Is the Epithelial-to-Mesenchymal Transition Clinically Relevant for the Cancer Patient?

Martin A. Krasnapolski*, Laura B. Todaro and Elisa Bal de Kier Joffé

Research Area, Institute of Oncology "Angel H. Roffo", University of Buenos Aires, Buenos Aires, Argentina

Abstract: Epithelial-to-mesenchymal transition (EMT) is a transdifferentiation process by which a fully differentiated epithelial cell acquires mesenchymal traits, and therefore, mesenchymal abilities such as motility and invasiveness. It is a pivotal physiological process involved in embryogenesis (Type 1 EMT) and in wound healing and tissue remodeling (Type 2 EMT), which, some authors claim, but there are still some controversies, has also been co-opted by tumor cells to increase their malignant potential (Type 3 EMT). Many biomarkers of Type 3 EMT have been characterized and classified into functional categories (*i.e.*, extracellular proteins, cell surface molecules, cytoskeletal markers, transcriptional factors, and, recently, micro RNAs). The extra and intracellular signals that lead to EMT are only starting to be understood, but there is a consensus that Ras and TGF-beta signaling must converge with NF-kB in order to achieve a full EMT. The most classical experimental model is the induction of EMT by TGF-beta in cultures of epithelial cells. Other pathways involving GSK3b, and Wnt/beta-catenin, are also implicated. Ultimately, every EMT-inducing pathway will activate any of the E-cadherin transcriptional repressors (ZEB1, ZEB2, Twist, Snail or Slug). Although in the pre-clinical setting, EMT has also been related to an accelerated tumor progression and to an increased resistance to conventional chemotherapy. In this sense, several groups are beginning to use EMT as a predictive marker of response to treatment. Finally, two chemicals targeting TGF-beta are in clinical trials and many laboratories have initiated studies to use other EMT-related molecules as a therapeutic target for the cancer patient with some modest, but encouraging results.

Keywords: EMT, biomarkers, molecular mechanism, cancer.

INTRODUCTION

What is the Epithelial-to-Mesenchymal Transition?

Epithelial-to-mesenchymal transition (EMT) is a transdifferentiation process undergone by some specialized embryonic cells. It can be viewed as a cell reprogramming that consists in switching the epithelial transcriptional program off while turning a mesenchymal transcriptional program on. This protein shifting leads to a profound morphological transformation mainly characterized by a change from a polyhedral to a spindle-like shape. But the implications of EMT are not only in morphology as transitional cells also have a different behavior. While epithelial cells, through their tight cell-to-cell adhesions, remain attached to the tissue where they belong, mesenchymal cells have the ability to detach and migrate.

EMT is a proven phenomenon presumed by many investigators worldwide to have been co-opted by different tumor cells to increase their metastatic ability [1-3]. Although there is strong evidence supporting a role for EMT in tumor progression, the literature is not completely uniform about this topic because some investigators, mainly pathologists, are not convinced about the actual occurrence of EMT in animal or human tumors. They claim that cancer cells only undergo a partial EMT [4]. This group is led by David Tarin, who even declared that the very concept of EMT in cancer is a "Fallacy" [5]. Although his arguments are worth listening, and may be true for some models and conditions, along this review we will present bibliography supporting the existence of EMT in cancer.

Classification of EMT

Depending on the setting EMT takes place in, it can be classified into 3 subtypes [6]. Type 1 EMT, executed by epithelial-like embryonic cells, is associated with implantation, embryo formation and organ development and generates mesenchymal cells (primary mesenchyma) that can invade contiguous structures but do not disseminate systemically [7]. Type 2 EMT, occurring during wound healing and tissue regeneration, is highly associated with inflammation and generates fibroblast-like and other related cells in order to reconstruct tissues following trauma and inflammatory injury [8]. Despite the upregulation of proteases and motility, this phenomenon is a local event and no dissemination is implicated. Finally, type 3 EMT is done by neoplastic cells that have previously undergone genetic and epigenetic changes, mainly in oncogenes and tumor suppressor genes [9]. Type 3 EMT is a gradual process with some cells retaining many epithelial traits while acquiring some mesenchymal ones and other cells becoming fully mesenchymal [10]. The few cells, generally located in the periphery of a tumor mass that go through this kind of EMT gain the ability to invade locally and systemically, finally leading to metastasis [11].

*Address correspondence to this author at the Research Area, Institute of Oncology "Angel H. Roffo", Av. San Martín 5481, (C1417DTB) Buenos Aires, Argentina;Tel: +5411 4504-7884; Fax: +5411 4580-2811;
E-mail: mkrasnapolski@gmail.com

1389-2010/11 $58.00+.00

EMT is Reversible

All three types of EMT can revert once the transitional cell has accomplished the task that mesenchymal features were needed for. This reverse process is called mesenchymal-to-epithelial transition (MET) and its failure may lead to pathological conditions. In type 1 EMT, after the transitional embryonic cells reached their target structure MET is undergone to generate secondary epithelia [12]. In type 2 EMT, once the inflammatory stimulus is attenuated, MET must occur, or chronic fibrosis might permanently damage the tissue under regeneration leading to organ fibrosis [13]. Fibroblast growth factor-1 was shown to be responsible for the MET in TGF-beta-induced lung fibrosis [14] In type 3 EMT, upon colonizing secondary organs, metastasizing carcinoma cells suffer MET generating epithelial micrometastases that resemble the primary tumor [15]. Obviously, the reexpression of adherent molecules such E-Cadherin and the reconstitution of tight junctions, as well as the downregulation of MMPs expression, are relevant effects to MET.

Biomarkers of Type 3 EMT

Biomarkers of EMT have been classified according different criteria. One of the more used classifications is based on whether the expression of a specific marker increases or decreases in the transitional cell. In this review we will use another one that integrates both, the functional category of the biomarker and its regulation Fig. (**1**), which we find more suitable and informative.

Extracellular Proteins

Extracellular matrix (ECM) is a protein meshwork that not only acts as a scaffold for cells but also establishes relevant interactions with specific membrane receptors to trigger several pathways involved in proliferation and survival. It is constituted by different molecules specifically expressed by epithelial or mesenchymal cells and EMT is characterized by a shift from a basement-type ECM to a fibrillar one. Indeed, an increase in fibronectin, a high molecular weight glycoprotein that is a main constituent of fibrillar ECMs, has been related to the induction of EMT and a migratory and invasive phenotype in diffuse-type gastric carcinoma-derived cell lines [16]. Even though fibronectin is associated with tissue fibrosis and the desmoplastic stroma in tumors, the utility of fibronectin as a type 3 EMT biomarker is limited because it is produced by various cell types, including actual fibroblasts, mononuclear cells, and some epithelial cells [17].

