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Involvement of epithelial-mesenchymal transition in urinary bladder cancer progression. A review

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Abstract
Epithelial-mesenchymal transition (EMT) is a morphogenetical process involved in embryogenesis and - as evidenced by a growing amount of reports - in the progression and metastasation of at least a subset of human malignant tumours. This review gives a brief description of the current EMT concept and summarizes findings relevant to urinary bladder cancer.

Key words: epithelial-mesenchymal transition, urinary bladder cancer, progression, metastasis

BACKGROUND

Epithelia are dynamic structures undergoing tissue growth, differentiation, cell division and apoptosis, yet maintaining tissue integrity and function. Epithelia are organized as sheets of cells attached firmly to an underlying extracellular matrix (ECM) called basement membrane. They are polarized along an apical-basal axis and connected to each other by multiple cell-cell adhesive junctions (1). These ensure mechanical integrity of epithelium and include adherens junctions (zonula adhaerens) and desmosomes (macula adhaerens). Both types of cell junctions recruit a protein named E-cadherin, which is central to the current EMT concept. Loss of E-cadherin results in disappearance of epithelial features and is observed in many human cancers (2). Mesenchymal cells do not form a cell layer neither are they attached firmly to a basement membrane. Instead, they contact other mesenchymal cells only focally and can migrate either as groups of cells or as individual cells through ECM. They exhibit a spindle-shaped, fibroblast-like morphology and front-back polarity (3).

Epithelial-mesenchymal transition (EMT) is a process, which allows to form more complex structures during the development of many species, including the human embryo. At the beginning of embryogenesis, it underlies germ layer reorganization and later on, plays a role in organogenesis. It has its counterpart in a process of mesenchymal-epithelial transition (MET) e.g. in kidney development. It is vital that the mechanisms involved in EMT occur in the correct place, at the right time and in the right sequence. They include specification of a cell to undergo EMT, disruption of the basement membrane, change in cell shape, withdrawal from the epithelial sheet, and differentiation to a mesenchymal cell (4). According to a growing amount of evidence, aberrant activation of EMT plays a role in many pathological processes including tumour metastasis. Although some authors deny the

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very existence of EMT (5), others imply that tumour progression is a tightly regulated morphogenetic process recapitulating early embryo- and histogenesis (6).

METHODS

The relevant articles were retrieved by a search in the PubMed database using search terms „urinary bladder cancer" or „urothelial carcinoma" and „epithelial-mesenchymal transition" without any time limit for the search.

RESULTS AND DISCUSSION

It is important to note that currently, no specific EMT marker exists. Cells considered to be undergoing EMT are determined as such based on their morphology, loss or acquisition of certain protein markers characteristic of either phenotype and by evidence of transcription of specific regulatory genes.
Morphological features defining a mesenchymal cell are arbitrary and have been postulated by EMT experts in 2003. They include: front end-back end polarity, elongate morphology, filopodia (front end cell membrane prominences interacting with the surrounding ECM) and invasive motility (2).
Cadherins (for calcium-dependent) are transmembrane glycoproteins responsible for cell-cell adhesion. Classic cadherin types E, N and P are composed of an extracellular region, a transmembrane region and an intracellular carboxy-terminal region. The cytoplasmic domain binds catenins (α, β and γ), which mediate the connection between epithelial E-cadherin and cytoskeletal protein F-actin in adherens junctions. Impaired cadherin or catenin function leads to loss of adhesion, a crucial moment in the initiation of EMT. While E-cadherin is one of the hallmarks of an epithelial phenotype, N-cadherin was found in fibroblasts and muscle, but not epithelial cells. A so called „cadherin switch" (from E- to N-cadherin) is typical of EMT. P-cadherin is localized to the basal cell compartment and its role in tumorigenesis remains unclear (7, 8).
One of the first relevant studies noted that loss of E-cadherin was associated with urinary bladder cancer grade and muscle invasiveness (9). The same was reported by Baumgart who also included N-cadherin to the investigation but found no relationship with tumour characteristics (10). E-cadherin immunohisto-chemical staining was present in 100% G1 human bladder carcinomas, the percentage decreased with grade 2 and 3. No G1 tumour expressed N-cadherin but its expression increased with grade and invasiveness. P-cadherin was confined to the basal cell layer in G1 and G2 tumours and dispersed throughout tumour tissue in G3 specimens. In one pT1 G2 lesion, all three cadherins were present (7). E-cadherin and N-cadherin were associated with grade and stage whereas P-cadherin was not in a recent study (8). N-cadherin expression detection rate was as high as 70% here by qRT-PCR, opposed to 39% on classical sections by immunohistochemistry (7) and 8% only by tissue microarray (TMA) (10); this may be due to the fact that N-cadherin expression is focal in tumours and TMA only processes a small amount of tissue. Interaction forces between two E-cadherin molecules are reported to be 200 nanoNewtons. Force required to separate N-cadherins is allegedly four times smaller while no detectable interaction exists between E- and N-cadherin molecules (11).
Catenin as well as E-cadherin staining outlines intercellular borders. This is because α and β catenins bind E-cadherin to the cytoskeleton. It is a common observation, that both E-cadherin and catenins in tumours show heterogenous rather than absent staining. E-cadherin and β and γ catenins expression were inversely correlated with grade and stage (10, 12). All had a similar prognostic value in bladder cancer in the study by Shimazui, but E-cadherin was not a predictor of survival in

