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REVIEW ARTICLE

Epithelial to Mesenchymal Transition in Renal Cell Carcinoma: Implications for Cancer Therapy

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Abstract Epithelial-to-mesenchymal transition (EMT) is a developmentally vital reversible process by which fully differentiated cells lose their epithelial features and acquire a migratory mesenchymal phenotype. EMT contributes to the metastatic potential of tumors. The expression profile and other biological properties of EMT suggest potential targets for cancer therapy, including in renal-cell carcinoma (RCC). The preclinical and clinical results have substantiated the promises that dysregulated elements leading to EMT can be a potential target in RCC patients. In this study, we illustrated the pathogenic and prognostic role of EMT in RCC. In addition, we reconstructed, by literature analysis, the different pathways implicated in the EMT process, thus supporting the rational for future EMT-directed therapeutic approaches for RCC patients.

Key points

Epithelial-to-mesenchymal transition (EMT) is a reversible process which leads to the loss of epithelial features and to the acquisition of a mesenchymal phenotype.

EMT plays a crucial role in RCC development and progression.

Targeting EMT components seems to represent an attractive therapeutic strategy in RCC patients.

1 Introduction

Renal-cell carcinoma (RCC) is the most common tumor of the kidney, clear-cell RCC (ccRCC) being the most frequent histological subtype. RCC is an immunogenic tumor and is characterized by chemo- and radio-resistance. Approximately 30 % of RCC tumors are already metastatic at initial diagnosis, with other 30–40 % of patients who develop distant metastases after initial nephrectomy [1].

Metastatic RCC dissemination is often initiated by reactivating an embryonic development program, such as the epithelial-to-mesenchymal-transition (EMT) [2]. Through the EMT process, fully differentiated cells lose their cell polarity and cell–cell adhesion features and gain a migratory and invasive mesenchymal phenotype. Loss of E-cadherin is considered to be a crucial event in EMT. This is the result of the increased expression of transcriptional repressors of E-cadherin expression, including ZEB1,

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ZEB2, Twist, Snail, and Slug. The existence of a dynamic and reversible EMT process suggests that epigenetic shifts are also involved in the acquisition of an alternative phenotype.

EMT plays an essential role in RCC pathogenesis, invasion and response to therapies. It has also been shown that EMT correlated with an increased recurrence risk and worst overall survival (OS) in patients with RCC [3]. Thus, targeting the molecular elements that give rise to this process can represent a key to improve the outcome of RCC patients.

In this study, we described the pathogenic, prognostic and therapeutic role of EMT. Furthermore, we reconstructed the different pathways, obtained by literature analysis, that lead to EMT in RCC, thus suggesting the rational for future EMT-tailored therapeutic strategies for these patients.

2 Role of EMT in RCC Development

During kidney development, the Wilms' tumor transcription factor 1 (WT1), which is not expressed in adult kidney, orchestrates the mesenchymal-to-epithelial transition (MET). However, in RCC it can induce an epithelial–mesenchymal hybrid transition (EMHT), marked out by both EMT and MET features (i.e. up-regulated Snail and maintained E-cadherin) and associated with tumor progression [4].

The inactivation of the von Hippel–Lindau (*VHL*) tumor suppressor gene that degrades hypoxia inducible factor 1α (HIF1α) constitutes a crucial step in RCC carcinogenesis. Interestingly, VHL loss induced EMT that is largely dependent on HIF1α-induced nuclear factor kappa B (NF-κB) [5]. However, additional genetic and epigenetic alterations are required for the malignant transformation to RCC.

It has been shown that chronic oxidative stress induces malignant transformation of renal epithelial cells via the acquisition of stem cell and EMT characteristics [6]. Huang and colleagues revealed that the down-regulation of miR-30c, a miRNA that is commonly repressed in several tumor types, by hypoxia led to overexpressed Slug and repressed E-cadherin, subsequently promoting EMT and cell migration [7]. However, the mechanisms by which hypoxia induces EMT have still to be completely clarified.

3 EMT in RCC Metastasis

EMT contributes to RCC metastasization by modifying tumor cell polarity and cell–cell adhesion and by increasing tumor migration and invasion. On this scenario, inflammation and genetic alterations, such as FOXO3A loss, are involved in modulating the role of EMT in RCC dissemination.