Laminins constitute a family of closely related heterotrimeric (alpha, beta, gamma) glycoproteins that compose basement membranes (a specialized type of ECM that harbors epithelial cells). Laminin alpha3-beta3-gamma2 (formerly laminin-5) can stimulate cell migration and/or invasion after having been cleaved by matrix metalloproteinases (MMPs) such as MMP-2 and MT1-MMP, and was found to be increased in ductal breast carcinomas [18], oral squamous cell carcinomas [19], and hepatocellular carcinoma [20]. In the same sense, an increase in alpha4 chain-containing laminins was reported in gliomas [21]. Also, in oral

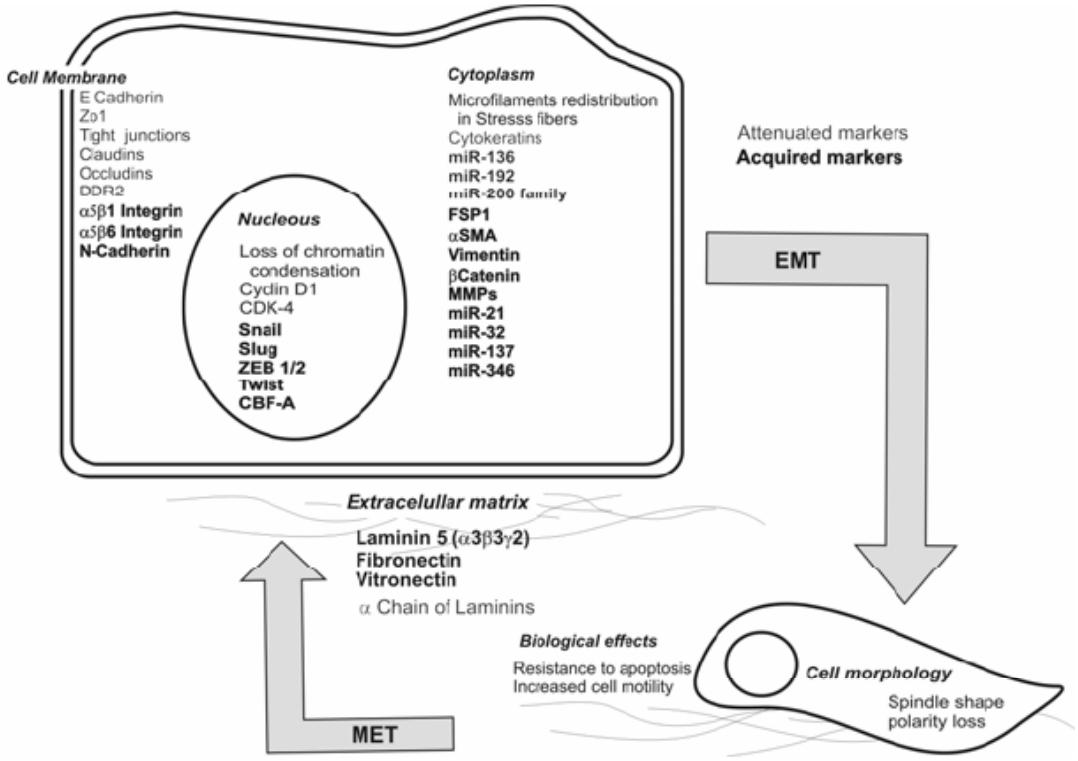

Fig. (1). Molecular and morphological changes in EMT. EMT involves a functional transition of a polarized epithelial cell into a mesenchymal cell. The epithelial and mesenchymal cell markers commonly used by EMT researchers and the redistribution of some subcellular structures are indicated. Acquired molecular markers are in bold and the attenuated ones are in grey.

squamous carcinomas cells that had undergone a full EMT *in vitro*, the downregulation of the isoform alpha5 and the concomitant upregulation of the alpha4 were detected and linked with a reduced adhesion to substrate that led to an increased invasive capacity [22].

Cell Surface Molecules

The Cadherin family comprises transmembrane calcium-dependent homotypic adhesion molecules. Historically, one of the most reliable biomarker of EMT has been the down-regulation of the expression of epithelial cadherin (E-cadherin, aka uvomorulin) [9]. The lack of this molecule, normally present in the *adherens* junctions, was related to an enhanced invasion in various tumor models [23, 24]. Furthermore, the loss of E-cadherin *per se* can induce EMT [25] which can be reversed by transfecting cells with its cytoplasmatic domain only [26]. Moreover it was reported the induction of a pathway related to EMT merely by blocking E-cadherin-mediated cell-cell contact [27]. Frequently, E-cadherin disappearance is followed by the emergence of another cadherin belonging to a different class in a process called cadherin-switch. Neural-cadherin (N-cadherin), initially discovered in neural tissue [28], is also normally present in mesenchymal cells, and is now being used as an EMT biomarker [29].

Claudins and occludins are integral membrane proteins localized at tight junctions that are responsible for the maintenance of epithelial cell polarity. The loss of their expression was found to be an event as early as the loss of E-cadherin during EMT [30].

Integrins are heterodimeric transmembrane heterotypic adhesion proteins involved in the anchorage to the surrounding tissues or the ECM. For this reason, the integrin subset each cell expresses is very sensitive to microenvironmental changes [31]. For example, alpha5beta1 integrin, the receptor for fibronectin, was found to be increased in Ha-Ras-transformed normal mammary epithelial cells EpH4 [31] and in hepatocellular carcinoma cells that had undergone EMT [32] . In the same sense, alpha(v)beta6, the receptor for vitronectin, was detected to be increased in invasive colorectal carcinoma cells that had suffered EMT [33]. Also, the forced expression of beta6 integrin in poorly invasive squamous cell carcinoma led to a fibroblast-like shape and to an increased invasiveness [34].

Another EMT marker that reflects adaptation to the altered ECM microenvironment associated with EMT is the collagen-specific receptor tyrosine kinase DDR2 (discoidin domain receptor tyrosine kinase 2). The shift from DDR1 to DDR2 in cancer cells correlates with increased invasiveness, demonstrating its utility in identifying type 3 EMT [35].

Cytoskeletal markers. Type 3 EMT is not a mechanism for forming fibroblasts, but a process used by tumor epithelial cells for movement, invasion, and metastasis. Therefore, the cytoskeleton is one of the cellular components that undergoes more remodeling, with changes in cortical actin and in actin stress fibers [36]. A prototypical fibroblast marker for detecting EMT in cancer is FSP1. This molecule is a member of the family of Ca^{2+}-binding S100 proteins. In addition, in models of cancer, metastatic cells often express FSP1 as part of the molecular program of type 3 EMT. Ectopic expression of FSP1 itself facilitates EMT in adult epithelial cells and cancer cells [33]. Another cytoskeletal marker related to EMT is alpha-type smooth muscle actin (alpha-SMA), one of the six actin family members [37].

A controversial marker of EMT is the intermediate filament vimentin, which is normally expressed in various cells, including fibroblasts, endothelial cells, cells of the hematopoietic lineages, and glial cells. However, vimentin is commonly used to identify cells undergoing type 3 EMT. This is based on a positive correlation of vimentin expression with increased invasiveness and metastasis [37].

Transcriptional Factors

The main transcription factors used as biomarkers of EMT are the transcriptional repressors of E-cadherin such as the bHLH protein Twist, the zinc finger proteins ZEB1 and ZEB2, and the Snail family of zinc finger proteins (*i.e.:* Snail and Slug) [37]. It´s interesting to note that the transcription factor Snail, besides being a suppressor of E-cadherin, regulates various other aspects of the EMT phenotype such as the decreased expression of claudins, occludins and cytokeratins, the inhibition of proliferation through suppression of cyclin D and cyclin-dependent kinase 4, as well as the increased expression of MMP, fibronectin and vitronectin.

Also, it has been described that siRNA knock-down of the transcription factor Rb in MCF-7 breast cancer cells led to EMT by inducing Slug and ZEB1 and further E-cadherin downregulation [38]. Although controversial, CBF-A, a member of nuclear ribonucleoprotein A/B (hnRNPA/B) family, can be observed in type 3 EMT associated with metastatic tumor formation. However, its expression is not confined to EMT because it is involved in various other cellular processes [39]. Finally, FOXC2 is a pleiotropic inducer of EMT, since when overexpressed, can *per se* induce EMT [40].

