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Review

Semaphorin-3A and urothelial carcinoma

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Urothelial cancer (UC) is a common malignancy with unique biology displaying a high tendency to recur despite complete resection of the primary lesion. In approximately 80% of the patients, UC is a non-curable chronic disease, however in the remaining ~20% of patients, the disease can progress and cause death. Despite the fact that UC in the USA is common and responsible for 82,000 new cases and 18,000 patients die from this disease annually, almost nothing has changed in the last decade regarding the diagnosis and treatment of UC. Hence, there is a burning need for non-invasive biomarkers for screening and follow up as well as to develop novel treatment strategies for targeted therapy. Recently we have discovered that Semaphorin-3A is involved in UC and may serve as a biomarker or a potential druggable target for therapy. Herein we review the current knowledge about Semaphorin-3A in UC.

Keywords

Urothelial cancer; noninvasive detection; urine; Semaphorin-3A; targeted therapy

1. Urothelial Cancer

1.1 Epidemiology

Urothelial cancer is the fourth most common malignancy in man and the eighth in women. In the USA, 82,000 new cases of bladder cancer were diagnosed in 2018 [1]. At the time of diagnosis, ~70% of the tumors were non-invasive, i.e. involving the epithelium or the lamina propria (stage Tis, Ta, T1), and the remaining ~30% of the patients suffered from invasive disease [2]. The current treatment of these tumors consists of trans-urethral resection (TURT), followed by intravesical treatment when indicated [2].

UC is a sporadic disease and is rarely a genetically inherited malignancy, where ~70% of the cases could be associated with exposure to carcinogens, especially aromatic amines [3]. In the past, occupational exposure was common, however today most of the cases are related to cigarette smoking [3]. A fact that may explain the increased rate of females who developed UC.

1.2 Symptoms and detection

The classical appearance of UC is painless hematuria, the majority (~85%) of new patients will be diagnosed with non-invasive UC, and following local resection with or without complementary intravesical treatment, they will need a lifelong follow-up to rule out tumor recurrence and progression [2]. Today, the follow up regimen includes urine cytology, endoscopy and upper tract imaging. This set of diagnostic tools has many disadvantages including: cytological analysis (which is the only noninvasive method) has a very low sensitivity (~50%) which is unacceptable [4]. Endoscopy and ionizing radiation imaging are invasive and carry discomfort and severe (even life threatening) side effects [5]. Molecular markers may have many advantages over conventional cytology, allowing earlier detection, higher overall sensitivity, and sensitivity that is tumor grade independent. However, although many studies were conducted to find a new noninvasive biomarker, clinicians continue to use urine cytology as the primary tool for non-invasive detection [4].

In spite of these advantages, there are few molecular markers available for clinical use [4]. Although most of the tested biomarkers demonstrated high sensitivity and specificity, examining the total number of patients participating in each study showed that the average size of the studied group is relatively low, hence the statistical power of these studies is very low, and recruitment of a larger number of patients may change the results [4]. Moreover, research laboratories are very different from a routine clinical laboratory; hence, despite the fact that one may have an excellent biomarker in hand, there still is a need to adjust the hardware to suit a hospital lab, an expensive procedure that may not suit the hospital setting or the limited budget of a medical system. Using the automated system, we tried to facilitate the use of microsatellite analysis in a routine lab using routine lab instruments with great success, however this process remains time consuming, expensive and mandates care by a specialist.

1.3 Treatment of non-invasive and invasive disease

The main goal in the treatment of non-muscle invasive disease is to reduce recurrence and progression. Various drugs have been used as an adjuvant treatment in order to reduce the recurrence and progression rate. In general, intravesical treatment reduces the recurrence rate from 50-70% to 15-30% and progression rate from 10-20% to $<$ 10% [2]. The most common agents used are:

Bacillus Calmette-Guerin (BCG), and Mitomycin C (MMC) [6, 7]. BCG elicits its therapeutic effect via nonspecific induction of the immune system in the bladder [6], whereas MMC is a chemotherapeutic alkylating agent that interferes with DNA replication in hyper-proliferating cancer cells [7]. In contrast to renal cell carcinoma and prostate cancer, in UC there is much slower progress in the molecular understanding of this disease, hence almost no new targeted drugs are currently available for these patients. Invasive disease is treated with neoadjuvant chemotherapy and radical surgery [8]. Chemotherapy is nonspecific and there are no specific targeted agents developed directly for metastatic UC.

