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LITERATURE REVIEW

Understanding Wnt5a Signaling Pathways in Urothelial Carcinoma Tumorigenesis

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Abstract

Urothelial carcinoma (UC) is the most common type (90%) of all cancers in the urinary bladder. Clinically, UC presents as a papillary tumor or as an invasive/aggressive tumor. Although the 5 year survival is close to 88% for patients with the papillary form stage I, the survival rate drops to 46% for patients with stage III.

Dysfunctional Wnt signaling has been implicated in pathological conditions, specifically over-expression of Wnt5a has been found in a variety of cancers. Recently, Wnt5a has been implicated in cancer progression as a potent enhancer of cell motility and invasiveness and Wn5a/ Ror2 signaling in particular has been involved in epithelial mesenchymal transition, promoting invasion and metastasis in several types of cancer. Our group reported a positive correlation between Wnt5a protein expression and the histopathological grade of UC in human samples. Also, we found that Wnt5a/Ror2 PCP signaling is a potential pathway functioning in aggressive UC cases.

This review summarizes the most relevant data of Wnt5a signaling pathways in urothelial carcinoma, with special emphasis on its potential applications as diagnostic and prognostic marker or eventual therapeutic target.

Introduction

As the life expectancy of humans extends in developed countries, bladder cancer incidence continues to rise as well. In the US around 79,000 new cases are reported annually, and 16,870 annual deaths are related to this cancer [1]. The American Cancer Society reported an incidence rate from 2009 to 2013 of 20.7 per 100,000 for the US population. This review summarizes the most relevant data of Wnt5a signaling pathways in urothelial carcinoma, with special emphasis on its potential applications as diagnostic and prognostic marker or eventual therapeutic target.

Urothelial carcinoma of the bladder

Urothelial carcinoma (UC) is the most common type (90%) of all cancers in the urinary bladder. The vast majority of UC arise in the transitional urothelium of the bladder; for this reason it has been designated as transitional cell carcinoma (TCC). Clinically, UC presents as a papillary tumor or as an invasive/aggressive tumor. Although the 5 year survival is close to 88% for patients with the papillary form stage I, it is because these tumors are usually of low grade and low pathological stage at diagnosis. The survival rate drops to 46% for patients with stage III. On the other hand, for those patients with invasive UC and either regional or distant metastasis, the survival rate is at best 50% and 6%, respectively [1].

A feature to be highlighted of UC is its great variety of morphological aspects. This particularity makes the diagnosis, treatment and prognosis of UC a challenge in the clinical practice. The first presentation for bladder cancer is often hematuria, as the UC usually is a highly vascularized tumor independent of the stage of the disease. The current standard diagnostic tool is the transurethral cystoscopy which provides opportunity for resection of the tumor and histological examination for accurate pathological grading and staging of the tumor. Although the histological grade and stage determined using hematoxylin and eosin staining and light microscopy is the standard method to classify the UC [2], the existence of subtypes and recurrence make difficult the prediction of the natural history of this cancer [3].

Papillary UC comprises the vast majority of cases and is usually resected via transurethral resection (TUR). Recurrence occurs in up to 75% of cases after first TUR. Additionally, cases of papillary UC with poor differentiation (20%) progress to invasive stages [4]. Invasive UC, usually derived from carcinoma in situ, shows an aggressive behavior with local invasion and metastasis and requires cystectomy and chemotherapy as treatment. This division in papillary, invasive or in situ has important clinical implications and it is associated with at least two different molecular pathways described for the oncogenesis of UC [4].

Wnt signaling

Wnt proteins are a family of secreted, highly conserved glycoproteins that play a critical role during embryogenesis and in some physiological aspects in the adult. The 19 proteins in this family are ligands that bind to a family of ten transmembrane receptors that include Frizzled (Fzd), other receptors such as receptor tyrosine kinase-like orphan receptor 1 and 2 (ROR1/2), receptor-like tyrosine kinase (RYK), and some co-receptors such as low density lipoprotein receptor-related protein (LRP5/6), collagen triple helix repeat-containing protein 1 (CTHRC1) and VANGL planar cell polarity protein 2 (Vangl2) [5]. By binding this vast repertoire of receptor/co-receptors, the Wnt proteins are capable of activating a wide variety of signaling pathways typically classified into two main categories, canonical and non-canonical [6].

