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Role of epithelial-to-mesenchymal transition (EMT) in drug sensitivity and metastasis in bladder cancer

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Abstract

Epithelial-to-mesenchymal transition (EMT) is a process that plays essential roles in development and wound healing that is characterized by loss of homotypic adhesion and cell polarity and increased invasion and migration. At the molecular level, EMT is characterized by loss of E-cadherin and increased expression of several transcriptional repressors of E-cadherin expression (Zeb-1, Zeb-2, Twist, Snail, and Slug). Early work established that loss of E-cadherin and increased expression of MMP-9 was associated with a poor clinical outcome in patients with urothelial tumors, suggesting that EMT might also be associated with bladder cancer progression and metastasis. More recently, we have used global gene expression profiling to characterize the molecular heterogeneity in human urothelial cancer cell lines (n=20) and primary patient tumors, and unsupervised clustering analyses revealed that the cells naturally segregate into two discrete “epithelial” and “mes-enchymal” subsets, the latter consisting entirely of muscle-invasive tumors. Importantly, sensitivity to inhibitors of the epidermal growth factor receptor (EGFR) or type-3 fibroblast growth factor receptor (FGFR3) was confined to the “epithelial” subset, and sensitivity to EGFR inhibitors could be reestablished by micro-RNA-mediated molecular reversal of EMT. The results suggest that EMT coordinately regulates drug resistance and muscle invasion/metastasis in urothelial cancer and is a dominant feature of overall cancer biology.

Keywords

Epithelial-to-mesenchymal transition; Epidermal growth factor receptor; Type-3 fibroblast growth factor receptor; Micro-RNA-mediated molecular reversal; Urothelial tumor

1 Epithelial-to-mesenchymal transition-background and relevance to cancer progression

The term epithelial-to-mesenchymal transition (EMT) refers to the complex reprogramming of epithelial cells that occurs on occasion during embryonic development and tissue repair in the adult organism [1]. Epithelial cells mediate important barrier functions and form tight homotypic interactions that contribute to the formation of these permeability barriers. E-cadherin plays a central role through its interaction with β-catenin and the actin cytoskeleton [1]. Thus, the most familiar change that occurs during EMT is downregulation of surface E-cadherin expression, resulting in loss of homotypic adhesion [1]. Other common features of

EMT include loss of basal-apical polarity genes and tight junction molecules and increased expression of vimentin, fibronectin, and the S100 proteins (Fig. 1) [1]. During wound healing, EMT facilitates repair by facilitating epithelial migration to the site of injury, and once repair is underway epithelial cells re-express E-cadherin and other epithelial markers via a process that is sometimes referred to as “mesenchymal-to-epithelial transition” (MET) [1]. There is evidence that cell division and invasion/migration are mutually exclusive processes [2, 3], so MET may be essential not only for reestablishing barrier function but also for enabling the cell proliferation that is required for complete wound closure.

EMT is controlled by a group of transcriptional repressors (Zeb-1, Zeb-2, Twist, Snail, and Slug) that recruit histone deacetylases to E-box elements that are located within the E-cadherin promoter and a variety of other genes [1]. Diverse upstream signals increase the expression of these E-cadherin repressors at the mRNA level. Of particular importance are the transforming growth factor-beta (TGFβ)/bone morphogenic protein (BMP) family of cytokines [1], although a variety of other developmental and inflammatory signals promote EMT as well (Fig. 1a). Upon binding their specific surface receptors, TGFβ and the BMPs promote the direct activation of a family of receptor-linked transcription factors (SMADs) [4], which translocate to the nucleus and regulate their target genes (including Snail) [5] in a phosphorylation-dependent manner. In addition, recent work demonstrated that SMADs 2 and 3 also exert effects on transcription and translation by regulating micro-RNA processing [6]. In normal epithelial cells, TGFβ simultaneously induces EMT by downregulating E-cadherin and upregulating Zeb-1, Zeb-2, and Snail and inhibits cell cycle progression by activating the p16/Rb checkpoint [1]. Increased TGFβ expression is a common feature of solid tumor progression. Although cancer progression often selects for the acquisition of defects on TGFβ signaling, in some cases, the mutations appear to selectively inactivate TGFβ's growth inhibitory effects without interfering with EMT signaling. An excellent example of this can be found in pancreatic cancer cells and other solid tumors that possess defects in SMAD4: recent work demonstrated that it is essential for TGFβ-induced growth inhibition but is dispensable for EMT [7].