MicroRNAs

MicroRNAs (miRs) are short (~22 bases) RNA molecules involved in the post-transcriptional regulation (mostly silencing) of several genes. The mechanism of their action involves binding to complementary sequences in the 3'-UTR of the target mRNA and recruitment of a multiprotein complex that, depending on the complementarity between the miRs and their binding sites in the target mRNAs, leads mRNAs either to degradation or to translation blocking [41]. In a human keratinocyte cell line that had underwent TGF-beta-1-induced EMT, Zabadil *et al.* described an EMT-specific microRNA signature that involved the upregulation of miR-21, miR-32, miR-137 and miR-346 and the down-regulation of miR-136, miR-192, miR-210 and miR-211 [42]. miR-205 and all five members of the miR-200 family of microRNAs (i.e. miR-200a, miR-200b, miR-200c, miR-141 and miR-429) participate in the maintenance of an epithelial phenotype by regulating the E-cadherin transcriptional repressors ZEB1/deltaEF1 and ZEB2/SIP1 [43]. The loss of its expression by epigenetic mechanisms as DNA methylation can lead to EMT and has also been linked to an aggressive cancer phenotype [44]. Also, miR-9, a microRNA that directly targets E-cadherin was found to be induced by c-Myc in breast cancer [45]. Finally, Li *et al.* showed that

miR-34a, a transcriptional target of p53, targets in turn, the expression of Notch and c-Met oncoproteins [46].

Many molecules with a wide range of functions and intracellular locations have been proposed as *in vitro* biomarkers of Type-3 EMT. Most of them, described in this section and depicted in Fig. (**1**), are involved in the underlying mechanism of EMT through reexpression or downregulation, but the majority of them await validation to be able to go through the clinical stage.

SIGNALING PATHWAYS LEADING TO EMT

Although the whole processes of EMT and MET are not fully understood, many extracellular signals have been characterized and many molecular pathways have been dissected that can lead to any of those processes.

The most classical model of EMT induction is the treatment of epithelial cells with TGF-beta. Upon factor addition, the receptor recruits and phosphorylates SMADs which translocate to the nucleus and act as transcription factors inducing or repressing the expression of specific genes involved in the maintenance of the mesenchymal and epithelial phenotypes.

Janda *et al.* have clearly shown that the synergistic effects of the signaling pathways elicited by the small-G-protein Ras and TGF-beta can lead to EMT, but each one individually is not sufficient to establish a full EMT and only leads to a reversible phenotypic change termed scattering that only morphologically resembles EMT [47]. At least for breast cancer, Huber *et al.* have shown that for inducing EMT, Ras and TGF-beta signaling must converge in NF-kB activation, or EMT will not be produced [48].

Activated Ras-triggered signaling pathway crosstalks with TGF-b signaling and leads to EMT. That is the case for fibroblast growth factor (FGF) [49], insulin-like growth factor (IGF) [50-52], epidermal growth factor (EGF) [53] and platelet-derived growth factor (PDGF). The same can be found upon activation of non-receptor kinases as Src [54].

Activation of the Wnt pathway by Frizzled agonists firstly results into the inhibition of Glycogen synthase kinase 3-beta (GSK3-beta) and the cytoplasmic release of the E-cadherin-sequestered beta-catenin and to its subsequently nuclear translocation which leads to the induction of several genes dependent of the T cell factor (TCF)/lymphocyte enhancer factor (LEF) transcriptional complex. The same cascade occurs upon E-cadherin loss, either by proteolytic degradation or transcriptional repression (*e.g.* by Snail): beta-catenin is released into the cytoplasm and translocates into the nucleus where activates TCF/LEF complex leading to the induction of several EMT-related genes [55].

GSK3-beta is a strong inhibitor of EMT as it targets beta-catenin for degradation by phosphorylating a domain shared with IKK [56], an inhibitor of the NF-kB inhibitor. In addition it inhibits Snail function by phosphorylating two conserved domains, one regulating Snail stability and the other its nuclear localization [57]. GSK3-beta can also be inhibited by AKT.

The downstream effectors of almost every EMT-related signaling pathway, such as TGF-b, the Wnt cascade, and PI3K /AKT axis, are the above mentioned transcriptional repressors of E-cadherin (ZEB1, ZEB2, Twist, Snail and Slug). These E-cadherin repressors might participate in the process of EMT as follows. First, Snail and ZEB2 would initiate the downregulation of E-cadherin and then, Slug and ZEB1 would maintain E cadherin repressed [58]. However, the effect of E-cadherin repressors on mesenchymal markers such as vimentin and N-cadherin remains unsolved. As already described, recent studies have demonstrated that these transcriptional repressors are also regulated by micro-RNAs.

Interestingly, EMT can also be induced by anticancer agents, as well as stress conditions as the exposure to radiation [59] and hypoxic conditions that promote HIF-1alpha stabilization [60].

EXPLOITING THE EMT IN THE CLINICAL SETTING

Several groups have looked at type 3 EMT-related molecules with a translational vision and tried to answer the question posed in the title of this review by using them as cancer clinical markers or therapeutic targets.

EMT as a Marker of Prognosis

Yoshida *et al.* used an *in silico* approach which analyzed the expression profiles of all genome-wide microarray assays of poor prognosis epithelial ovarian carcinoma published up to July 2008. They found that 70% of genes upregulated or downregulated in those patients were also related to EMT [61].

In colorectal cancer, alpha(v)beta6 integrin, a marker of EMT, was associated to a faster progression to terminal disease [33]. Finally, several transcriptional factors such as Snail, Slug, and Twist have been useful markers to predict prognosis in various human carcinomas. For example, Yang *et al.* showed that *in vivo* the co-expression of HIF-1alpha, Twist and Snail in primary tumors of patients with head and neck cancers correlated with metastasis and the worst prognosis [62].

In spite of these encouraging results, Logullo *et al.* examined the expression of E-cadherin, beta-catenin, Snail, TGF-b and c-Met by immunohistochemistry in samples of breast carcinoma *in situ* and in primary invasive tumors but found no prognostic association [63].

Taken together, these findings support the idea that the well-characterized EMT features *in vitro* may be determinants of tumor progression and suggest their potential use as clinical prognostic markers, at least for epithelial cancers of certain localizations.

EMT as a Predictive Marker of Response to Treatment

Cells undergoing EMT have shown an acquired resistance to many traditional anticancer agents. In this sense, this process was related to the resistance to Gemcitabine in a pancreatic carcinoma cell line [64]. Also, EMT was related to resistance to new molecularly-targeted agents such as Gefitinib (Iressa), in a non-small cell lung cancer cell line [65] and in a hepatocellular model [66]. In the same sense, lapatinib-resistant endometrial cell lines exhibited epithelial-

to-mesenchymal transition features [67]. Also, a strong multigene signature indicative of EMT was identified in 47 non-small cell lung cancer cell lines as a determinant of insensitivity to Erlotinib [68].