Canonical or β-catenin pathway is mediated by proteins such as Wnt1, Wnt3a and Wnt10b and has been extensively studied. It is well recognized for its role in colon cancer. In the absence of canonical Wnt signaling activation, β-catenin is bound to E-cadherin at the cell membrane and any excess of cytoplasmic β-catenin is ubiquitinated followed by proteasomal degradation. When canonical Wnt ligands bind Fdz receptor and LRP5/6 co-receptor, the outcome of Wnt signaling activation leads to stabilization and accumulation of β-catenin in the cytoplasm, its translocation into the nucleus of the cell, and binding of β-catenin to T-cell factor/lymphoid enhancer-binding factor, and finally the transcription of canonical Wnt target genes.

The non-canonical or β-catenin independent Wnt signaling pathway is mediated by proteins such as Wnt4, Wnt11 and Wnt5a. The non-canonical pathway is less known but recently has been more studied. At least two signaling pathways, Wnt/Ca2+ and planar cell polarity (PCP) can be activated once the Wnt proteins bind the appropriate receptors/co-receptors.The Ca2+ pathway involves activation of Ca2+ dependent molecules such as protein kinase C (PKC), Ca2+/calmodulin-dependent protein kinase II(CaMKII) and nuclear transcription factor of activated T cell (NFAT). The PCP pathway can be

activated through binding of several Fzd receptors which activate the small GTP binding proteins RhoA and Rac and their downstream signaling molecules Rho-kinase and c-Jun N-terminal Kinases (JNKs). It is important to mention that Wnt5a activates or inhibits β-catenin signaling depending on receptor context [7]. Also, the Ca2+ pathway can block the β-catenin pathway through phosphorylation of TCF/LEF transcription factors via activation of the TGF-β activated kinase 1 (TAK1)-Nemo-like Kinase (NLK) cascade [8].

Although Wnt5a shares common characteristics with other members of the Wnt family of proteins, in recent years it has been an important topic of research due to several interesting features associated with the Wnt5a signaling pathways: (a) it is highly expressed/secreted by macrophages, a critical cell involved in innate immunity and (b) two isoforms of Wnt5a have been described (long and short composed of 380 and 365 amino acids, respectively) with potentially different functions in cancer and inflammation[9].

Wnt5a in health and disease

Dysfunctional Wnt signaling has been implicated in pathological conditions such as cancer and inflammatory diseases[10-14]. Specifically, over-expression of Wnt5a has been found in a variety of cancers [11]. Interestingly, the role of Wnt5a in the pathogenesis of cancer is still unclear and controversial, and opposing roles as tumor promoter and tumor suppressor have been reported [15,16]. Up-regulation of Wnt5a has been reported in lung, stomach, colon, breast, pancreas and prostate cancers, where it is thought to be a tumor promoter[12,17,18], while Wnt5a has been described as a tumor suppressor in hematopoietic, brain, and thyroid cancers [16,19]. The role of Wnt5a as tumor suppressor by antagonism of the canonical Wnt/β-catenin signaling pathway has been demonstrated in colorectal carcinoma [19].

Recently, Wnt5a has been implicated in cancer progression as a potent enhancer of cell motility and invasiveness in different type of cancers, such as malignant melanoma [17] and pancreatic cancer [20]. Also, Wnt5a has been associated with epithelial mesenchymal transition (EMT) in a PKC-dependent manner in melanoma [21], epidermoid carcinoma and osteosarcoma [22], prostate cancer [23] and human gastric carcinoma cells [24]. In particular, the Wnt5a/Ror2 signaling pathway has been reported as an important pathway in motility and invasiveness of carcinoma cells [22,25].

Wnt5a is a protein secreted by different types of cells present in the tumor microenvironment such as tumor associated macrophages (TAM), fibroblasts, and endothelial cells. An upregulation of Wnt5a in TAM was reported in breast [26] and colon cancers [27]. Interestingly, Wnt5a is a ligand that works in an autocrine and paracrine manne r[7,13,28,29]. In summary, all of these functional characteristics of Wnt5a highlight the high complexity of the potential roles of Wnt5a in tumorigenesis.