Exciting recent work suggests that members of the miR200 family of micro-RNAs play central roles in mediating the effects of TGFβ and other EMT regulators in normal and malignant epithelial cells [8–11]. Members of the family bind directly to the mRNAs encoding Zeb-1 and Zeb-2, promoting their degradation and blocking translation. TGFβ downregulates miR200 family micro-RNAs via poorly characterized mechanism(s), leading to accumulation of Zeb-1 and Zeb-2, suppression of E-cadherin expression, and increased motility and invasiveness. Interestingly, Zeb-1 and Zeb-2 can also suppress miR200 family expression, indicating that feedback loops are in place to fine tune EMT-related signaling.

The parallels between EMT and the processes that underlie various aspects of cancer progression and metastasis have not been lost on cancer researchers [1]. It has long been recognized that E-cadherin expression is decreased in metastatic cancers [12]. Fidler's group demonstrated that loss of E-cadherin accompanied by increased expression of matrix metalloproteinases (which mediate invasion and are markers of EMT) (Fig. 1b) was a better predictor of poor prognosis in several models as compared to downregulation of E-cadherin or upregulation of MMPs alone [13–22] (based on this work, Slaton et al. demonstrated that

a low E-cadherin-to-MMP9 ratio predicted poor outcome in patients with bladder cancer [23]). Studies in preclinical models demonstrated that loss of E-cadherin directly promoted an EMT phenotype and reprogrammed global gene expression [24]. Aside from promoting adhesion-related signaling, E-cadherin re-expression also promotes relocalization of β-catenin, which can function as a transcriptional co-activator, from the nucleus to the plasma membrane [25]. Finally, recent analyses of the changes in micro-RNA expression in a transgenic mouse model of lung cancer demonstrated that downregulation of miR200 family expression plays a major role in disease progression [26]. These observations have stimulated strong interest in defining the molecular mechanisms underlying this epithelial-to-mesenchymal "switch" during tumor progression. Although autocrine or paracrine cytokine (especially TGFβ family) signaling seems an obvious place to start, the E-cadherin promoter is commonly silenced by chromatin methylation in solid tumor cells [27], and whether this is facilitated by the same transcriptional repressors that down-regulate E-cadherin is not yet clear. In addition, E-cadherin is infrequently inactivated by mutation [27], and under these circumstances, there may or may not be added selective pressure on the miR200 family or the EMT-mediating E-cadherin repressors.

2 EMT and the two tracks of bladder cancer progression

As discussed throughout this themed issue, one of the distinguishing features of urothelial cancer is that it progresses along two pathways that appear to be distinct at the phenotypic and molecular levels [28]. The fact that they are distinguished by their relative propensities to become muscle-invasive strongly suggests that the EMT-related programming of the two types of cancer is vastly different. Indeed, independent global gene expression profiling studies by Waldman's [29] and Cordon-Cardo's [30] groups demonstrated that superficial and muscle-invasive tumors fall into two distinct subgroups when analyzed by unsupervised clustering. We downloaded their data and performed an *in silico* analysis of the relationship between EMT marker expression (E-cadherin, Zeb-1, and Zeb-2) and disease subtype. The results confirmed that there was a highly significant enrichment for Zeb-1 and Zeb-2 expression in the muscle-invasive tumors (Fig. 2) (W. Choi et al., manuscript submitted), supporting the concept that EMT might underlie urothelial cancer invasion and metastasis.