In hepatocellular carcinoma cells, the inhibitor of the vascular endothelial growth factor receptor ZD6474 could inhibit the growth of tumor cells, but, such effect was reverted by the addition of the EMT-upregulated, laminin alpha3-beta3-gamma2 (formerly laminin-5) but not by other extracellular matrix proteins[69], suggesting the participation of EMT in the acquisition of resistance to this compound. Using a panel of 17 human bladder cancer cell lines, Shrader *et al.* could correlate the presence of low levels of E-cadherin and high levels of vimentin with resistance to Gefitinib [70] but, since they found exceptions and the correlation was not perfect, they suggested that these markers cannot be used by themselves to prospectively predict EGF receptor (EGFR)-dependent growth. In an attempt to correlate EMT-related markers with the *in vitro* resistance to EGFR-targeted teraphy, Frederick *et al*, identified the loss of E-cadherin, claudin 4 and claudin 7 as a signature of resistance to Gefitinib in cell lines derived from human non-small cell lung cancer and head and neck squamous cell carcinoma [71].

The up-regulation of Twist was also associated with cellular resistance to paclitaxel in human nasopharyngeal, bladder, ovarian, and prostate cancers. In colorectal cancer, stable oxaliplatin-resistant cells can acquire the ability to migrate and invade with phenotypic changes resembling EMT (spindle-cell shape, loss of polarity, intercellular separation and pseudopodia formation) [72]. Stable pancreatic [73] and ovarian [74] cancer cell lines resistant to gemcitabine and paclitaxel show some features related to EMT including an increased expression of Snail and Twist.

Analyzing the expression of the epithelial marker E-cadherin by immunohistochemistry on primary tumor samples, Yauch *et al.* could correlate E-cadherin positive staining with good clinical response to Erlotinib in non-small cell lung cancer patients [68].

As seen before, EMT can lead to resistance to multiple drugs and allows rapid progression of the tumor, two clinical findings directly linked with the inherent characteristics of EMT. Clarifying the underlying mechanism linking EMT and drug resistance may help clinicians to select an optimal anticancer drug treatment [36].

EMT as a Therapeutic Target

Many research groups are employing *in vitro* and animal models to use some EMT-related molecules to finally achieve a therapeutic effect in the cancer patient. So far, with the exception of TGF-beta, as will be mentioned below, none has passed on to the clinical stage, but many encouraging results have come out. The following paragraphs and the Table **1** will summarize some of the more promising preclinical attempts.

The approach selected by some authors was to restore the cellular expression of different molecules lost in EMT. For instance, Witta *et al.* found that the re-expression of E-Cadherin by transfection or by pretreating gefitinib-resistant non-small cell lung cancer cells with the histone deacetylases inhibitor, MS-275 increased its *in vitro* sensitivity to gefitinib [75]. Moreover, Kent *et al.* demonstrated that the re-expression of miR-34a in pancreatic ductal adenocarcinoma cell lines presented anti-proliferative activity for this malignancy [76]. Also trying to induce the reexpression of mIRs, Li *et al.* using natural agents (3,3'-diindolylmethane and isoflavone) induced the re-expression of miR-200b and miR-200c, which led to MET and abrogated *in vitro* the Gemcitabine resistance of pancreatic cancer cells [77].

On the other hand, others tried to inhibit *in vitro* some molecules upregulated during EMT. For example, the treatment with Dasatinib, a Src and Abl kinase inhibitor could prevent the *in vitro* growth of a basal-type "triple-negative" breast cancer cell lines with EMT-related features [78]. Also. the inhibition of hedgehog signaling using Cyclopamine could prevent human pancreatic cancer cells from undergoing EMT *in vitro*, and also diminished its metastatic potential in an orthotopic xenograft model [79]. Finally, Yang *et al.* using siRNA against Twist in HIF-1alpha-overexpressing or in hypoxic cells managed to reverse EMT and the metastastic phenotype [60].

In addition, a group of papers has showed that targeting EMT-related molecules can lead to a reduced malignancy *in vitro* which correlated with the *in vivo* behavior in xenograft models. Such is the case for Olmeda *et al.* who showed that silencing Snail by shRNA induced MET *in vitro* and a reduction of tumor growth and invasiveness of two carcinoma cell lines *in vivo* [80]. And for Fuchs *et al.* who demonstrated that the inhibition of integrin-linked kinase (ILK) restored the sensitivity of erlotinib- gefitinib- and cetuximab-resistant hepatocarcinoma cells that had undergone EMT to EGFR-targeted therapy both *in vitro* and in an *in vivo* xenograft model [81].

Finally, Moore *et al.* using a siRNA targeted at stroma-derived TGF-b could reduce metastasis in an *in vivo* model of breast cancer [82].

Although not intentionally aiming to the EMT, two compounds that target the TGF-beta signaling are now in clinical trials [83]. GC1008 is a human IgG4 kappa monoclonal antibody capable of neutralizing all mammalian isoforms of TGFbeta (i.e., beta1, beta 2 and beta 3). It has just finished a still unpublished Phase I clinical trial for renal cell carcinoma and malignant melanoma patients with encouraging results [83].

LY2157299, the first clinical selective chemical TGF-beta type I receptor kinase inhibitor, has just finished a still unpublished multi-center phase I trial for colon cancer, prostate cancer, adrenocortical carcinoma, breast cancer and malignant melanoma patients. Using a daily oral administration, LY2157299 was well tolerated and no drug-related grade three or four toxicities were observed [83]

CONCLUSION

The concept of EMT, as developed in the field of embryology has, with divided opinions, been extended to cancer progression and metastasis. Accordingly, molecular analyses based on EMT in embryology have been applied to cancer progression and data from *in vitro* and experimental animal models now support for an actual role of EMT as a metasta-

Table 1. Preclinical Experimental Approaches to use EMT as a Therapeutical Target

Molecular Target	Therapeutic Approach	Model	Outcome	Ref
E-Cadherin	Reexpression by transfection or by treatment with the histone deacetylases inhibitor MS-275	Gefitinib-resistant non-small cell lung cancer cell lines	Increased *in vitro* sensitivity to gefitinib	[75]
miR-200b and miR-200c	Reexpression by treatment with 3,3'-diindolylmethane or isoflavone	Gemcitabine-resistant pancreatic cancer cells	Increased *in vitro* sensitivity to gemcitabine	[77]
miR-34a	Reexpression by transfection	Pancreatic ductal adenocarcinoma cell lines	*In vitro* cell growth inhibition	[76]
Src and Abl kinase	Inhibition by desatinib	Basal-type "triple-negative" breast cancer cell lines with EMT-related features	*In vitro* growth inhibition	[78]
hedgehog	Inhibition by ciclopamine	EMT-prone human pancreatic cancer cells	EMT prevention *In vitro* and metastatic diminishing in an orthotopic xenograft model	[79]
Twist	Inhibition by siRNA	HIF-1alpha-overexpressing or hypoxic cells	*In vitro* EMT prevention	[58]
Snail	Silencing by shRNA	Carcinoma cell lines	*In vitro* MET induction and *in vivo* reduced tumor growth and invasiveness	[80]
Integrin-linked kinase (ILK)	Transfection with kinase-inactive ILK	Human hepatoma cell lines insensitive to anti-EGFR therapy	Increased sensitivity to EGFR inhibitors both, *in vitro* and in nude mice xenografts	[81]
Stroma-derived TGF-b	*In vivo* silencing by siRNA	Human MDA-MB-435 cell line	Reduction of metastasis in nude mice xenografts	[82]

sis-promoting mechanism. This concept is also supported by the worst prognosis of the patients bearing tumors with EMT-related traits. In fact, cancer cells undergoing type 3 EMT can acquire invasive properties and enter the surrounding stroma, resulting in the creation of a favorable microenvironment for cancer progression and metastasis. The biology of EMT has been clarified in tumor samples through the use of EMT-associated markers, such as mesenchymal specific markers (*e.g.* vimentin and fibronectin), epithelial specific markers (*e.g.* E-cadherin and cytokeratins), and transcription factors (*e.g.* Snail and Slug). Studying the EMT signaling pathways in tumors may make it possible to identify novel pathways specific to cancer progression and to suggest new therapeutic strategies in cancer therapy. Also, since EMT features are so strongly related to acquire resistance to conventional and molecularly-targeted therapies, any treatment intended to preventing or reducing EMT, or directly to inducing MET would be very beneficial for the cancer patient.