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recent studies (10, 13). The role of β-catenin in colorectal cancer was thoroughly studied by Brabletz et al. Whereas membranous E-cadherin staining was present in central tumour areas and absent at the invasive front of the tumour, β-catenin staining was membranous and cytoplasmic in central areas, but predominantly nuclear at the invasive front (14). Intracellular distribution of β-catenin impacts the cell phenotype and behaviour. When cytoplasmic β-catenin is increased, it associates with DNA binding proteins of LEF/TCF1 family, is transported to the nucleus and regulates transcription of EMT-related genes (slug, uPAR, c-jun, VEGF, FGF-2, fibronectin, VEGF) (6). LEF/TCF (lymphoid enhancer factor/T cell factor) are transcription factors partnering with other regulators (β-catenin, SMAD) to alter E-cadherin expression (1). Colorectal cancer cell line LIM1863 cells form three-dimensional spherical structures where central lumen is surrounded by differentiated columnar and goblet cells while undifferentiated cells are located at the periphery of the spheres. Any cell type removed and cultured was able to reorganize to identical spherical so called organoids. (15).

Plakoglobin (=γ-catenin) is found in desmosomes. It was associated with grade and stage of bladder tumours and also with E-cadherin expression and survival (10, 12).

Novel expression of vimentin was increased in invasive and grade 3 bladder tumours compared to superficial and G1+G2 cancers (10). Vimentin expression (as well as that of ZEB1, SIP1, metalloproteinase MMP-2 and MMP-9) was increased in muscle-invasive compared to superficial tumours (16).

Fibroblast growth factor receptor (FGFR) family plays a role in cellular differentiation and tumour phenotype in bladder cancer. Bladder tumours with low E-cadherin expression had low levels of FGFR2b, too (9). In a bladder carcinoma cell line TSU-Pr1, a reduction of FGFR2IIIc expression caused a switch to a mesenchymal-like morphology (17).

Epidermal growth factor receptor (EGFR) has been correlated with the transition from a superficial to invasive bladder cancer. Its two specific ligands, EGF and TGFα are significantly more present in malignant than normal bladder tissue samples. In a human bladder carcinoma cell line CAL 29 they were able to induce EMT (as evidenced by novel vimentin expression) and cell scattering. Scattering, but not EMT could be elicited also by HGF (hepatocyte growth factor, also known as scatter factor, SF) and aFGF (acidic fibroblast growth factor, also known as FGF-1). This study was interesting in that only 6% of TGFα-treated cells became vimentin positive, which might reflect a real-life situation where only a small number of tumour cells are destined to undergo EMT (18).

While the description of cellular markers of EMT is quite easy, the molecular pathways and genes involved in EMT create an almost impenetrable jungle of interconnected, mutually dependent factors and cascades, which are difficult to understand for a non-expert. The mosaic is only slowly assembling and today's knowledge of the diverse implicated players is fragmented. Let us try to describe the most important points.