Two other recent studies provide further support for this concept. In the first, Baumgart and coworkers characterized the expression of E-cadherin, β-catenin, plakoglobin, and vimentin in a series of 825 primary tumors displayed on 10 tissue microarrays. Downregulation of epithelial markers was associated with disease progression in terms of both grade and stage (i.e., superficial versus muscle-invasive cancer), and in univariate analyses, downregulation of either β-catenin or plakoglobin was associated with shorter disease-specific survival [31]. In the second study, Sayan and coworkers assessed expression of the EMT regulators Zeb-1 and Zeb-2 in a panel of human urothelial cancer cell lines and primary human tumors. They confirmed that Zeb-1 protein expression was associated with increased invasion/ migration *in vitro* and Zeb-2 was enriched in muscle-invasive cancers [32]. Importantly, overexpression of Zeb-2 in "epithelial" cell lines rendered them resistant to radiation-induced apoptosis, and overexpression of Zeb-2 in radiation-exposed primary tumors was also associated with poor clinical outcome [32]. Radiation resistance was linked to increased

DNA repair capacity as measured by clearance of radiation-induced H2AX foci in cell lines *in vitro* [32].

Prompted by these observations, we recently measured EMT marker expression in a panel of 20 human urothelial cell lines and a set of 114 primary urothelial tumors for which outstanding clinical outcome information was available (W. Choi et al., manuscript submitted). We observed a strong inverse correlation between the expression of E-cadherin and Zeb-1, Zeb-2 and vimentin in the cell lines and the tumors, and expression of the mesenchymal markers was confined to muscle-invasive disease. Included in our panel of markers was p63 (Fig. 1b), a member of the p53 family that Cordon-Cardo's group [33, 34] and others [35–41] showed was downregulated as a function of progression, particularly in muscle-invasive tumors. Expression of p63 and E-cadherin were closely correlated in the cell lines and the primary tumors, strongly suggesting that it is a marker of the “epithelial” phenotype (W Choi et al., manuscript submitted). Strikingly, however, high-level expression of p63 in muscle-invasive (T2–T4) tumors was significantly associated with poor clinical outcome. This relationship was even apparent in the T1 tumors, a transitional subset in which clinical outcome is particularly difficult to predict. Our data create an important paradox and a conceptual inconsistency with the recent published literature [31, 32]. On the one hand, our data strongly support the previous implication of EMT in muscle-invasive disease, the “track” that is responsible for essentially all bladder cancer mortality. However, they also suggest that within this subset, retention, or reversion to a more “epithelial” phenotype is associated with even worse outcome. Whether this represents a “MET” that selects for increased proliferation once cancer cells have metastasized is unclear. In ongoing studies, we are aggressively pursuing the mechanistic basis for the link between p63 expression and the “bad” biology observed within the muscle-invasive subset. One possibility is that it is somehow linked to urothelial cancer “stemness,” since p63 expression tracks closely with markers such as cytokeratins 5 and 17 that identify the “basal” cells within the bladder (M. Tran, unpublished observations; see chapter by Brandt et al. in this volume), and p63 clearly plays an essential role in maintaining “stemness” in the epithelial (basal) compartments of the prostate [42–44] and skin [45], and it also localizes to the basal layer of the normal urothelium [46] (M. Tran, unpublished observations).

3 Role of EMT in EGFR dependency

Inhibitors of the epidermal growth factor receptor (EGFR) were among the first targeted agents to be developed for cancer therapy [47]. The rationale underlying this effort came from a large number of studies that documented that the EGFR tends to be overexpressed as a function of progression in solid tumors, including urothelial cancer [48, 49]. Preclinical studies provided further support for the idea that EGFR inhibitors might exert unique potency in bladder cancer. For example, work from our group demonstrated that EGFR blockade inhibited the growth and metastasis of orthotopic human 253J B-V bladder xenografts, effects that were linked to inhibition of tumor production of angiogenic factors (VEGF, bFGF, and IL-8) [50], and overexpression of the EGFR promotes tumorigenesis in transgenic mice [51]. These studies and others prompted the clinical evaluation of small molecule and antibody-based inhibitors of the EGFR in patients with muscle-invasive urothelial cancers. Although it is still too early to evaluate whether or not the approach has