3.1 Role of Inflammation

Pro-inflammatory cytokines and chemokines contribute to RCC tumorigenesis by facilitating cancer proliferation and metastasis. Tumor necrosis factor (TNF-α) is produced by a number of cancer cells, including RCC. The serum levels of TNF-α correlated with tumor size in RCC [8, 9]. In addition, TNF-α has been shown to induce EMT [by inhibiting glycogen synthase kinase 3β (GSK-3β) activity] and promote RCC metastasization by suppressing E-cadherin expression, up-regulating vimentin expression and activating Matrix metalloproteinase 9 (MMP9) [10].

As for interleukin-15 (IL-15), it is a powerful immunomodulatory factor with structural similarity to IL-2. RCC expresses a particular form of membrane-bound IL-15 (mb-IL-15) that seems to be directly involved in RCC progression. Indeed, its stimulation via the soluble IL-15 receptor α (s-IL-15Rα) induces EMT in renal cancer cells [11].

Also EMT-related microRNA-200 family, which includes miR-200a/b/c, miR-141 and miR-429, is involved in RCC, in which they act as tumor-suppressive miRNAs and are frequently down-regulated [12].

3.2 Role of FOXO3A Loss

Ni et al. investigated the role of FOXO3A in ccRCC metastasis. They observed that the loss of FOXO3A induced EMT of tumor cells by up-regulating Snail, which promoted tumor cells metastasis in vitro and in vivo [13]. Snail indirectly increases MUC1 expression, which also contributes to EMT [14]. Snail protein expression levels were significantly associated with pathological tumor stage and histological grade, as well as with the presence of sarcomatoid differentiation [15]. In fact, EMT orchestrates the sarcomatoid conversion of ccRCC, characterized by E-to N-cadherin switching, dissociation of β-catenin from the membrane, and increased expression of Snail and Sparc [16, 17].

4 Prognostic Role of EMT in RCC Patients

EMT seems to correlate with the outcome of RCC patients. In a series of 47 RCC patients who underwent nephrectomy, the rate of spindle-cell EMT was 96.4 % in patients with shorter OS (3–6 months), whilst it was 42.1 % in patients with longer OS (>6 months), supporting the use of spindle-cell EMT rate as a prognostic factor in RCC [18].

Harada et al. [19] evaluated by immunohistochemistry the expression of 11 EMT markers in a series of specimens from 122 patients who underwent radical nephrectomy for clinically localized RCC. They found that the expression levels of Clusterin and Twist, as well as C-reactive protein (CRP) and microvascular invasion independently predicted disease recurrence at multivariate analysis. Significant differences were found in terms of recurrence-free survival based on the number of positive independent factors present in each patient. Indeed, disease recurrence occurred in 7.7 % of patients negative for any risk factor, 31.5 % of patients with one or two risk factors and 60.9 % of patients with three or four risk factors [19]. In addition, a better outcome was observed in patients with low levels of CXCR4, Vimentin, Fibronectin and TWIST1 mRNA [20].

ZEB2 is implicated in the modulation of EMT in RCC. When down-regulated, ZEB2 decreases RCC migration and invasion in vitro [21]. In addition, Fang and colleagues assessed ZEB2 expression by IHC and western blot analyses in 116 RCC patients treated with radical nephrectomy. They found that high ZEB2 expression correlated with worst OS and progression-free survival (PFS) [21]. Similarly, high Snail expression is more frequent in high-grade RCC and correlated with worse disease-free and disease-specific survival in these patients [15].

5 EMT and the Response to Targeted Agents

Vascular endothelial growth factor receptor-tyrosine kinase inhibitors (VEGFR-TKIs) and the inhibitors of the mammalian target of rapamycin (mTOR) have dramatically revolutionized the therapeutic scenario of metastatic RCC. However, patients progress along treatments, and the rate of complete responses remains poor [22].

The mechanisms of primary and acquired resistance to TKIs are poorly understood. The close association of the reversible EMT process with both cancer stem cells (CSCs) [3] and tumor-related inflammation [23] may also lead to the development of acquired resistance [24]. Indeed, Hammers et al. revealed that the resistance to sunitinib, a VEGFR-TKI inhibitor, was reversible and EMT-dependent in xenografts from a patient progressed during this agent [25].

5.1 Reconstructed EMT Pathway in RCC

Figure 1 shows the pathway reconstruction of EMT in RCC obtained by literature analysis. There are different pathways, interconnected among them, that lead to EMT. One of them originates from Wnt signaling, that promotes transcription of Snail by blocking GSK-3β and increasing β-catenin.