A loss or impaired function of E-cadherin is considered to be sufficient in triggering changes in cell shape and behaviour associated with EMT. Therefore, factors and genes implicated in E-cadherin signaling are at center of researchers' interest. The reader can most often encounter the following.

Twist is a basic helix-loop-helix (hence the alternative name bHLH) transcriptional activator that mediates its effect via E-boxes of target genes (the same is true for snail, slug and ZEB; see below). It is expressed in neural crest cells during embryogenesis. It promotes loss of epithelial markers including E-cadherin, expression of mesenchymal markers such as N-cadherin and intravasation of cells (1). It was increased in bladder tumour tissues compared with nonmalignant controls with a correlation between grade, stage and staining intensity. Twist specifically enhances intravasation step of metastasis and is involved in acquired cisplatin resistance in advanced bladder cancer. Its antiapoptotic effect is based on antagonising the p53 pathways (19).

Snail, a zinc-finger transcriptional repressor, plays key roles in epithelial remodeling in

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many organisms. It cooperates with FGF signaling in the induction of EMT (1). Snail blocks cell-cycle progression by repressing CyclinD expression and increasing the expression of cell-cycle inhibitor p21; it promotes angiogenesis and resistance to apoptosis (20). Glycogen synthase kinase 3β (GSK-3β)-mediated phosphorylation of snail leads to its nuclear export, increased ubiquitination and proteolytic destruction (11). Snail was demonstrated at the invasive front of colorectal tumours and in gastric, mammary and endometrial neoplasms (11).

Slug (also called Snail2) is a member of the same family. Its action is mediated by a target gene RhoB, otherwise involved in cell motility (3).

Snail, but not slug was associated with bladder tumour recurrence (21). In another study, slug but not snail was a predictor of survival and twist was associated with grade, stage and prognosis (22).

ZEB1 (=δEF1) was described as a transcriptional repressor of E-cadherin and other regulators of epithelial phenotype and cell polarity in vitro; in vivo, it is associated with progression of colon, lung and endometrioid carcinoma (23). No ZEB1 expression was found in normal urothelial tissue compared to 22% positivity in carcinomatous tissue; staining showed association with neither clinical and pathological variables nor alternative EMT markers (E-cadherin, plakoglobin and vimentin) (24). Another ZEB family member, SIP1 (=ZEB2) also binds with E-boxes of E-cadherin gene repressing its transcription, as well as that of other junctional complexes and cell polarity genes. In normal urothelium ZEB1 and SIP1 expression was absent; 7,5% and 24% of bladder tumours expressed ZEB1 and SIP1, respectively. Both correlated inversely with E-cadherin expression and SIP1 correlated with cancer-specific survival (13).

Rb tumour suppressor protein regulates E-cadherin expression. Rb associates with the promoter region of E-cadherin gene together with E-cadherin promoting factor AP-2α; Rb depletion induces Slug and ZEB1 expression. In breast cancer cells, down-regulation of Rb expression was associated with decrease in E-cadherin and mesenchymal conversion of cancer cells (25).

Integrin-linked kinase (ILK) participates in signaling cascades via phosphorylation of protein kinase Akt and GSK-3β resulting in E-cadherin repression. ILK was only marginally expressed in normal mouse urothelium but strongly expressed in invasive bladder cancer, particularly at the invasive front. In vitro, bladder cancer cell lines with elevated ILK expression levels had decreased E-cadherin and vice versa. Increased ILK activity promoted cell invasion, which was inhibited by ILK knockdown (26).

Thymosin β4 was shown to up-regulate ILK and thus decrease E-cadherin expression. Inhibition of thymosin β4 reversed EMT of the cells, inhibited their migration and promoted apoptosis (27). NF-κB is a transcription factor involved in cell-survival and immune response regulation. In response to cytokines, it translocates to the nucleus and mediates expression of target genes including snail, slug, twist and SIP1, promoting E-cadherin down-regulation (1).