been successful, the preliminary results suggest that benefit will be limited to a relatively small subset of patients, if indeed the approach proves to add benefit at all (discussed in a subsequent article by Dovedi and Davies). One of us (A. Siefker-Radtke) is currently leading three such studies. The first is an MD Anderson-based, SPORE-supported investigator-initiated trial in which the small molecule EGFR tyrosine kinase inhibitor gefitinib is given only after conventional combination chemotherapy has been administered to maximum benefit (i.e., in the setting of minimal residual disease), and the second is a Southwest Oncology Group trial in which the blocking anti-EGFR antibody cetuximab is administered in combination with gemcitabine and cisplatin, where we will be measuring EMT markers as a component of the correlative studies attached to the trial. Finally, in another SPORE trial we are performing a neoadjuvant study with erlotinib, where the main objective is to determine whether EMT markers correlate with biological response to the drug (measured by Ki-67 and p27 immunohistochemistry).

However, our expectations for the potential efficacy of EGFR-directed therapy in muscle-invasive disease have changed markedly since we conducted some of the first xenograft studies with this class of drugs almost a decade ago. This change has been driven largely by the results of clinical trials with EGFR inhibitors in other solid malignancies [52–54]. The EGFR is also overexpressed in advanced lung, colon, pancreas, and head and neck cancers, and small molecules (gefitinib, erlotinib) and blocking antibodies have been evaluated extensively in patients with lung and colon cancer. Originally, patient enrollment was guided by immunohistochemical detection of high level EGFR expression in pretreatment tumor tissues, but it has become clear that EGFR expression does not correlate clearly with response [53, 54]. Rather, in lung cancer, the presence of activating kinase domain mutations, which are found in approximately 15% of patients in the USA (closer to 25% in Japan), are clearly associated with response to the small molecule inhibitors [53, 55], and there is also evidence that lung and colon cancers that display EGFR gene amplification are also sensitive [56–59]. Conversely, the presence of activating Kras mutations renders lung and colon cancers resistant to EGFR-directed therapy [53, 60, 61]. Overall, what is becoming increasingly apparent is that sensitivity or resistance to targeted therapy is complex and not tightly linked to overexpression of the target.

We, therefore, designed experiments to define the potential heterogeneity in EGFR inhibitor responsiveness more comprehensively. We assembled a relatively large set of unique human urothelial cancer lines (n=20) and screened them for sensitivity to gefitinib-induced growth arrest (as measured by ^{3}H-thymidine incorporation) [62, 63]. In parallel, we characterized their baseline gene expression profiles using the Illumina platform. We found that approximately 30% of the lines displayed at least 50% growth arrest at clinically relevant concentrations of the drug (<1 μM) [64]. Sensitivity was only loosely associated with surface EGFR levels but was clearly linked to autocrine signaling, as measured by the coupling of EGFR tyrosine kinase activity to downstream ERK and AKT pathway activation [62, 64]. Strikingly, all of the drug-sensitive cells expressed E-cadherin [64] and the panel displayed indistinguishable patterns of sensitivity and resistance to cetuximab [65], demonstrating that the effects of gefitinib were related to intrinsic EGFR dependency and not to other effects of the drug. Importantly, RNAi-mediated knockdown of E-cadherin

rendered cells resistant to cetuximab [65], demonstrating that E-cadherin plays a causal role in maintaining EGFR inhibitor sensitivity.