1.4 Semaphorins

Semaphorins are classified into eight sub-classes based on their structural domains [9]. Semaphorins play a significant role in cell attachment, migration and motility[10]. Semaphorins have a role in organogenesis, vascularization, angiogenesis, apoptosis, and neoplastic transformation [11, 12, 13, 14, 15, 16, 17]. Furthermore, recent studies pointed to the involvement of Neuropilin-1 and certain Semaphorins in the regulation of the immune system, and thus these Semaphorins are termed "immune Semaphorins" [18, 19]. The seven class-3 Semaphorins (Sema3s), designated by the letters A-G, are the only vertebrate secreted Semaphorins. Neuropilins (NRPs) and the type A/D family Plexins (Plexin-A1, -A2, -A3, and Plexin-D1) act as receptors for Semaphorin 3 [13, 20]. Each Semaphorin 3 family member shows distinct binding selectivity for NRPs [13, 20]. Each Sema3-NRP complex associates with specific plexins to mediate downstream signaling. Most membrane-bound vertebrate Semaphorins directly bind plexins, while Sema3s require NRPs as obligatory co-receptors.

Semaphorin-3A (Sema3A), a class-3 secreted member of the Semaphorin family, has been established as an axonal guidance factor during development [11, 17]. Interestingly, several lines of evidence suggest that Sema3A also affects immune cell functions [18]. Sema3A was shown to be expressed by activated T cells and to inhibit T cell proliferation and cytokine secretion [19]. Moreover, the expression of Sema3A, Neuropilin 1 (NRP-1), Neuropilin 2 (NRP-2), and Plexins was found to be increased in differentiating macrophages and in activated T cells [19]. In another study, kidney biopsies from lupus glomerulonephritis (LGN) patients showed stronger staining with anti-NRP-1, anti-Sema3A and anti-Semaphorin4A antibodies as compared with either normal biopsies or biopsies derived from patients with primary nephropathy and proteinuria [15]. Subsequent studies have shown that Sema3A serum levels in systemic lupus erythematosus (SLE) patients are significantly lower than in healthy individuals [18, 19, 21].

1.5 Semaphorins and Sema3A in cancer

A major question that emerges is the molecular mechanism underlying the association of semaphorins and cancer. Recently, expression profiles of Sema3s and their association with patient survival and tumor microenvironment were studied in 31 cancer types using the TCGA data [22]. The expression of Sema3 family members varied in distinct cancer types. It was shown that Sema3A, Sema3C, and Sema3F were upregulated in cancer cells, whereas the remaining of Sema3s were down-regulated in tumor specimens. An association with survival was also noted; Sema3A and Sema3E were associated with a poor prognosis and survival, whereas Sema3G was associated with survival advantage. This study further suggested that Sema3 genes, Sema3C and Sema3F in particular, may contribute to drug-induced cancer cell resistance.

The literature provides very conflicting evidences showing that Sema3a may on one hand promote cancer and on the other hand may interfere with cancer formation and progression [12, 19]. One of the well-known mechanisms of cell plasticity in cancer development is the epithelial-mesenchymal transition (EMT), which is a pivotal process during embryonic development and is commonly utilized by cancer cells to gain invasiveness [23, 24]. Specifically, EMT is a process by which cancer cells lose their epithelial characteristics, their cytoskeletal structure is re-organized, their cell shape changes and cells activate genes that promote the mesenchymal phenotype, all of which lead to an increased cell motility and metastatic dissemination of tumor cells to distant organs. Sema3A was found to inhibit EMT and pro-invasive mechanisms induced by cancer cells (e.g. pancreatic neuroendocrine tumors and HPV16/E2 cervical carcinomas) by inhibiting NF-κB and SNAIL2 expression. Thus, in these reports Sema3A is thought to be a tumor suppressor gene. Sema3A was found to impede tumor cell migration and lymph node metastasis in patients with prostate cancer [16]. The findings of the this study suggested that Sema3A, 3B, 3C, and 3E immunostaining in prostate cancer biopsies, as supplements to clinic-pathological parameters, could be used for predicting biochemical recurrence in low- and intermediate-risk prostate cancer patients after radical prostatectomy. Specifically, concurrent Sema3C-positive and Sema3A-negative, Sema3B-negative, Sema3E-negative staining was associated with an adverse prognosis. Another immunohistochemistry study suggested that decreased expression of Sema3A and a higher expression of matrix metalloproteinase 14 (MMP14) may promote the occurrence and development of non-small cell lung cancer (NSCLC) and that the combined detection of both Sema3A and MMP14 protein may be a useful tool in predicting the prognosis of NSCLC [25].

Semaphorins were also studied as potential druggable targets [22, 26]. Regarding the urinary system, our group has recently reported that Sema3A is overexpressed in urothelial cancer patients, both in urine and in bladder tissue[27].