E2A proteins reduce E-cadherin, induce invasion and migration and promote EMT (1). Transforming growth factor β (TGFβ) cytokine family bind to transmembrane receptor serine/threonine kinases and via their signaling cascades induce the expression of snail, slug, SIP1 and E2A, thus inducing loss of E-cadherin and triggering EMT (1). TGFβ binds to specific receptors, TGFBR I and II, which phosphorylate SMAD proteins. Phosphorylated SMAD bind with SMAD4 and translocate to the nucleus to activate target genes expression. In bladder cancer cell line T24, TGFβ1 enhanced migration and invasiveness of T24 cells and up-regulated TGFBR I and matrix metalloproteinase MMP-9 (28).

Wnt signalling pathway controls the specification, maintenance and activation of stem cells in many tissues. Activating mutations trigger ligand-independent Wnt signaling implicated in carcinogenesis. Deregulated Wnt leads (via binding Wnt1 and Wnt3a glycoproteins to the Frizzled receptor which produces Dishevelled, an inhibitor of GSK-3β that normaly phosphorylates β-catenin before its ubiquitin-mediated

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destruction) to cytoplasmic accumulation of β-catenin and LEF1/TCF with their subsequent nuclear translocation and gene regulation (1, 2). APC (adenomatous polyposis coli) mutation results in the same effect (6).

CONCLUSIONS

Few carcinoma cell types with an epithelial phenotype are capable of full EMT in vitro (3). In vivo, the study of EMT is more difficult, but will be indispensable in order to clarify whether this transition really occurs in clinical tumour progression. Because of the transient and focal nature of the phenomenon, whole-tumour samples seem to be the best type of material for histopathological and other laboratory investigations. While in colorectal and some other cancers, research has already brought some clues concerning EMT in tumour development, the same is not true in urothelial cancer and other urological malignancies. Therefore, we must conclude our concise review with the mostly over-used cliché stating that „more research in the field is needed". And we mean it.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

References

[1] Baum B, Settleman J, Quinlan MP. Transitions between epithelial and mesenchymal states in development and disease. *Semin Cell Dev Biol.* 2008;19:294–308.

[2] Hay ED. The Mesenchymal Cell, Its Role in the Embryo, and the Remarkable Signaling Mechanisms That Create It. *Dev Dyn.* 2005;233:706–720.

[3] Thiery JP. Epithelial-mesenchymal transitions in tumour progression. *Nat Rev Cancer.* 2002;2(6):442-54.

received July 24th 2011
accepted August 5th 2011
published August 13th 2011

[4] Shook D, Keller R. Mechanisms, mechanics and function of epithelial–mesenchymal transitions in early development. *Mech Dev.* 2003;120:1351-83.

[5] Tarin D. The Fallacy of Epithelial Mesenchymal Transition in Neoplasia. *Cancer Res.* 2005;65:5996-6001.

[6] Brabletz T, Hlubek F, Spaderna S, et al. Invasion and Metastasis in Colorectal Cancer: Epithelial- Mesenchymal Transition, Mesenchymal-Epithelial Transition, Stem Cells and β-Catenin. *Cells Tissues Organs.* 2005;179:56–65.

[7] Rieger-Christ KM, Cain JW, Braasch JW, et al. Expression of Classic Cadherins Type I in Urothelial Neoplastic Progression. *Hum Pathol.* 2001;32:18-23.

[8] Jäger T, Becker M, Eisenhardt A, et al. The prognostic value of cadherin switch in bladder cancer. *Oncol Rep.* 2010;23:1125-32.

[9] De Medina SG, Popov Z, Chopin DK, et al. Relationship between E-cadherin and fibroblast growth factor receptor 2b expression in bladder carcinomas. *Oncogene.* 1999;18: 5722-26.

[10] Baumgart E, Cohen MS, Neto BS, et al. Identification and Prognostic Significance of an Epithelial-Mesenchymal Transition Expression Profile in Human Bladder tumours. *Clin Cancer Res.* 2007;13(6):1685-94.

[11] Lee JM, Dedhar S, Kalluri R, Thompson EW. The epithelial–mesenchymal transition: new insights in signaling, development, and disease. *J Cell Biol.* 2006;172:973-81.

