Expression and Prognostic Significance of a Comprehensive Epithelial-Mesenchymal Transition Gene Set in Renal Cell Carcinoma

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Purpose: Epithelial-mesenchymal transition enhances tumor cell motility and has a critical role in invasion and metastasis in a number of carcinomas. A set of transcription factors acts as a master regulator of the epithelial-mesenchymal transition process. To our knowledge it is unknown whether epithelial-mesenchymal transition is important for clear cell renal cell carcinoma progression. Therefore, we comprehensively assessed mRNA levels of epithelial-mesenchymal transition associated genes in renal cell carcinoma as well as their prognostic relevance.

Materials and Methods: We determined the expression of a set of 46 epithelial-mesenchymal transition related genes by oligonucleotide microarray and gene set enrichment analyses using RNA from 14 samples each of normal kidneys, and G1 and G3 primary renal cell carcinomas. Expression of select epithelial-mesenchymal transition genes was validated by real-time polymerase chain reaction in normal kidneys, primary renal cell carcinomas and metastases in an independent cohort of 112 patients. Results were combined with followup data for survival analysis.

Results: The epithelial-mesenchymal transition gene set was preferentially expressed in primary renal cell carcinoma compared to normal tissue (false discovery rate 0.01). No difference was found between G1 and G3 tumors. Quantitative reverse transcriptase-polymerase chain reaction revealed down-regulation of critical epithelial-mesenchymal transition genes such as *CDH2* and *ZEB1* in metastases, suggesting epithelial-mesenchymal transition reversal during metastasis. Kaplan-Meier analysis demonstrated a better outcome in patients with low CXCR4, vimentin, fibronectin and TWIST1 mRNA levels. Multivariate analyses revealed that *CXCR4* and *VIM* up-regulation represents an independent prognostic marker for poor cancer specific survival in patients with renal cell carcinoma.

Conclusions: Taken together, our data provide strong evidence that epithelial-mesenchymal transition occurs in renal cell carcinoma. Thus, interference with epithelial-mesenchymal transition in renal cell carcinoma might represent a future therapeutic option.

Key Words: kidney; carcinoma, clear cell; epithelial-mesenchymal transition; prognosis; gene expression

Abbreviations and Acronyms

CXCR = CXC chemokine receptor
EMT = epithelial-mesenchymal transition
ES = enrichment score
fc = fold change
FDR = false discovery rate
FN1 = fibronectin
GSEA = gene set enrichment analysis
MET = mesenchymal-epithelial transition
PCR = polymerase chain reaction
RCC = renal cell carcinoma
TGF-β = transforming growth factor-β
VIM = vimentin
ZEB1 = zinc finger E-box binding homeobox 1

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Carcinoma cells can undergo a characteristic change from an epithelial to a mesenchymal cell-like phenotype called EMT during cancer progression.[1,2] This kind of cell phenotype change can facilitate tumor cell migration and invasion at an early stage of tumor development.[3] There is increasing evidence that the dynamic process of EMT gives rise to tumor cell dissemination and together with MET (the reversal of EMT) finally leads to metastatic outgrowth.[4,5] More recently, it was noted that the EMT phenotype is shared by cancer stem cells.[6] This is clinically highly relevant since cancer stem cells are thought to selectively survive chemotherapy due to drug insensitivity, which can lead to disease relapse.[7] In addition, EMT genes attract interest for their clinical significance as prognosis predictors or potential targets of therapy.[7,8]

EMT is mostly under transcriptional control. Master transcription factors are important, such as ZEB and SNAIL family members, which can induce EMT upon over expression.[9] Hallmarks of EMT that facilitate the invasive phenotype include loss of expression of the cell adhesion molecule E-cadherin, mediated by transcriptional repression of *CDH1* (encoding E-cadherin) through ZEB and SNAIL transcription factors, and induction of extracellular matrix degrading metalloproteinases such as MMP2 and MMP9.[9] The EMT process can be induced by cytokines and growth factors such as TGF-β, which are produced by tumor stroma or possibly tumor cells.[10]

It is widely accepted that EMT has an important role in a number of solid tumors.[1,8] However, it has not been comprehensively investigated in RCC. A few studies of EMT in RCC focused on the expression of single EMT genes or limited sets of EMT related genes mostly at the protein level using immunohistochemistry.[11,12] To our knowledge no extensive quantitative gene expression studies have been done at the mRNA level to assess EMT in RCC. Moreover, to our knowledge the expression of EMT related genes in the metastases of patients with RCC has not been analyzed until now.

Given the essential role of EMT for tumor progression in other types of carcinoma and the limited information available on RCC, we comprehensively analyzed EMT associated gene expression in RCC. Since EMT is a transcriptionally regulated differentiation process, we used transcriptome analysis and real-time reverse transcriptase-PCR to quantify EMT related gene expression. We assembled a list of 46 genes reported to be up-regulated during EMT in different epithelial cancers. Using oligonucleotide microarray data we evaluated the preferential expression of EMT genes in primary RCC compared to normal kidneys. A panel of 13 genes from the gene list was validated by real-time PCR using tissues from a different cohort of patients with long-term followup. By comparing normal kidney, primary RCC and metastases, and correlating results with patient cancer specific survival we identified the differential expression pattern of EMT genes during tumor progression as well as their potential as independent outcome predictors for RCC.

PATIENTS, MATERIALS AND METHODS

Patient Tissues

Primary tumors and metastasis samples were collected from patients with clear cell RCC undergoing surgical resection of RCC at one of 2 centers. A total of 42 samples, including 14 normal tissues from tumor bearing kidneys and 28 primary RCCs (14 each of grade G1 and G3) were used for microarray analysis, which was done at the University of Rostock department of urology. Another 112 tissue samples from a total of 82 patients with clear cell RCC were used for survival analysis by real-time PCR, including 19 normal tissues from tumor bearing kidneys, 55 primary RCCs and 38 metastases from various sites. One tissue sample from each patient and site was analyzed.

Patients underwent tumor nephrectomy, partial nephrectomy or metastasis resection between 1992 and 2011 at the University of Munich department of urology. Table 1 lists patient characteristics. Median followup was 54 months. Staging was performed according to the TNM classification.[13] Normal tissues were taken from tumor bearing kidneys and confirmed by a pathologist to be histologically normal. All patients provided informed consent and the research was approved by the local ethics committee.

Table1. *Characteristics of Munich patient cohort*

	No. Primary Tumors (%)	No. Metastases (%)
T stage:		
T1-T2	29 (52.7)	12 (31.6)
T3-T4	26 (47.3)	26 (68.4)
N stage:		
N0	41 (74.5)	22 (57.9)
N+	10 (18.2)	11 (28.9)
NX	4 (7.3)	5 (13.2)
Metastasis:		
M0	26 (47.3)	16 (42.1)
M1	29 (52.7)	22 (57.9)
Grade:		
G1-G2	45 (81.8)	22 (57.9)
G3	10 (18.2)	16 (42.1)
Age:		
Less than 60	22 (40.0)	23 (60.5)
60 or Greater	33 (60.0)	15 (39.5)
Male	32 (58.2)	23 (60.5)
Female	23 (41.8)	15 (39.5)
Metastatic site:	—	
Adrenal gland		12 (31.6)
Lymph node		12 (31.6)
Lung		7 (18.4)
Liver		4 (10.5)
Vena cava		2 (5.3)
Brain		1 (2.6)