Wnt and urothelial carcinoma

The role of Wnt signaling in the pathogenesis of UC is not fully understood, with conflicting results from different studies. Mutations in the adenomatous polyposis coli (APC) or β-catenin (CTNNB1) genes are frequent causes of aberrant canonical Wnt pathway activation in colon cancer, but this mechanism is rare in urogenital cancers [30-32]. Likewise, the role of non-canonical signaling in the carcinogenesis of UC is still unclear. As was mentioned above, Wnt5a shows paradoxical effects in various type of cancers. It is well known that the malignant transformation is a stepwise progression that involves activation of a variety of molecular events during carcinogenesis. Wnt5a has been described as a tumor suppressor and tumor promoter in different malignancies at the initial stages of malignant transformation [33]. In other cancers the role of Wnt5a has been associated with the metastatic process via EMT, a final event in the malignant transformation [22,34].

Our group reported a positive correlation between Wnt5a protein expression and the histopathological grade of UC in human samples. Using immunostaining in UC tissue obtained via TUR, we found that the intensity of the immunostaining for Wnt5a correlated with the

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Page 3/8

histological grade and pathological stage described in the pathology report. These results let us to conclude that Wnt5a plays some role in the pathogenesis of UC with a potential application as a diagnostic/prognostic marker for this cancer. In the same study we found that Wnt5a increases migration, interestingly with different rates, of T24 and J82 cells, two UC cell lines [35].

In our second study we investigated more specifically the expression of Wnt5a and its receptor Ror2,involved in PCP, as a potential pathway playing a role in those aggressive UC cases. Interestingly, we found by immunohistochemistry that the correlation between Ror2 and pathological grade is even stronger than the correlation between Wnt5a and pathological grade. This result led us to speculate that Wnt5a/Ror2 PCP signaling is a potential pathway functioning in the mechanism of aggressive UC cases. Also, the high expression of CTHRC1 in those tumors with high Ror2 expression supports the idea that the PCP pathway is participating in the aggressiveness of UC.