[12] Shimazui T, Schalken JA, Giroldi LA, et al. Prognostic Value of Cadherin-associated Molecules (α-, β-, and γ-Catenins and p120cas) in Bladder tumours. *Cancer Res.* 1996;56:4154-8.

[13] Sayan AE, Griffithsa TR, Pala R, et al. SIP1 protein protects cells from DNA damage-induced apoptosis and has independent prognostic value in bladder cancer. *PNAS.* 2009;35(106):14884–9.

[14] Brabletz T, Jung A, Simone Reu S, et al. Variable β-catenin expression in colorectal cancers indicates tumour progression driven by the tumour environment. *PNAS.* 2001;98:10356-61.

[15] Vincan E, Brabletz T, Faux MC, Ramsay RG. A Human Three-Dimensional Cell Line Model Allows the Study of Dynamic and Reversible Epithelial-Mesenchymal and Mesenchymal-Epithelial Transition That Underpins Colorectal Carcinogenesis. *Cells Tissues Organs* .2007;185:20–28.

[16] McConkey DJ Lee S, Choi W, et al. Molecular genetics of bladder cancer: Emerging mechanisms of tumour initiation and progression. *Urol Oncol Semin Invest.* 2010;28:429-40.

[17] Chaffer CL, Brennan JP, Slavin JL, Blick T, Thompson EW, Williams ED. Mesenchymal-to- Epithelial Transition Facilitates Bladder Cancer Metastasis: Role of Fibroblast Growth Factor Receptor-2. *Cancer Res.* 2006;66(23):11271-8.

[18] Cattan N, Rochet N, Mazeau C, et al. Establishment of two new human bladder carcinoma cell lines, CAL 29 and CAL 185. Comparative study of cell scattering and epithelial to mesenchyme transition induced by growth factors. *Br J Cancer.* 2001;85(9):1412-17.

[19] Wallerand H, Robert G, Pasticier G, et al. The epithelial-mesenchymal transition-inducing factor TWIST is an attractive target in advanced and/or metastatic bladder and prostate cancers. *Urol Oncol Semin Invest.* 2010;28:473-9.

[20] Nieto MA: Epithelial-Mesenchymal Transitions in development and disease: old views and new perspectives. *Int J Dev Biol.* 2009;53:1541-7.

[21] Bruyere F, Namdarian B, Corcoran NM, et al. Snail expression is an independent predictor of tumour recurrence in superficial bladder cancers. *Urol Oncol: Semin Invest.* 2010;28:591–96.

[22] Yu Q, Zhang K, Wang X, Liu X, Zhang Z. Expression of transcription factors snail, slug, and twist in human bladder carcinoma. *J Exp Clin Cancer Res.* 2010;29:119.

[23] Aigner K, Dampier B, Descovich L, et al. The transcription factor ZEB1 (δEF1) promotes tumour cell dedifferentiation by repressing master regulators of epithelial polarity. *Oncogene* 2007;26:6979–88.

[24] Kenney PA, Wszolek MF, Rieger-Christ KM, et al. Novel ZEB1 expression in bladder tumorigenesis. *BJU Int.* 2010;107:656-63.

[25] Arima Y, Inoue Y, Shibata T, et al. Rb Depletion Results in Deregulation of E-Cadherin and Induction of Cellular Phenotypic Changes that Are Characteristic of the Epithelial-to-Mesenchymal Transition. *Cancer Res.* 2008;68:5104-12.

[26] Matsui Y, Assi K, Ogawa O, et al. The importance of integrin-linked kinase in the regulation of bladder cancer invasion. *Int J Cancer.* 2011, doi: 10.1002/ijc.26008.

[27] Wang ZY, Zeng FQ, Zhu ZH, et al. Evaluation of thymosin 4 in the regulation of epithelial- mesenchymal transformation in urothelial carcinoma. *Urol Oncol.* 2010, doi:10.1016/j.urolonc.2010.02.009

[28] Li Y, Yang K, Mao Q, Zheng X, Kong D, Xie L. Inhibition of TGF-b receptor I by siRNA suppresses the motility and invasiveness of T24 bladder cancer cells via modulation of integrins and matrix metalloproteinase. *Int Urol Nephrol.* 2010;42:315-23.

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