HIF1α transcription factor transcriptionally activates C-MET by binding its promoter and enhances C-MET pathway. This activates pathways of cellular proliferation, motility, migration and invasion. For example, the downstream Slug transcription factor represses E-cadherin transcription, causing desmosomal disruption and cell spreading. The downstream STAT3 transcription factor has multiple roles promoting tumor cell proliferation, survival, invasion, angiogenesis, immune evasion, regulating epigenetic mechanisms and mitochondrion functions. Vimentin, another protein activated by C-MET pathway, is a cytoskeletal structural protein associated with increased migratory and invasive capacity [26]. HIF1α transcription factor promotes MUC1 transcriptional up-regulation by binding with two HRE cis-elements within the MUC1 promoter [27]. MUC1 can functionally suppress E-cadherin in breast cancer cell lines [28] and suppress E-cadherin protein expression in pancreatic and breast cell lines [29]. E-cadherin expression is repressed also by Snail that directly binds E-cadherin promoter in the renal carcinoma cell lines [14]. In human pancreatic cancer cell lines, MUC1 enhances the Wnt/β-catenin signaling pathway probably because MUC1 C-terminal domain (MUC1-C) enhances the activity of β-catenin [30] and promotes the localization of β-catenin on the Snail promoter [14]. MUC1-C and β-catenin increase Snail transcription [14], whereas *Snail* does not directly interact with MUC1 promoter (dash line in Fig. 1) [14]. Snail transcription factor represses epithelial maintenance genes as occludin, desmoplakin and E-cadherin so causing adherens junction disassembly and EMT. Snail promotes EMT also increasing both directly the transcription of vimentin, fibronectin and N-cadherin and, indirectly, of