These results from formalin-fixed, paraffin-embedded human UC samples are in line with our in vitro studies. Using reverse transcription-polymerase chain reaction (RT-PCR) of RNA extracted from RT4, J82 and T24 cells, three urothelial carcinoma cell lines isolated from UC cancers with different pathological grade, we demonstrated a similar trend for Wnt5a and Ror2 transcription[36]. The positive rate of Wnt5a expression associated with advanced stage of cancer also has been also described for gastric, pancreatic and melanoma cancers[12, 21,37]. Furthermore, our previous report of a role for Wnt5a in cell migration in pancreatic cancer cell lines [20] was later confirmed by Bo H. et al. in 134 surgically resected pancreatic adenocarcinomas [34]. Recently, Wn5a/ Ror2 signaling has been implicated as an important pathway in EMT and promotion of invasion and metastasis in epidermoid carcinoma [22] and pancreatic cancer [20,34]. Our results described above suggest a possible role of Wnt5a/planar cell polarity pathway through the Ror2 signaling pathway in a late stage of carcinogenesis, the development of metastasis in bladder UC [36] (Figure 1).
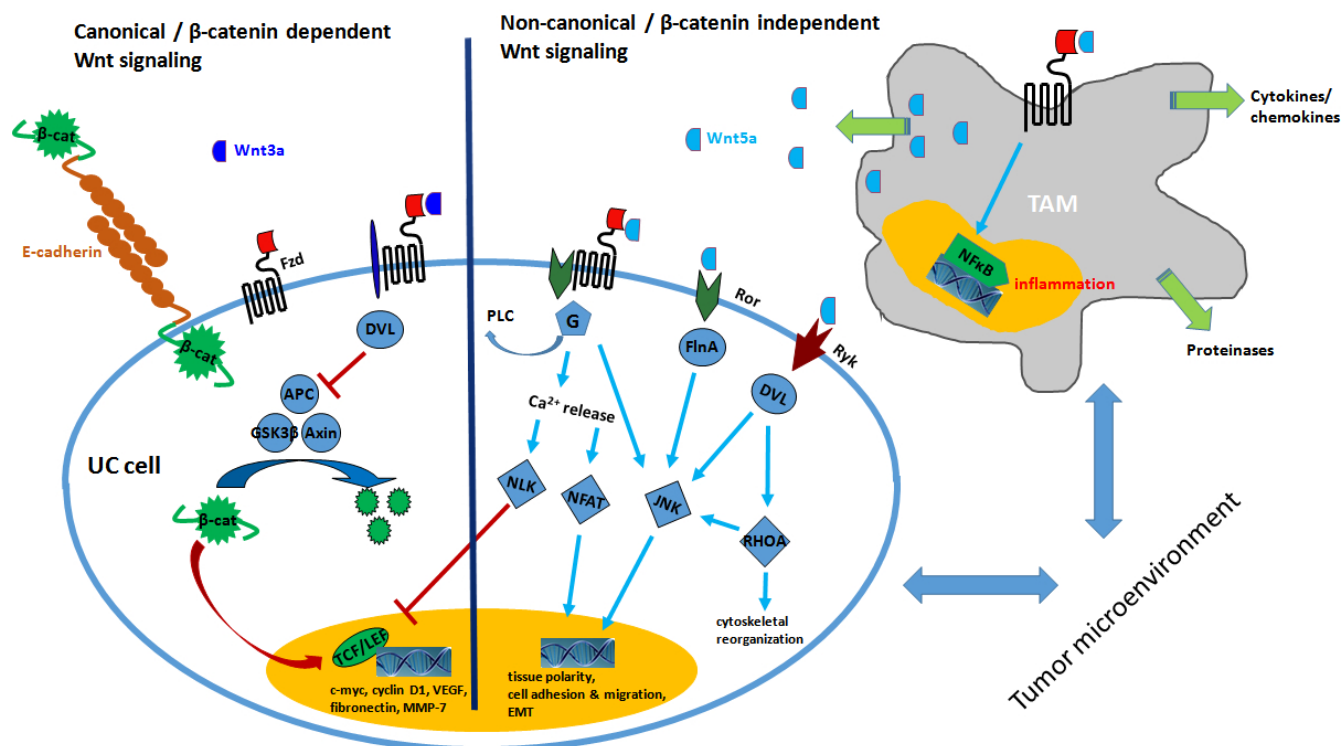
It is important to remember that by binding different receptors/co-receptors on the cell membrane, and through a variety of intracellular signaling pathways, Wnt5a is capable of inducing diverse effects on tumor cells, such as (1) senescence of cancer stem cell chemoresistance, (2) altering tumor microenvironment via activation of tumor associated macrophages, and (3) migration-invasion-metastasisvia the EMT process [33]. These characteristics of Wnt5a make it capable of acting at different levels/steps in the process of carcinogenesis.

Wnt5a signaling as a biomarker for UC

Cystoscopy and urine cytology have been the gold standard techniques for the diagnosis of bladder cancer. The accurate pathological grading and staging of the UC are critical for defining the treatment and prognosis. The challenges are the identification of those papillary tumors with poor differentiation (high grade) that will potentially progress to invasive stages and the early detection of the aggressive UC associated with carcinoma in situ as a precursor lesion.

Despite concerted efforts in developing specific markers and a large number of studies published about diagnostic/prognostic tools, there are still several problems to address. In the last decade multiple biomarkers have been described and used for diagnosis or grading the UC, with many of them demonstrating their value as prognostic markers. Among others, p53, cytokeratin 20, E-cadherin, Ki67, CD44 and survivin have been studied as potential candidates with conflicting results when considering histological grade, stage or even variants of UC [2,38].

