migratory and invasive abilities of bladder cancer cells and contributes to their tumorigenesis potential [40]. The putative CSCs are believed to have their niches on basal layer of urothelium. Many study groups reported the successful identification of putative bladder cancer stem cells based on surface markers restricted to basal cells. However it is still unclear whether these cells are in fact cancer stem cells or they are sub-population of cancer cells with tumor formation capacity known as tumor initiating cells [41].

He et al. also targeted highly tumorigenic urothelial cancer cells using CK17, which is considered as basic marker for identifying urothelial basal cells [42]. Chan et al. expanded the range of markers used in order to detect bladder cancer stem cells in primary bladder cancer samples and noticed expression of CD44, CK5, and the lack of expression of CK20 both on urothelial basal cells and highly proliferative subpopulation of bladder cancer cells [43]. Authors noticed ability of these cells to induce xenograft tumors *in vivo* and recapitulate heterogeneity of the original tumor. The tumor forming potential could be linked to high expression of CD47 molecule protein that provides an inhibitory signal for macrophage phagocytosis [44]. In different study Yang et al. demonstrated that CD44 cells possess the ability for colony-forming, self-renewal and proliferation and therefore they might be attractive candidates for urothelial cancer stem cells [45]. Bentivegene et al. provided detailed evaluation of cancer cell population that corresponds to cancer stem cells. These

cells were detected using well-established normal stem cells markers such as (CD133, OCT-3/4, NESTIN) and seemed to show the most stemness characteristic among all investigated putative cancer stem cells isolated from bladder cancer species. In culture they form spheres and show capacity to differentiate into cells identified with UroVysion test. However, during *in vitro* maintenance these cells gradually decrease proliferation rate and lose self-renewal ability [46].

The results of published studies did not confirm the existence of cancer stem cells within urothelial tumor in urinary bladder. The heterogenous nature of TCC hinders the comparison of results and mentioned studies. None of the authors provided a convincing characterization of cells, which could be doubtfully defined to be cancer stem cells.

It is very likely that bladder cancer stem cells might be undetected subpopulation of tumor initiating cells and further research should be done to provide more detailed molecular characteristic of bladder cancer cells showing tumorigenic potential.

Renal cancer CSC – still a lot of confusion

Renal Cell Cancer (RCC) recapitulates epithelium lining proximal convoluted tubules [47]. Renal epithelial cells are derived from mesenchyme and may present dual character of mesenchymal and epithelial cells. Under normal condition about 1% tubular cells proliferate in order to replace damaged or dead cells throughout life. In response to acute damage, these cells begin to proliferate to repopulate and restore epithelium of injured tubules. During this burst of cellular proliferation renal epithelial cells may easily oscillate between mesenchymal and epithelial state which is known as Mesenchymal to Epithelium Transition (MET) and Epithelium to Mesenchymal Transition (EMT) (Figure 4 A) [48]. Renal epithelium progenitor cells that are generated after renal injury do not display features of stem cells. These cells showed directed differentiation potential into renal epithelial lines. The ambiguous nature of renal epithelium may determinate the characteristic features of renal cancer including extensive angiogenesis and spontaneous regression of metastatic lesions as well.

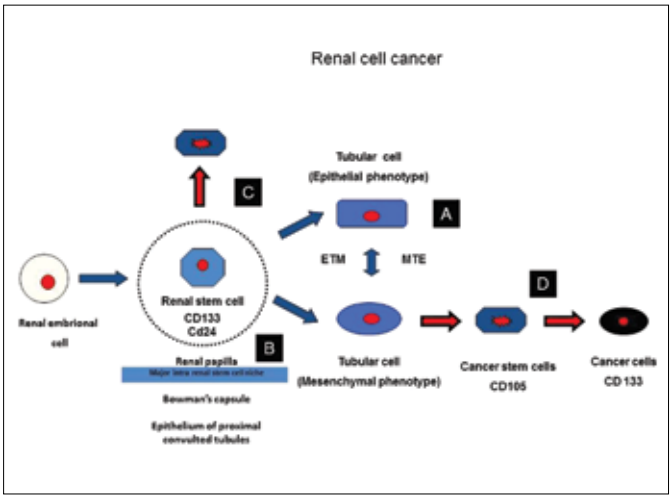

Figure 4. *The figure presents the model of cellular hierarchy of renal epithelium. In adult kidney there are three stem cell niches: renal papilla, bowman's capsule and epithelial layer of proximal convoluted tubules. Cancer stem cell origin may encompass natural stem cells reservoirs within kidney or epithelial progenitor cells that are derived during epithelium regeneration.*

Renal stem cells. Renal epithelium

Renal stem cells are detected within papilla and Bowman's capsule using CD133 and CD24 antibody (Figure 4 B). CD133 cells from renal papilla were also found to express nestin, Nanog, SOX2, and OCT3/4 that are associated with pluripotent embryonic stem cells.