Finally, to try to answer the question that triggered the writing of this review, we can conclude that we are only at the dawn of the era of the clinical management of EMT in the cancer patient. Although there are many basic papers in this area, with the exception of TGF-beta, most of the genes and pathways mentioned in this article are not currently part of any clinical trial nor have specific drugs targeting them. Nevertheless, some alternatives to specifically drug EMT can be proposed. Some possible and still unexplored opportunities could be targeting the ECM, the Wnt pathway or re-expressing EMT-downregulated microRNAs (as the miR-200 family). Another possibility would be blocking EMT-inducing microRNAs (as miR-21 or miR-346) with new pharmacological agents such as naked [84], or lentivirus-delivered antagomirs [85]. We envision that this new drugs will come up, hopefully, sooner than later.

ABBREVIATIONS

EMT = Epithelial-to-mesenchymal transition
MET = Mesenchymal-to-epithelial transition
ECM = Extracellular matrix
E-cadherin = Epithelial cadherin
N-cadherin = Neural-cadherin
miR = microRNA
FGF = Fibroblast growth factor
IGF = Insulin-like growth factor
EGF = Epidermal growth factor
EGFR = Epidermal growth factor receptor
PDGF = Platelet-derived growth factor
alpha-SMA = Alpha-type smooth muscle actin

REFERENCES

[1] Voulgari, A.; Pintzas, A. Epithelial-mesenchymal transition in cancer metastasis: mechanisms, markers and strategies to overcome drug resistance in the clinic. *Biochim. Biophys. Acta*, **2009**, *1796*(2), 75-90.

[2] Wu, Y.; Zhou, B. P. New insights of epithelial-mesenchymal transition in cancer metastasis. *Acta Biochim. Biophys. Sin., (Shanghai)* **2008**, *40*(7), 643-650.

[3] Tse, J. C.; Kalluri, R. Mechanisms of metastasis: epithelial-to-mesenchymal transition and contribution of tumor microenvironment. *J. Cell Biochem.*, **2007**, *101*(4), 816-829.

[4] Garber, K. Epithelial-to-mesenchymal transition is important to metastasis, but questions remain. *J. Natl. Cancer Inst.*, **2008**, *100*(4), 232-233, 239.

[5] Tarin, D.; Thompson, E. W.; Newgreen, D. F. The fallacy of epithelial mesenchymal transition in neoplasia. *Cancer Res.*, **2005**, *65*(14), 5996-6000; discussion 6000-5991.

[6] Kalluri, R.; Weinberg, R. A. The basics of epithelial-mesenchymal transition. *J. Clin. Invest*, **2009**, *119*(6), 1420-1428.

[7] Perez-Pomares, J. M.; Munoz-Chapuli, R. Epithelial-mesenchymal transitions: a mesodermal cell strategy for evolutive innovation in Metazoans. *Anat. Rec.*, **2002**, *268*(3), 343-351.

[8] Flanders, K.C. Smad3 as a mediator of the fibrotic response. *Int. J. Exp. Pathol.*, **2004**, *85*(2), 47-64.

[9] Thompson, E. W.; Torri, J.; Sabol, M.; Sommers, C. L.; Byers, S.; Valverius, E. M.; Martin, G. R.; Lippman, M. E.; Stampfer, M. R.; Dickson, R. B. Oncogene-induced basement membrane invasiveness in human mammary epithelial cells. *Clin. Exp. Metastasis*, **1994**, *12*(3), 181-194.

[10] Turley, E. A.; Veiseh, M.; Radisky, D. C.; Bissell, M. J. Mechanisms of disease: epithelial-mesenchymal transition--does cellular plasticity fuel neoplastic progression? *Nat. Clin. Pract. Oncol.*, **2008**, *5*(5), 280-290.

[11] Xue, C.; Plieth, D.; Venkov, C.; Xu, C.; Neilson, E.G. The gatekeeper effect of epithelial-mesenchymal transition regulates the frequency of breast cancer metastasis. *Cancer Res.*, **2003**, *63*(12), 3386-3394.

[12] Acloque, H.; Adams, M. S.; Fishwick, K.; Bronner-Fraser, M.; Nieto, M. A. Epithelial-mesenchymal transitions: the importance of changing cell state in development and disease. *J. Clin. Invest.*, **2009**, *119*(6), 1438-1449.

[13] Kalluri, R.; Neilson, E. G. Epithelial-mesenchymal transition and its implications for fibrosis. *J. Clin. Invest.*, **2003**, *112*(12), 1776-1784.

[14] Ramos, C.; Becerril, C.; Montano, M.; Garcia-De-Alba, C.; Ramirez, R.; Checa, M.; Pardo, A.; Selman, M. FGF-1 reverts epithelial-mesenchymal transition induced by TGF-{beta}1 through MAPK/ERK kinase pathway. *Am. J. Physiol. Lung Cell Mol. Physiol.*, **2010**, *299*(2),L222-231

[15] Wells, A.; Yates, C.; Shepard, C. R. E-cadherin as an indicator of mesenchymal to epithelial reverting transitions during the metastatic seeding of disseminated carcinomas. *Clin. Exp. Metastasis*, **2008**, *25*(6), 621-628.

[16] Yang, Z.; Zhang, X.; Gang, H.; Li, X.; Li, Z.; Wang, T.; Han, J.; Luo, T.; Wen, F.; Wu, X. Up-regulation of gastric cancer cell invasion by Twist is accompanied by N-cadherin and fibronectin expression. *Biochem. Biophys. Res. Commun.*, **2007**, *358*(3), 925-930.

[17] Zeisberg, M.; Strutz, F.; Muller, G.A. Renal fibrosis: an update. *Curr. Opin. Nephrol. Hypertens*, **2001**, *10*(3), 315-320.

[18] Carpenter, P. M.; Wang-Rodriguez, J.; Chan, O. T.; Wilczynski, S. P. Laminin 5 expression in metaplastic breast carcinomas. *Am. J. Surg. Pathol.*, **2008**, *32*(3), 345-353.

[19] Giannelli, G.; Milillo, L.; Marinosci, F.; Lo Muzio, L.; Serpico, R.; Antonaci, S. Altered expression of integrins and basement membrane proteins in malignant and pre-malignant lesions of oral mucosa. *J. Biol. Regul. Homeost Agents*, **2001**, *15*(4), 375-380.

[20] Giannelli, G.; Fransvea, E.; Bergamini, C.; Marinosci, F.; Antonaci, S. Laminin-5 chains are expressed differentially in metastatic and nonmetastatic hepatocellular carcinoma. *Clin. Cancer Res.*, **2003**, *9*(10 Pt 1), 3684-3691.

[21] Ljubimova, J. Y.; Lakhter, A. J.; Loksh, A.; Yong, W. H.; Riedinger, M. S.; Miner, J. H.; Sorokin, L. M.; Ljubimov, A. V.; Black, K. L. Overexpression of alpha4 chain-containing laminins in human glial tumors identified by gene microarray analysis. *Cancer Res.*, **2001**, *61*(14), 5601-5610.