mucin 1 (MUC1), Nanog, gastrin, ornithine decarboxylase 1 (ODC1), hyaluronan synthase 2 (HAS2) and thrombospondin-1 (THBS1). In particular, Nanog transcription factor causes the oncogenic reprogramming and confers cancer cells a stem-like phenotype, as self-renewal and long-term proliferative ability. ODC1 enhances transcription, translation and replication, gastrin is implicated in resistance to apoptosis and neoplastic transformation, HAS2 induces migratory phenotype and anchorage-independent growth, THBS1 is a glycoprotein that mediates cell interactions with other cells or matrix, so is involved in cell adhesion and angiogenesis. Signaling from GF receptor, through PI3K/AKT, activates MDM2 proto-oncogene that, by ubiquitination, blocks p53 [31], reduces miR-192 transcription, increases the level of ZEB2. It is zinc-finger transcription factor that represses E-cadherin, causing trans-endothelial migration [32, 33]. Secondly PI3K/AKT activates mTORC1, c-Myc, Cyclin D1, Nibrin (NBN) and ODC1 and represses p27, Bad, FOXO and GSK-3β, leading to cell proliferation and survival. Moreover, protein kinase D1 (PKD1), by phosphorylation, and E3 ubiquitin ligases SCF-FBXO11, by ubiquitination, target and block Snail in RCC [34]. The integrin-linked kinase (ILK) has oncogenic effect
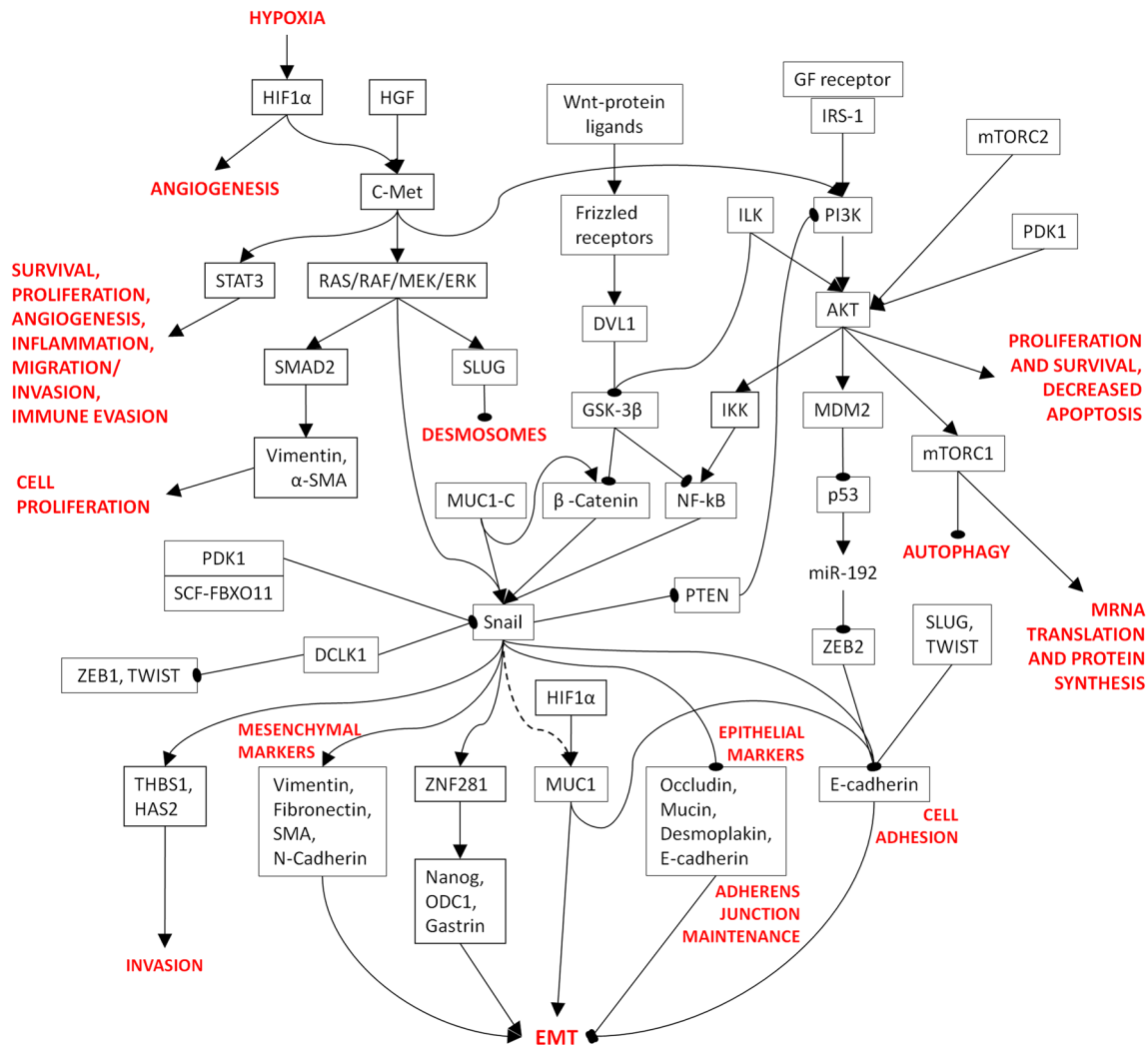
Fig. 1 Pathway reconstruction of EMT obtained by literature analysis shows the different pathways, interconnected among them, that lead to EMT in RCC. *DCLK1* doublecortin-like kinase 1, *EMT* epithelial–mesenchymal transition, ILK integrin-linked kinase, *MUC1* mucin 1, *NBN* nibrin, *ODC1* ornithine decarboxylase 1, *PKD1* protein kinase D1, *RCC* renal-cell carcinoma. See *text* for additional abbreviations. *Dash line* means an indirect interaction