Not all of the “epithelial” bladder cancer cell lines in our panel responded to EGFR tyrosine kinase inhibitors or cetuximab. Reanalysis of our gene expression profiling data revealed that the levels of FGFR3 were higher in the “epithelial” as compared to the “mesenchymal” cells [66] (W. Choi, unpublished observation), and direct sequencing of FGFR3 demonstrated that four of our cell lines possessed activating FGFR3 mutations (P. Black, manuscript in preparation). As discussed in previous chapters of this volume, activating FGFR3 mutations are very common in superficial tumors [67], and more recent work has demonstrated that RNAi-mediated knockdown of FGFR3 [68] or exposure to a clinical FGFR3 inhibitor [69] blocked proliferation in these cells. We have confirmed that several of the “epithelial” lines that do not respond to EGFR antagonists are strongly inhibited by a selective small molecule inhibitor of FGFR3 (A. Kwan, I. Lee, unpublished observations). Interestingly, some of the inhibitor-sensitive cell lines (i.e., RT4, UM-UC1, and RT112) express only wild-type FGFR3 [69], indicating that FGFR3 inhibitor sensitivity is not limited to the mutant tumors. However, as is true for EGFR antagonists, all of the cells that are sensitive to FGFR3 inhibitors are confined to the “epithelial” subset of cell lines. We are currently in the process of determining whether a molecular signature for FGFR3 dependency can be identified that can distinguish FGFR3-dependent from EGFR-dependent cells.

We also screened our cell lines and a relatively large set of primary human tumors for the presence of EGFR gene amplification, the presence of activating kinase domain mutations (akin to those described in lung cancer), and expression of a variant form of the EGFR (type III) that has been implicated in EGFR dependency in gliomas [70, 71]. We found only one cell line (UMUC5) that possessed high level amplification of the EGFR, but otherwise, none of the other specimens possessed these features of EGFR dependency [72]. We also found that two cell lines that possess activating Ras mutations (T24-H-Ras, UMUC3-K-Ras) are completely resistant to EGFR or FGFR3 inhibitors and cluster with our “mesenchymal” cell lines. Therefore, it is not clear to us that activating Ras mutations and activation of the EGFR or FGFR3 play redundant roles in driving the growth of the “epithelial” subset of urothelial cancer cells.

4 Relationship between EMT and TRAIL sensitivity

As discussed by Rosevear and colleagues in a subsequent article, Bacillus Calmette-Guerin (BCG) is the current frontline therapy for high-risk superficial bladder cancer and carcinoma *in situ*, but many patients ultimately fail BCG therapy and no molecular markers are available that can distinguish BCG-responsive from BCG-refractory disease. BCG appears to act (at least in part) by triggering neutrophils to secrete TNF-related apoptosis-inducing ligand (TRAIL) [73–77]. Another immunomodulator that has promising anti-tumor activity in bladder cancer is interferon-α (IFNα) [78, 79], which blocks tumor growth in preclinical models via direct induction of tumor cell death and inhibition of angiogenesis [80–86]. We, therefore, performed experiments to identify the molecular mechanisms that underlie IFNα's anti-tumoral effects. Our results demonstrate that like BCG, the direct tumoricidal effects of

IFNα are dependent on autocrine TRAIL production [80–82], whereas the inhibition of angiogenesis involves parallel downregulation of bFGF and IL-8 [85, 86].

With these observations in mind, we have also characterized the heterogeneity in TRAIL responsiveness across our panel of human urothelial cancer cell lines. The results demonstrate again that sensitivity to TRAIL tracks closely with an “epithelial” phenotype (T24, which is TRAIL-sensitive but mesenchymal, is the “outlier”) (F. Martin, manuscript in preparation). We have not yet identified a molecular explanation for the link between EMT and TRAIL resistance, but the “mesenchymal” lines express higher levels of the apoptosis inhibitors XIAP and BCL-2 and compounds that target these factors increase TRAIL sensitivity in the resistant cells. Notably, a recent screen by a group from Genentech demonstrated that expression of Golgi enzymes involved in protein glycosylation and fucosylation correlated with TRAIL sensitivity in a much larger set of human solid tumor cell lines [87]. Strikingly, the expression of two of these enzymes (FUT1 and GALNT14) correlates closely with E-cadherin and inversely with Zeb-1 in our cell lines and the public gene expression profiling datasets (W. Choi, unpublished observations), suggesting that in addition to their potential direct roles in regulating TRAIL sensitivity, the fucosylation and glycosylation enzymes identified by the Genentech group are also excellent surrogate markers of an “epithelial” phenotype and that this programming probably also plays a central role in maintaining TRAIL sensitivity.