[22] Takkunen, M.; Ainola, M.; Vainionpaa, N.; Grenman, R.; Patarroyo, M.; Garcia de Herreros, A.; Konttinen, Y. T.; Virtanen, I. Epithelial-mesenchymal transition downregulates laminin alpha5 chain and upregulates laminin alpha4 chain in oral squamous carcinoma cells. *Histochem. Cell Biol.*, **2008**, *130*(3), 509-525.

[23] Lu, Z.; Ghosh, S.; Wang, Z.; Hunter, T. Downregulation of caveolin-1 function by EGF leads to the loss of E-cadherin, increased transcriptional activity of beta-catenin, and enhanced tumor cell invasion. *Cancer Cell*, **2003**, *4*(6), 499-515.

[24] Lin, C.Y.; Lin, C. J.; Chen, K. H.; Wu, J. C.; Huang, S. H.; Wang, S. M. Macrophage activation increases the invasive properties of hepatoma cells by destabilization of the adherens junction. *FEBS Lett.*, **2006**, *580*(13), 3042-3050.

[25] Huber, M. A.; Kraut, N.; Beug, H. Molecular requirements for epithelial-mesenchymal transition during tumor progression. *Curr. Opin. Cell Biol.*, **2005**, *17*(5), 548-558.

[26] Kuphal, S.; Bosserhoff, A.K. Influence of the cytoplasmic domain of E-cadherin on endogenous N-cadherin expression in malignant melanoma. *Oncogene*, **2006**, *25*(2), 248-259.

[27] Knirsh, R.; Ben-Dror, I.; Spangler, B.; Matthews, G.D.; Kuphal, S.; Bosserhoff, A.K.; Vardimon, L. Loss of E-cadherin-mediated cell-cell contacts activates a novel mechanism for up-regulation of the proto-oncogene c-Jun. *Mol. Biol. Cell*, **2009**, *20*(7), 2121-2129.

[28] Walsh, F. S.; Barton, C. H.; Putt, W.; Moore, S. E.; Kelsell, D.; Spurr, N.; Goodfellow, P. N. N-cadherin gene maps to human chromosome 18 and is not linked to the E-cadherin gene. *J. Neurochem.*, **1990**, *55*(3), 805-812.

[29] Shintani, Y.; Fukumoto, Y.; Chaika, N.; Svoboda, R.; Wheelock, M.J.; Johnson, K.R. Collagen I-mediated up-regulation of N-cadherin requires cooperative signals from integrins and discoidin domain receptor 1. *J. Cell Biol.*, **2008**, *180*(6), 1277-1289.

[30] Ikenouchi, J.; Matsuda, M.; Furuse, M.; Tsukita, S. Regulation of tight junctions during the epithelium-mesenchyme transition: direct repression of the gene expression of claudins/occludin by Snail. *J. Cell Sci.*, **2003**, *116*(Pt 10), 1959-1967.

[31] Maschler, S.; Wirl, G.; Spring, H.; Bredow, D. V.; Sordat, I.; Beug, H.; Reichmann, E. Tumor cell invasiveness correlates with changes in integrin expression and localization. *Oncogene*, **2005**, *24*(12), 2032-2041.

[32] Xu, Z.; Shen, M. X.; Ma, D. Z.; Wang, L. Y.; Zha, X. L. TGF-beta1-promoted epithelial-to-mesenchymal transformation and cell adhesion contribute to TGF-beta1-enhanced cell migration in SMMC-7721 cells. *Cell Res.*, **2003**, *13*(5), 343-350.

[33] Bates, R. C.; Mercurio, A. M. The epithelial-mesenchymal transition (EMT) and colorectal cancer progression. *Cancer Biol. Ther.*, **2005**, *4*(4), 365-370.

[34] Ramos, D. M.; Dang, D.; Sadler, S. The role of the integrin alpha v beta6 in regulating the epithelial to mesenchymal transition in oral cancer. *Anticancer Res.*, **2009**, *29*(1), 125-130.

[35] Maeyama, M.; Koga, H.; Selvendiran, K.; Yanagimoto, C.; Hanada, S.; Taniguchi, E.; Kawaguchi, T.; Harada, M.; Ueno, T.; Sata, M. Switching in discoid domain receptor expressions in SLUG-induced epithelial-mesenchymal transition. *Cancer*, **2008**, *113*(10), 2823-2831.

[36] Iwatsuki, M.; Mimori, K.; Yokobori, T.; Ishi, H.; Beppu, T.; Nakamori, S.; Baba, H.; Mori, M. Epithelial-mesenchymal transition in cancer development and its clinical significance. *Cancer Sci.*, **2010**, *101*(2), 293-299.

[37] Zeisberg, M.; Neilson, E.G. Biomarkers for epithelial-mesenchymal transitions. *J. Clin. Invest.*, **2009**, *119*(6), 1429-1437.

[38] Arima, Y.; Inoue, Y.; Shibata, T.; Hayashi, H.; Nagano, O.; Saya, H.; Taya, Y. 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*(13), 5104-5112.

[39] Ramaswamy, S.; Ross, K. N.; Lander, E. S.; Golub, T. R. A molecular signature of metastasis in primary solid tumors. *Nat. Genet.*, **2003**, *33*(1), 49-54.

[40] Mani, S. A.; Yang, J.; Brooks, M.; schwaninger, R.; Zhou, A.; Miura, N.; Kutok, J.; Hartwell, K.; Richardson, A. L.; Weinberg, R. A. Mesenchyme forkhead 1 (FOXC2) plays a key role in metastasis and is associated with aggressive basal-like breast cancers Proc. Natl. Acad. Sci. USA, **2007**, 104, 10069-10074.

[41] Scherr, M.; Venturini, L.; Eder, M. Lentiviral Vector-Mediated Expression of pre-miRNAs and AntagomiRs. *Methods Mol. Biol.*, **2010**, *614*, 175-185.

[42] Zavadil, J.; Narasimhan, M.; Blumenberg, M.; Schneider, R. J. Transforming growth factor-beta and microRNA:mRNA regulatory networks in epithelial plasticity. *Cells Tissues Organs.*, **2007**, *185*(1-3), 157-161.

[43] Gregory, P. A.; Bert, A. G.; Paterson, E. L.; Barry, S. C.; Tsykin, A.; Farshid, G.; Vadas, M. A.; Khew-Goodall, Y.; Goodall, G. J. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. *Nat. Cell Biol.*, **2008**, *10*(5), 593-601.

[44] Vrba, L.; Jensen, T. J.; Garbe, J. C.; Heimark, R. L.; Cress, A. E.; Dickinson, S.; Stampfer, M. R.; Futscher, B. W. Role for DNA methylation in the regulation of miR-200c and miR-141 expression in normal and cancer cells. *PLoS ONE*, **2010**, *5*(1), e8697.

[45] Khew-Goodall, Y.; Goodall, G. J. Myc-modulated miR-9 makes more metastases. *Nat. Cell Biol.*, **2010**, *12*(3), 209-211.

[46] Li, Y.; Guessous, F.; Zhang, Y.; Dipierro, C.; Kefas, B.; Johnson, E.; Marcinkiewicz, L.; Jiang, J.; Yang, Y.; Schmittgen, T.D.; Lopes, B.; Schiff, D.; Purow, B.; Abounader, R. MicroRNA-34a inhibits glioblastoma growth by targeting multiple oncogenes. *Cancer Res.*, **2009**, *69*(19), 7569-7576.