by activating AKT and inhibiting GSK-3β, this last stabilizes the proto-oncogenic β-Catenin [35]. In fact, in vivo knockdown of ILK in nude mice down-regulated EMT markers such as Snail, ZEB1, vimentin, and E-cadherin [36]. Also doublecortin-like kinase 1 (DCLK1) is involved in renal EMT since following its knockdown, SNAI1, SNAI2, TWIST1 and ZEB1 were significantly decreased [3].

6 Potential Therapeutic Targets for EMT in RCC Patients

Current therapeutic strategies for metastatic RCC patients are focused on the role of angiogenesis in the carcinogenesis and progression of this tumor. In the last decade, several agents targeting VEGF (bevacizumab), VEGF receptor (sorafenib, sunitinib, pazopanib, axitinib), mTOR pathway (temsirolimus and everolimus) and, more recently, programmed death-1 (PD-1) immunocheckpoint, have been developed for these patients.

Based on the role of EMT in RCC progression and drug resistance, targeting EMT may represent an effective strategy in this context. Although present data do not allow a clear comprehension of the best setting for EMT-tailored approaches, several targets such as Snail, DCLK1, ILK and miRNAs seem to merit careful consideration as potential future targets for RCC patients.

6.1 Snail

Snail is implicated in the EMT process and correlates with stemness and with the ability of RCC to invade and

metastasize [37]. Zheng et al. [34] investigated the role of SCF-FBXO11, an E3 ligase that is able to ubiquitinate and degrade Snail. They found that FBXO11-induced degradation of Snail is dependent on PKD1 phosphorylation of Snail. As a consequence EMT, tumor development and metastatis were inhibited in breast cancer models [34], supporting the potential of Snail-targeted approaches also in RCC patients.

6.2 DCLK1 and ILK

DCLK1 is a serine/threonine kinase involved in microtubule-mediated neuronal migration and morphogenesis [38]. DCLK1 is overexpressed and dysregulated in over 93 % of RCC tumors [39] and its knockdown of DCLK1 by siRNA leads to a decreased expression of EMT and CSC markers. This event is associated with inhibited invasion, migration and drug resistance.

Concerning ILK, it is a serine/threonine kinase implicated in modulating cancer cell growth, survival and invasion, as well as EMT and tumor angiogenesis [35]. In nude mice, ILK knockdown reduced the invasion and metastasis of primary RCC by down-regulating EMT markers [36]. These data support the potential of DCLK1- and ILK-targeted therapeutic strategies in RCC patients [3].

6.3 miRNAs

Recent studies suggest that miRNAs act as regulators of RCC metastasis and are implicated in EMT. Among them, miR-21 [40], miR-145 [41], miR-629 [42], miR-22 [43], miR-134 [44], miR-141 [45], miR200s [12], miR-218 [46] and miR-30c [7] seem to play a crucial role in modulating EMT in RCC. However, no data are yet available on their use in patients with RCC.

6.4 Immunotherapy

Based on the evidence that TNF-α induces EMT, the efficacy and safety of anti-human TNF-α mAb infliximab should be investigated in patients stratified by the expression of EMT markers. In addition, the relationship between IL-15 and EMT suggests that individualized immunotherapeutic approaches should be evaluated in patients with high expression of EMT markers.

6.5 Natural Compounds

Among emerging natural compounds, honokiol, isolated from *Magnolia* spp. bark, has been found to suppress RCC metastasis via dual-blocking EMT and CSC properties.

Indeed, honokiol could up-regulate miR-141, which targets and modulates ZEB2 expression [45]. The use of honokiol in RCC patients seems to merit further evaluation.

7 Discussion

EMT is involved in a variety of cancer cell functions, thus representing a promising therapeutic target for RCC. Our results showed that EMT is a multifactorial process that involves several pathways, such as Wnt signaling and PI3K/AKT/mTOR axis, as well as DCLK1, PKD1 and ILK kinases, interleukins, growth factors and miRNAs. The complexity of this scenario makes the identification of a single key factor more challenging. However, research to delineate the precise role of EMT in RCC carcinogenesis and metastasis and within the therapeutic armamentarium for patients with RCC remains encouraging.

Testing EMT by immunohistochemistry and gene expression arrays should be further investigated and validated for patients with metastatic disease. Based on the role of EMT in RCC metastatic spread and on its relationship with disease free survival (DFS), EMT markers should be tested by pathologists also in patients who underwent nephrectomy for localized RCC, in order to exploit them as predictive markers of recurrence. However, further clinical studies are still required in order to state these markers as predictive factors for disease recurrence.

Furthermore, the correlation between the expression of EMT markers and the development of acquired reversible resistance to VEGFR-TKIs support the need to evaluate the role of EMT testing in guiding the selection of the best therapeutic sequence (VEGFR-TKI—VEGFR-TKI or VEGFR-TKI—mTOR inhibitor) for individual RCC patients.

In summary, the recent discoveries on the EMT process have shed new light on the role of this pathway in RCC. If modulating EMT is to become a viable strategy, a better perception of the EMT process is required in order to guide clinicians in the selection of the most appropriate therapeutic approach for an individual patient. These strategies could include synergic drug combinations, obtained by the association of anti-angiogenic agents or immunotherapy with EMT-targeting approaches in order to reduce the development of drug resistance and optimize patient outcome. Due to the present low rate of complete responses in patients with metastatic RCC treated with targeted agents, our increasing understanding of the pathogenic mechanisms underlying this tumor brings hope for new strategies to target the key features that make RCC such a difficult disease to treat.

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