5 Molecular control of EMT in urothelial cancer cells

As discussed above, EMT is controlled by antagonistic interactions between members of the miR200 family of micro-RNAs and transcriptional repressors of E-cadherin expression including the direct miR200 targets Zeb-1 and Zeb-2 [9, 10]. We are, therefore, exploring how these proteins regulate invasion/migration and EGFR dependency in urothelial cancer cells. As discussed earlier, overexpression of Zeb-2 in “epithelial” urothelial cells renders them more invasive and resistant to radiation-induced apoptosis, effects that are associated with enhanced DNA repair [32]. Conversely, we have found that knockdown of Zeb-1 in “mesenchymal” cell lines inhibits tumor cell invasion and migration, although the effects are highly cell type-dependent. For example, in UMUC3 cells, Zeb-1 knockdown results in strong upregulation of E-cadherin mRNA and protein levels and parallel strong inhibition of invasion (A. Das et al, manuscript in preparation). However, in KU7 or T24 cells, Zeb-1 knockdown weakly upregulates E-cadherin mRNA expression and does not appreciably affect protein expression, and as a result, invasion is not attenuated. Importantly, enforced overexpression of E-cadherin does inhibit invasion in the KU7 cells, indicating that the block in E-cadherin expression is probably responsible for the inability of Zeb-1 knockdown to decrease invasion and migration. In ongoing studies, we are investigating whether other known EMT-related repressors (Zeb-2, Snail) are bound to the E-cadherin promoter in the KU7 and T24 cells. Intriguingly, our preliminary studies suggest that additional chromatin-modifying enzymes may also be involved. By analyzing E-cadherin promoter histone methylation by chromatin immunoprecipitation, we have found that a repressive histone methylation mark (H3K27me3) is present at much higher levels in the KU7 and T24 cells as compared to UMUC3 (A. Das, manuscript in preparation). Therefore, it seems likely that

enzymes that regulate histone methylation reinforce the EMT phenotype in the KU7 and T24 cells.

In a parallel study, we compared the global micro-RNA expression profiles in representative "epithelial," EGFR inhibitor-sensitive (UMUC5) and "mesenchymal," EGFR inhibitor-resistant (KU7) cell lines to define the potential roles of differential miRNA expression in the regulation of EGFR inhibitor sensitivity [11]. The results demonstrated that members of the miR200 family were among the most differentially expressed micro-RNAs [11], present at very high levels in UMUC5 and almost undetectable levels in KU7. We have since measured all five members of the miR200 family by quantitative real-time PCR in our whole panel of cell lines and have confirmed that there is a strong inverse association between miR200 expression and the "mesenchymal" phenotype (M. Williams, manuscript in preparation). Overexpression of miR200c in the UMUC3 cells suppressed expression of Zeb-1 and Zeb-2, restored E-cadherin expression, and inhibited invasion and migration, effects that were similar to those observed with Zeb-1 knockdown [11]. Strikingly, however, overexpression of miR200c in UMUC3 or miR200b in T24 also increased cell sensitivity to EGFR inhibitors [11], effects that we have not been able to reproduce in the Zeb-1-silenced cells. Because E-cadherin restoration was similar in the miR200c and Zeb-1 knockdown cells, the most attractive explanation for the discrepancy in phenotypes is that there are additional miR200 targets (aside from Zeb-1 and Zeb-2) that play important role(s) in regulating EGFR-mediated signal transduction. Consistent with this idea, we have shown that miR200c directly targets the mRNA encoding ERRFI-1, an inhibitor of EGFR signaling, and that knockdown of ERRFI-1 in UMUC3 also increases cellular sensitivity to EGFR inhibitors [11].