[47] Janda, E.; Lehmann, K.; Killisch, I.; Jechlinger, M.; Herzig, M.; Downward, J.; Beug, H.; Grunert, S. Ras and TGF[beta] cooperatively regulate epithelial cell plasticity and metastasis: dissection of Ras signaling pathways. *J. Cell Biol.*, **2002**, *156*(2), 299-313.

[48] Huber, M. A.; Azoitei, N.; Baumann, B.; Grunert, S.; Sommer, A.; Pehamberger, H.; Kraut, N.; Beug, H.; Wirth, T. NF-kappaB is essential for epithelial-mesenchymal transition and metastasis in a model of breast cancer progression. *J. Clin. Invest.*, **2004**, *114*(4), 569-581.

[49] Acevedo, V. D.; Gangula, R. D.; Freeman, K. W.; Li, R.; Zhang, Y.; Wang, F.; Ayala, G. E.; Peterson, L. E.; Ittmann, M.; Spencer, D.M. Inducible FGFR-1 activation leads to irreversible prostate adenocarcinoma and an epithelial-to-mesenchymal transition. *Cancer Cell*, **2007**, *12*(6), 559-571.

[50] Graham, T. R.; Zhau, H. E.; Odero-Marah, V. A.; Osunkoya, A. O.; Kimbro, K. S.; Tighiouart, M.; Liu, T.; Simons, J. W.; O'Regan, R. M. Insulin-like growth factor-I-dependent up-regulation of ZEB1 drives epithelial-to-mesenchymal transition in human prostate cancer cells. *Cancer Res.*, **2008**, *68*(7), 2479-2488.

[51] Sivakumar, R.; Koga, H.; Selvendiran, K.; Maeyama, M.; Ueno, T.; Sata, M. Autocrine loop for IGF-I receptor signaling in SLUG-mediated epithelial-mesenchymal transition. *Int. J. Oncol.*, **2009**, *34*(2), 329-338.

[52] Kim, H. J.; Litzenburger, B. C.; Cui, X.; Delgado, D. A.; Grabiner, B. C.; Lin, X.; Lewis, M. T.; Gottardis, M. M.; Wong, T. W.; Attar, R. M.; Carboni, J. M.; Lee, A.V. Constitutively active type I insulin-like growth factor receptor causes transformation and xenograft growth of immortalized mammary epithelial cells and is accompanied by an epithelial-to-mesenchymal transition mediated by NF-kappaB and snail. *Mol. Cell Biol.*, **2007**, *27*(8), 3165-3175.

[53] Lo, H. W.; Hsu, S. C.; Xia, W.; Cao, X.; Shih, J. Y.; Wei, Y.; Abbruzzese, J. L.; Hortobagyi, G. N.; Hung, M. C. Epidermal growth factor receptor cooperates with signal transducer and activator of transcription 3 to induce epithelial-mesenchymal transition in cancer cells via up-regulation of TWIST gene expression. *Cancer Res.*, **2007**, *67*(19), 9066-9076.

[54] Mandal, M.; Myers, J. N.; Lippman, S. M.; Johnson, F. M.; Williams, M. D.; Rayala, S.; Ohshiro, K.; Rosenthal, D. I.; Weber, R. S.; Gallick, G. E.; El-Naggar, A. K. Epithelial to mesenchymal transition in head and neck squamous carcinoma: association of Src activation with E-cadherin down-regulation, vimentin expression, and aggressive tumor features. *Cancer*, **2008**, *112*(9), 2088-2100.

[55] Kim, K.; Lu, Z.; Hay, E. D. Direct evidence for a role of beta-catenin/LEF-1 signaling pathway in induction of EMT. *Cell Biol. Int.*, **2002**, *26*(5), 463-476.

[56] Sanchez, J. F.; Sniderhan, L. F.; Williamson, A. L.; Fan, S.; Chakraborty-Sett, S.; Maggirwar, S. B. Glycogen synthase kinase 3beta-mediated apoptosis of primary cortical astrocytes involves inhibition of nuclear factor kappaB signaling. *Mol. Cell Biol.*, **2003**, *23*(13), 4649-4662.

[57] Zhou, B. P.; Deng, J.; Xia, W.; Xu, J.; Li, Y. M.; Gunduz, M.; Hung, M. C. Dual regulation of Snail by GSK-3beta-mediated phosphorylation in control of epithelial-mesenchymal transition. *Nat. Cell Biol.*, **2004**, *6*(10), 931-940.

[58] Peinado, H.; Olmeda, D.; Cano, A. Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? *Nat. Rev. Cancer*, **2007**, *7*(6), 415-428.

[59] Jung, J. W.; Hwang, S. Y.; Hwang, J. S.; Oh, E. S.; Park, S.; Han, I.O. Ionising radiation induces changes associated with epithelial-mesenchymal transdifferentiation and increased cell motility of A549 lung epithelial cells. *Eur. J. Cancer*, **2007**, *43*(7), 1214-1224.

[60] Yang, M. H.; Wu, M. Z.; Chiou, S. H.; Chen, P. M.; Chang, S. Y.; Liu, C. J.; Teng, S. C.; Wu, K. J. Direct regulation of TWIST by HIF-1alpha promotes metastasis. *Nat. Cell Biol.*, **2008**, *10*(3), 295-305.

[61] Yoshida, S.; Furukawa, N.; Haruta, S.; Tanase, Y.; Kanayama, S.; Noguchi, T.; Sakata, M.; Yamada, Y.; Oi, H.; Kobayashi, H. Expression profiles of genes involved in poor prognosis of epithelial ovarian carcinoma: a review. *Int. J. Gynecol. Cancer*, **2009**, *19*(6), 992-997.

[62] Yang, M. H.; Wu, K. J. TWIST activation by hypoxia inducible factor-1 (HIF-1): implications in metastasis and development. *Cell Cycle*, **2008**, *7*(14), 2090-2096.

[63] Logullo, A. F.; Nonogaki, S.; Pasini, F. S.; Osorio, C. A.; Soares, F. A.; Brentani, M. M. Concomitant expression of epithelial-mesenchymal transition biomarkers in breast ductal carcinoma: association with progression. *Oncol. Rep.*, **2010**, *23*(2), 313-320.

[64] Wang, Z.; Li, Y.; Kong, D.; Banerjee, S.; Ahmad, A.; Azmi, A. S.; Ali, S.; Abbruzzese, J. L.; Gallick, G. E.; Sarkar, F. H. Acquisition of epithelial-mesenchymal transition phenotype of gemcitabine-resistant pancreatic cancer cells is linked with activation of the notch signaling pathway. *Cancer Res.*, **2009**, *69*(6), 2400-2407.

[65] Rho, J. K.; Choi, Y. J.; Lee, J. K.; Ryoo, B. Y.; Na, II.; Yang, S. H.; Kim, C. H.; Lee, J.C. Epithelial to mesenchymal transition derived from repeated exposure to gefitinib determines the sensitivity to EGFR inhibitors in A549, a non-small cell lung cancer cell line. *Lung Cancer*, **2009**, *63*(2), 219-226.

[66] Giannelli, G.; Azzariti, A.; Fransvea, E.; Porcelli, L.; Antonaci, S.; Paradiso, A. Laminin-5 offsets the efficacy of gefitinib ('Iressa') in hepatocellular carcinoma cells. *Br. J. Cancer*, **2004**, *91*(11), 1964-1969.