Increased expression of Ki67 and p53 has been used as markers of high grade UCs. E-cadherin, a membrane glycoprotein involved in cell to cell adhesion, has been proposed as a prognostic marker. Several studies have shown that a reduction and change in expression pattern of E-cadherin is associated with aggressive UC [39]. Related to this, loss of β-catenin expression from the cell surface, has been reported as a marker of EMT in invasive carcinomas [40]. Also, survivin has been targeted as a prognostic marker for UC. Ying-bei Chen et

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Page 4/8

Figure 1: Roles of Wnt5a signaling pathways in the UC cell.

al. [41] reported that positive nuclear staining for survivin was significantly higher in high grade tumors compared to low grade. They suggested that immunostaining for survivin could be a useful adjunct tool for grading UC. In line with this study Shannon in 2001[42] reported the detection of survivin in urine as a predictor/prognostic diagnostic tool. They reported for the urine survivin test 100% sensitivity for detection of new or recurrent bladder cancer and 95% specificity. Recently, low expression of COX2 in the tumor together with the absence of tumor infiltrating lymphocytes has been associated with increased recurrence in cases of solitary non-invasive bladder cancer [43]. Coleman and Hansel have reviewed in-depth the use of diagnostic and prognostic markers in UC and highlight three important aspects related to its application in UC: (a) currently, the biomarkers are used within panels; (b) many of the markers are specific for a subtype of UC; and (c) the use of a diagnostic marker should be used in combination with light microscopy for confirmation [38].

Our group reported that Wnt5a staining intensity correlated positively with histological grade and stage of the UC; even more, our results suggest that Wnt5a, acting through the PCP pathway, could be involved in the metastasis process in UC [35,36]. Interestingly, Sin ML et al., using RNA detection from exfoliated cells in the urine, described Wnt5a together with ROBO1 and CDC42BPB as potential important markers for UC [44].

All the findings described above suggest that further research in this area is warranted, not only to identify novel prognostic markers to characterize subtypes and predict the clinical outcome of UC but also to understand in-depth the molecular mechanisms involved in the carcinogenesis of UC in order to develop novel therapeutic strategies.

Wnt5a signaling as a therapeutic target

Currently, there is a large amount of research focused on modulating Wnt5a signaling as a potential therapeutic

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Page 5/8

target for those diseases in which this signaling is involved [45]. At least four methods have been used to modulate the Wnt signaling: antibodies, RNA mediators, polypeptides and chemicals. Different targets in the signaling pathway, such as Wnt ligands, Fzd receptors and co-receptors, have been targeted for signaling inhibition or signaling enhancement. As was previously mentioned, a major limitation on modulating Wnt5a is the complexity of its signaling in vivo.

There is some evidence that modulation of Wnt5a signaling could be a novel target for therapeutic drug development [46,47]. Using antisense Wnt5a and dominant negative Wnt5a vectors or anti-Fz5 antibodies, Sen et al. described decreased activation of Wnt5a signaling in rheumatoid synoviocyte activation as a potential therapeutic target for rheumatoid arthritis [48]. More recently, use of siRNA to silence Wnt5a was shown to delay atherosclerosis development in an animal model [49]. Due to the variety of roles that Wnt5a plays in physiological processes, it is critical to avoid off-target effects when this signaling pathway is manipulated.

Conclusions, Remarks and Future Perspectives

As summarized above, research on new molecular pathways involved in UC is critical in order to find new targets for diagnosis, prognosis and treatment of UC. This review emphasized the involvement of Wnt5a in the development and progression of UC. Further research is needed to explore the exact role of Wnt5a, which is likely to be broad and complex. The complexity of the Wnt5a signaling pathway results from many factors, such as the large number of receptors and co-receptors involved in a variety of downstream effects, modulation of Wnt signaling by the tumor microenvironment, and finally the cross-talk of Wnt signaling with other cellular signaling pathways. In this scenario it is critical to develop animal models in which this complexity can be addressed. Targeting Wnt signaling pathways may be useful in treatment of UC. However, it is important to avoid off-target effects associated with manipulation of the Wnt5a

signaling pathway.

Figure 1 Roles of Wnt5a Signaling Pathways in the UC Cell

The diagram represents a summary of some of the signaling pathways activated by Wnt5a in the context of tumor microenvironment with likely effects on tumorigenesis and cancer progression. UC cell, urothelial carcinoma cell; TAM, tumor associated macrophage; DVL, disheveled; FlnA, filamin A; G, G protein; JNK, c-Jun N terminal protein kinase; RHOA, rho-associated kinase; NFAT, nuclear transcription factor of activated T cell; NLK, Nemo-like kinase; NFκB nuclear transcription factor κB.

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