6 Conclusions and future directions

Our experience with urothelial cancer cell lines indicates that "epithelial" and "mesenchymal" gene expression signatures exert extremely strong, global effects on cell biology. We have made progress in defining the molecular mechanisms underlying the stable signatures but much more work needs to be done. We do not yet know if TGFβ family cytokines or other autocrine mechanisms maintain EMT-related gene expression in the "mesenchymal" urothelial cancer cell lines. This seems to be a likely possibility given the central roles these cytokines play in normal tissue biology and cancer metastasis in other models, but it is also possible that the genetic alterations that underlie the two tracks of bladder cancer are involved as well. Related to this question, it appears clear that members of the miR200 family of micro-RNAs control the EMT phenotype in bladder cancer cells, but what controls expression of the miR200 family itself needs to be defined. Again, the TGFβ family of cytokines is an attractive place to start.

There is also a need to place EMT within the context of other processes that are known to regulate urothelial cancer progression and metastasis. The earlier studies demonstated that VEGF levels and the ratio of E-cadherin to MMP9 expression predicted poor outcome [23], and our real-time quantitative RT-PCR assessment of MMP2 and MMP9 levels in our cohort of patient tumors confirms that both are elevated in muscle-invasive disease. However, what is not clear is whether the global regulators of EMT also control expression of VEGF, the

MMPs, and possibly other factors relevant to the biology of this disease subset. With respect to EGFR signaling, our preliminary assessments suggest that ligand (TGFα) expression correlates with an “epithelial” phenotype (Fig. 1b) but whether this is restricted to EGFR-dependent (as opposed to FGFR3-dependent) “epithelial” lines requires additional investigation.

Another important objective for future study is to determine how having tumor EMT information can be used to improve clinical outcome. There is arguably limited value in being able to distinguish superficial from muscle-invasive disease using primary tumor tissue. However, if confirmed in patients, the observation that EGFR inhibitors selectively target “epithelial” urothelial cells could be used to identify the subset of muscle-invasive tumors that will respond better to EGFR-directed therapy. The information could also prove valuable within the setting of high-risk superficial disease and CIS, where an EMT signature would also predict *de novo* resistance to BCG and other TRAIL-based therapies. Here these agents should probably be combined either with another modality that inactivates the specific resistance mechanism(s) that are in place (i.e., combination therapy with BCG and an XIAP or BCL-2 inhibitor) or an agent that selectively kills the “mesenchymal” cells, or some attempt could be made to reverse the EMT signature altogether. Along these lines, recent work has demonstrated that clinical histone deacetylase (HDAC) inhibitors are capable of restoring E-cadherin expression and EGFR inhibitor sensitivity in lung cancer cells [88], prompting interest in performing clinical trials with EGFR inhibitor/HDAC inhibitor combinations in patients.

Finally, it will be important to determine how EMT affects tumor responses to other conventional and investigational therapies. The recently published work demonstrating that Zeb-2 expression is associated with poor outcome in patients receiving radiation therapy [32] suggests that EMT might contribute to a more global therapeutic resistance than has been described here, or it may at least suppress DNA damage-induced cell death. The observation that Zeb-2 promotes DNA repair and suppresses radiation-induced apoptosis in urothelial cancer cells *in vitro* provides further support for this concept [32], as does recent work in other models directly linking EMT to cancer “stemness” [89] and resistance to conventional chemotherapy [90, 91]. It should be possible to identify agents that selectively target “mesenchymal” cancer cells, as was done recently by another group [91]. Overall, understanding how EMT and other developmental pathways control cancer cell biology should greatly facilitate the objective of designing more personalized cancer therapy.