[67] Konecny, G. E.; Venkatesan, N.; Yang, G.; Dering, J.; Ginther, C.; Finn, R.; Rahmeh, M.; Fejzo, M. S.; Toft, D.; Jiang, S.W.; Slamon, D. J.; Podratz, K. C. Activity of lapatinib a novel HER2 and EGFR dual kinase inhibitor in human endometrial cancer cells. *Br. J. Cancer*, **2008**, *98*(6), 1076-1084.

[68] Yauch, R. L.; Januario, T.; Eberhard, D. A.; Cavet, G.; Zhu, W.; Fu, L.; Pham, T. Q.; Soriano, R.; Stinson, J.; Seshagiri, S.; Modrusan, Z.; Lin, C. Y.; O'Neill, V.; Amler, L. C. Epithelial versus mesenchymal phenotype determines *in vitro* sensitivity and predicts clinical activity of erlotinib in lung cancer patients. *Clin Cancer Res.*, **2005**, *11*(24 Pt 1), 8686-8698.

[69] Giannelli, G.; Azzariti, A.; Sgarra, C.; Porcelli, L.; Antonaci, S.; Paradiso, A. ZD6474 inhibits proliferation and invasion of human hepatocellular carcinoma cells. *Biochem. Pharmacol.*, **2006**, *71*(4), 479-485.

[70] Shrader, M.; Pino, M.S.; Brown, G.; Black, P.; Adam, L.; Bar-Eli, M.; Dinney, C. P.; McConkey, D. J. Molecular correlates of gefitinib responsiveness in human bladder cancer cells. *Mol. Cancer Ther.*, **2007**, *6*(1), 277-285.

[71] Frederick, B. A.; Helfrich, B. A.; Coldren, C. D.; Zheng, D.; Chan, D.; Bunn, P. A. Jr.; Raben, D. Epithelial to mesenchymal transition predicts gefitinib resistance in cell lines of head and neck squamous cell carcinoma and non-small cell lung carcinoma. *Mol. Cancer Ther.*, **2007**, *6*(6), 1683-1691.

[72] Yang, A. D.; Fan, F.; Camp, E. R.; van Buren, G.; Liu, W.; Somcio, R.; Gray, M. J.; Cheng, H.; Hoff, P.M.; Ellis, L.M. Chronic oxaliplatin resistance induces epithelial-to-mesenchymal transition in colorectal cancer cell lines. *Clin. Cancer Res.*, **2006**, *12*(14 Pt 1), 4147-4153.

[73] Shah, A. N.; Summy, J. M.; Zhang, J.; Park, S. I.; Parikh, N. U.; Gallick, G. E. Development and characterization of gemcitabine-resistant pancreatic tumor cells. *Ann. Surg. Oncol.*, **2007**, *14*(12), 3629-3637.

[74] Kajiyama, H.; Shibata, K.; Terauchi, M.; Yamashita, M.; Ino, K.; Nawa, A.; Kikkawa, F. Chemoresistance to paclitaxel induces epithelial-mesenchymal transition and enhances metastatic potential for epithelial ovarian carcinoma cells. *Int. J. Oncol.*, **2007**, *31*(2), 277-283.

[75] Witta, S. E.; Gemmill, R. M.; Hirsch, F. R.; Coldren, C. D.; Hedman, K.; Ravdel, L.; Helfrich, B.; Dziadziuszko, R.; Chan, D.C.; Sugita, M.; Chan, Z.; Baron, A.; Franklin, W.; Drabkin, H. A.; Girard, L.; Gazdar, A. F.; Minna, J. D.; Bunn, P. A. Jr. Restoring E-cadherin expression increases sensitivity to epidermal growth factor receptor inhibitors in lung cancer cell lines. *Cancer Res.*, **2006**, *66*(2), 944-950.

[76] Kent, O. A.; Mullendore, M.; Wentzel, E. A.; Lopez-Romero, P.; Tan, A. C.; Alvarez, H.; West, K.; Ochs, M. F.; Hidalgo, M.; Arking, D. E.; Maitra, A.; Mendell, J. T. A resource for analysis of microRNA expression and function in pancreatic ductal adenocarcinoma cells. *Cancer Biol. Ther.*, **2009**, *8*(21), 2013-2024.

[77] Li, Y.; VandenBoom, T. G^{2nd}.; Kong, D.; Wang, Z.; Ali, S.; Philip, P. A.; Sarkar, F. H. Up-regulation of miR-200 and let-7 by natural agents leads to the reversal of epithelial-to-mesenchymal transition in gemcitabine-resistant pancreatic cancer cells. *Cancer Res.*, **2009**, *69*(16), 6704-6712.

[78] Finn, R. S.; Dering, J.; Ginther, C.; Wilson, C. A.; Glaspy, P.; Tchekmedyian, N.; Slamon, D. J. Dasatinib, an orally active small molecule inhibitor of both the src and abl kinases, selectively inhibits growth of basal-type/"triple-negative" breast cancer cell lines growing *in vitro*. *Breast Cancer Res. Treat.*, **2007**, *105*(3), 319-326.

[79] Feldmann, G.; Dhara, S.; Fendrich, V.; Bedja, D.; Beaty, R.; Mullendore, M.; Karikari, C.; Alvarez, H.; Iacobuzio-Donahue, C.; Jimeno, A.; Gabrielson, K.L.; Matsui, W.; Maitra, A. Blockade of hedgehog signaling inhibits pancreatic cancer invasion and metastases: a new paradigm for combination therapy in solid cancers. *Cancer Res.*, **2007**, *67*(5), 2187-2196.

[80] Olmeda, D.; Jorda, M.; Peinado, H.; Fabra, A.; Cano, A. Snail silencing effectively suppresses tumour growth and invasiveness. *Oncogene*, **2007**, *26*(13), 1862-1874.

[81] Fuchs, B.C.; Fujii, T.; Dorfman, J. D.; Goodwin, J. M.; Zhu, A. X.; Lanuti, M.; Tanabe, K. K. Epithelial-to-mesenchymal transition and integrin-linked kinase mediate sensitivity to epidermal growth factor receptor inhibition in human hepatoma cells. *Cancer Res.*, **2008**, *68*(7), 2391-2399.

[82] Moore, L.D.; Isayeva, T.; Siegal, G.P.; Ponnazhagan, S. Silencing of transforming growth factor-beta1 in situ by RNA interference for breast cancer: implications for proliferation and migration *in vitro* and metastasis *in vivo*. *Clin. Cancer Res.*, **2008**, *14*(15), 4961-4970.

[83] Tan, A. R.; Alexe, G.; Reiss, M. Transforming growth factor-beta signaling: emerging stem cell target in metastatic breast cancer? *Breast Cancer Res. Treat.*, **2009**, *115*(3), 453-495.

[84] Krutzfeldt, J.; Rajewsky, N.; Braich, R.; Rajeev, K. G.; Tuschl, T.; Manoharan, M.; Stoffel, M. Silencing of microRNAs *in vivo* with 'antagomirs'. *Nature*, **2005**, *438*(7068), 685-689.

[85] Scherr, M.; Venturini, L.; Battmer, K.; Schaller-Schoenitz, M.; Schaefer, D.; Dallmann, I.; Ganser, A.; Eder, M. Lentivirus-mediated antagomir expression for specific inhibition of miRNA function. *Nucleic Acids Res.*, **2007**, *35*(22), e149.

Received: April 05, 2010 **Accepted: May 20, 2010**