Renal papilla was proposed to be stem cells niche where true renal stem cells with retained embryonic character resided. $CD133^{+}$ cells isolated from renal papilla had capacity of spontaneous tubulogenesis and differentiation into mature renal epithelial cells [49]. In recent study conducted by Lindgren et al. similar cells were isolated from epithelium of proximal tubules. It is the only research that revealed the stem cells niches might be also localized in intact epithelium of renal tubules [50].

Renal stem cells were reported to show multipotent differentiation potential after observed their ability to enhance tubular epithelium regeneration *in vivo* after injury and capacity to differentiate *in vitro* into osteogenic-like, adipocyte-like, and neuronal-like cells [51]. However the role of intra renal stem cells in kidney healing and regeneration was not resolved so far. There are no definitive data published that renal progenitor cells outside of the tubular cells themselves play a major role in the normal maintenance of the glomerulus or tubular epithelium [52].

Renal cancer stem cells

The major concept of renal cancers stem cells takes into account that normal stem cells may undergo transformation into malignant cells CD 133 and CD 24 positive cells. Thus, renal stem cells are

ideal targets to undergo "first hit" during cancerogenesis (Figure 4 C). Some of renal cancer cells retain mesenchymal character manifested in multilineage mesenchymal differentiation potential after *in vitro* cultivation in conditioned media[53]. Zhong et al. showed that samples of RCC included cellular population capable of growing as tumor spheres in serum-free medium supplemented with EGF and bFGF. Authors showed that 3 x 10^6 SK-RC-42 cells are needed to form new tumor in mice. However in this study any particular population of cells was not described as renal cancer stem cells [54].

Glycoprotein CD133 is well-established surface marker of cancer stem cells in epithelial tumors including breast and colon cancer. CD133 positive cells derived from renal cancer were not proven to have tumorigenic potential but rather contribute to enhance tumor development and growth by stimulating neovascularization (Figure 4 D) [55]. Many authors have documented important role of hypoxia depended pathways in renal tumor development and progression [56]. Bruno et al. showed that hypoxia stimulated clonogenicity, proliferation of CD133 cells and modulate their differentiation into vessels [57].

Bussolati et al. proposed that CSC arise from renal stem cells and have mesenchymal origin as the only isolated and describe the cell population meeting the criteria for cancer stem cells CD105 was used as a major antigen distinguishing cancer stem cells from the rest of tumor cellular mass. CD105 cells derived from RCC initiated tumor formation with 100% efficiency after allotransplantation [58].

Better understanding of renal cancer origin and development may lead to new approaches to treatment in the near future. Azzi et al. basing on the latest advances in the field of cancer stem cell research, proposed to use IL-15 for renal cancer stem cell depletion and generation of differentiated non-tumorigenic cells that are sensitive to chemotherapeutic agents [59]. Clinical and molecular stratification of disease severity may also be revolutionized very soon, due to introducing new cell markers which may predict cancer progression. D'Alterio et al. evaluated the expression of CD133 on samples of human renal clear cell carcinoma, finding no correlation between CD133 expression and pathological features of tumor or influence on patients prognosis [60]. On the other hand da Costa et al. reported that low expression of CD133 is associated with higher probability of disease progression and death from cancer [61]. These contradictory results of preliminary research indicate how puzzling biology of renal cancer stem cells is and how carefully we should apply experimental research data to clinical practice.

CONCLUSIONS

CSC identification and *in vitro* cultivation makes possible to develop new therapies it precisely targeting cells responsible for tumor re–growth and metastasis formation.

There is convincing evidence that kidney, prostate, bladder, and testicular cancers are clones of cells that originate from CSC. Their successful identification among population of primary cancer cells may provide new targets for further therapies.

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