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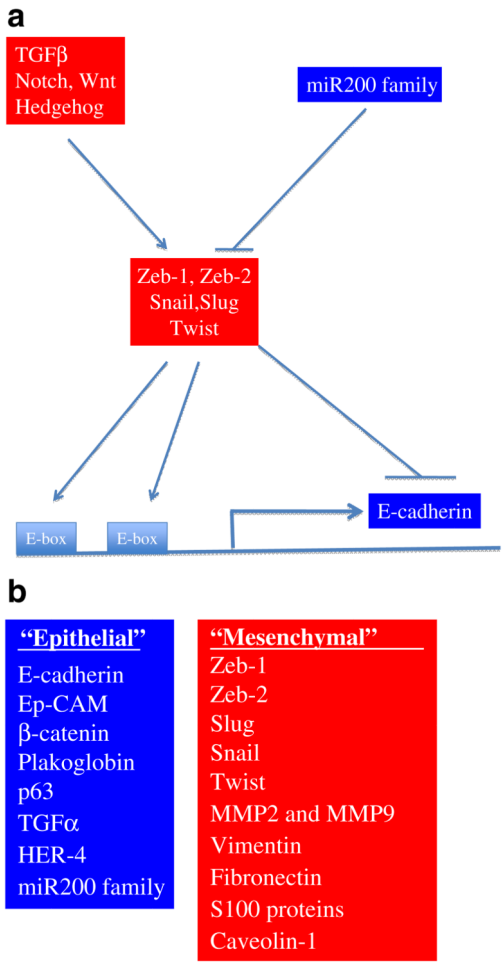

Fig. 1.

Molecular markers of EMT. Epithelial markers are displayed in *blue*, mesenchymal in *red*. a Regulation of E-cadherin expression. Upstream signals, and in particular TGFβ, increase expression of transcriptional repressors of E-cadherin (Zeb-1, Zeb-2, Twist, Snail, and Slug) that function by binding to two so-called "E-box" elements that are located within the E-cadherin promoter. This results in recruitment of histone deacetylases and possibly histone and DNA methyltransferases to the E-cadherin promoter, resulting in transcriptional silencing. Members of the miR200 family of micro-RNAs directly antagonize this process by binding to multiple copies of specific "seed" sequences located within the mRNAs encoding Zeb-1 and Zeb-2, resulting in transcript degradation and inhibition of translation. b. Other representative markers of the "epithelial" and "mesenchymal" states. The lists provided are not comprehensive, and the potential mechanistic relationships between these markers and E-cadherin signaling has in most cases not been determined

	Symbol	UniGene ID	Description	Blaveri (Ref29)		Sanchez-Carbayo (Ref30)	
				P-value	Fold Change *	P-value	Fold Change *
"M" Markers	FN1	Hs.203717	Fibronectin 1	< 1e-07	7.48	< 1e-07	7.59
	MMP2	Hs.513617	Matrix metalloproteinase 2	< 1e-07	4.33	2.4E-06	2.37
	ZEB2	Hs.34871	Zinc finger E-box binding homeobox 2	< 1e-07	2.45	2.5E-06	3.68
	ZEB1	Hs.124503	Zinc finger E-box binding homeobox 1	3.52E-05	1.47	< 1e-07	3.63
"E" Marker	CDH1	Hs.461086	Cadherin 1, type 1, E-cadherin (epithelial)	4.61E-05	0.43	0.0010	0.36

Fig. 2.

Relationship of EMT to the two tracks of bladder cancer progression. We compared the expression of four “mesenchymal” markers and E-cadherin in gene expression profiles obtained from superficial and muscle-invasive urothelial cancers using data from two publically available datasets. Fold changes and statistical signficance are indicated. Note that upregulation of the mesenchymal markers and downregulation of E-cadherin are significantly associated with muscle-invasive disease

*Fold change: Invasive tumors/Superficial tumors.