Sema3A was also reported to be important in hematological malignancies; it was found to be expressed at lower levels in acute lymphoid and myeloid leukemia (ALL/AML) and chronic myelogenous leukemia (CML) cells, in serum of these malignancies as well as in multiple myeloma compared to hematopoietic cells found in the normal bone marrow and in the serum of healthy volunteers [15]. As a putative tumor suppressor molecule, it was found that Sema3A (via interaction and formation of receptor complexes with NRP1 and Plexin-A1) could promote Fas translocation into membrane rafts, thereby increasing apoptosis of leukemic cells via Fas-mediated apoptosis.

Lepelletier et al., demonstrated that Sema3A is expressed by activated dendritic cells (DC) and T cells, and that its secretion in co-cultures of DC and T cell was delayed [19]. Sema3A-NRP1 interaction down-modulated T cell activation since addition of Sema3A in DC+T cell co-cultures markedly blocked allogeneic T cell proliferation. Furthermore, neutralization by blocking antibodies or by antagonistic peptide of endogenous Sema3A

produced by DC+T cell co-cultures revealed a 130% increase in T cell proliferation. Remarkably, Sema3A acted directly on T cells, since it blocked anti-CD3/CD28-stimulated proliferation of T cells. Mechanistically, the immunomodulatory functions of Sema3A were based on the blockage of actin cytoskeleton reorganization and impaired T cell receptor polarization. These results show that Sema3A secretion and the resulting Sema3A-NRP1 interaction, are involved in a late negative feedback loop.

The ability to suppress the immune system is one of the basic principles in cancer formation and progression. We know that BCG is a most effective modality in the adjuvant treatment of UC; its mechanism of action against UC is via recruitment of immune cells to the bladder wall, hence it can be postulated that higher levels of Sema3A will be associated with increased immunosuppression and more aggressive tumors. Indeed, our previous studies demonstrated elevated levels of Sema3A in more aggressive UC lesions as observed in immunohistochemical staining of various UC specimens [18].

Other evidences showed that Sema3A has antitumor activity which include inhibiting angiogenesis by competing with VEGF , which constitute the fundamental activities required for malignant tumor development [18]. Sema3A as a NRP1 ligand, is an anti-angiogenic agent [28]. How could this fact comply with our results that Sema3A levels are elevated in more aggressive UC lesions is yet unclear. There is also cumulative evidence that a systemic delivery of Sema3A *in vivo* inhibited tumor growth and tumor progression [17, 29], and that even low levels of Sema3A were found in NSCLC and malignant melanoma [25]. One can postulate that each malignant cell is fighting for its own nutritional supply and therefore secretes Sema3A in order to suppress adjacent competitors.

1.6 Sema3A and Urothelial cancer

We have previously conducted two studies that demonstrated the relevance of Sema3A in UC [21]. Our main study included 183 patients and aimed to evaluate Sema3A as a potential non-invasive biomarker for UC. Urine was obtained from: a) patients with known bladder tumor; b) patients with non-malignant urological conditions; c) healthy volunteers. Higher Sema3A levels were significantly correlated ($P = 0.006$) with the presence of UC, as determined by positive cystoscopy or urethroscopy and pathological biopsy. The combination of Sema3A levels and cytology increased sensitivity (66% *vs.* 33%) with a small reduction of specificity (77% *vs.* 90%). Immunohistochemical staining was positive in tumors and almost universally negative in normal tissues [14].

An additional study aimed to evaluate the utility of Sema3A as a biomarker for diagnosis and management of upper tract urothelial carcinoma (UTUC). Diagnosis and follow-up upper tract UC is challenging and patients should undergo invasive ureteroscopic procedures mandating frequent anesthesia which carries high potential for severe side effects. A specific and sensitive biomarker should provide a significant advantage for these UTUC patients. In this study we evaluated ten patients with current or past UTUC. In 80%, Sema3A levels were high, confirming the presence of UC in the upper tract. Urine cytology was positive only in 5 patients. Sema3A showed higher sensitivity than cytology, and high levels of it correlated well with UTUC. Combination of cytology and Sema3A showed 100% sensitivity.

2. Conclusions

Sema3A is a novel, independent, potential biomarker for the detection and follow up of UC. Combination of both Sema3A and cytology may increase sensitivity. Sema3A presence and levels correlated with the higher stage and grade of UC. The exact mechanism by which Sema3A is influencing the evolution and progression of UC remains to be elucidated, following which there will be a possibility to consider it as a druggable target for targeted therapy.

Conflict of interest

There are no conflicts of interest to disclose.

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