

# Loss of E-cadherin expression and epithelial mesenchymal transition (EMT) as key steps in tumor progression

## Abstract

The development of a regulatory program referred to as Epithelial Mesenchymal Transition (EMT) has been implicated as a means by which transformed epithelial cells may acquire the ability to invade, resist apoptosis, and disseminate.<sup>1-3</sup> It would appear that the EMT inducing factors involved would have the ability to orchestrate most of the steps in the cascade of invasion and metastasis. It is still necessary to know that invasive carcinomatous cells acquire their capacity to do so through the activation of parts of the EMT program.<sup>4</sup> In tumor types where they have been identified, cancer precursor cells have shown resistance to current therapies and are associated with recurrence of the disease. Thus, by forcing these cells back into their epithelial state, they could be made more receptive to current therapies.

**Keywords:** epithelial mesenchymal transition, EMT, E-cadherin, cancer, invasion, metastasis

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Mariano N Carrizo

Medical Oncologist, Regional Medical Leader LATAM, Argentina

**Correspondence:** Mariano Carrizo. Rawson 3150, B1610BAL  
 Ricardo Rojas, Tigre, Buenos Aires, Argentina,  
 Email mariano220877@yahoo.com.ar

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## Objectives

Systematic bibliographic review of mechanisms involved in EMT, considering individual relevance of each in the process of tumor progression in relation to the ability to invade, resist apoptosis and disseminate.

## Introduction

In 2000 the underlying mechanisms of invasion and tumor metastasis were an enigma; it was clear that carcinomas arising from epithelial tissues progressed to higher histological grades of malignancy, reflected in local invasion and the ability to metastasize. Tumor cells typically develop alterations in their form as well as their adhesion to other cells and to the extracellular matrix.<sup>4</sup> One of the most primitive divergences in early cellular phenotypes in organisms is the distinction between epithelial and mesenchymal cells. Epithelial cells maintain cell-cell adhesion essential for the integrity of organisms, while the mesenchymal phenotype cell provides support and structure to the epithelial cell primarily through the production of extracellular matrix, and unlike the confined and immobile epithelial cell, these are highly mobile and invasive.<sup>5</sup> The best characterized alteration involves the loss of E-Cadherin by the cells of the carcinomas, key element for the cell-cell molecular adhesion. Research on the ability to invade and metastasize has accelerated dramatically in recent years and critical regulatory genes have been identified.<sup>1,6,7</sup>

The Epithelial Mesenchymal Transition (EMT) as a regulated developmental program has become one of the most involved processes as a means by which transformed epithelial cells acquire the ability to invade, resist apoptosis, and disseminate. During this process, epithelial cells repress cell-cell adhesion structures, reorganize their skeletal structure, become isolated and become mobile.<sup>1</sup> This multifaceted process may be activated transiently or stably and to varying degrees by carcinomatous cells during the invasion and metastasis process.

A set of transcriptional factors involved, including Snail, Slug, Twist, and Zeb 1/2 are able to orchestrate most of the steps in the

invasion cascade until colonization, at which point the cell could undergo a reverse process called Mesenchymal Epithelium Transition (MET), this plasticity being important for the formation of new tumor colonies acquiring characteristics similar to the cells of the primary tumor, which did not go through the EMT process.<sup>6,8,9</sup>

The concept of EMT may be simplistic in light of all the processes involved, but it is a first approximation since the process of metastasis can be separated into two important parts, first the physical dissemination of cells from the primary tumor to distant tissues and according to the adaptation of those cells to a foreign tissue microenvironment to generate successful colonization. Understanding programs that allow for dissemination and colonization represents a major challenge for ongoing and future research.

## Discussion

### Cell adhesion: E-cadherin

Most malignant human tumors are of epithelial origin, being the main characteristic of the epithelia: high cell adhesion, extracellular matrix in the form of basement membrane, cellular polarity and capacity of regeneration.<sup>5</sup> Tumors of epithelial origin develop from benign precursor lesions to invasive carcinomas and then metastasize, and in certain epithelial tumors the activation of the EMT program is crucial for dissemination.<sup>6</sup> The most important event of this program is the loss of E-Cadherin which was showed as a prerequisite for invasion, E-Cadherin being encoded by the CDH1 gene and having a dual epithelial function on the one hand the molecular adhesion Cell and on the other as a negative regulator of the Wnt signaling cascade, in particular of its central mediator  $\beta$  Catenin.<sup>8,10</sup>

### Physiology of E-cadherin

The milestone of epithelial cell layers is the expression of E-Cadherin located on the lateral basement membrane at the adherent junctions defining apico-basal polarity, connecting neighboring epithelial cells by homotropic calcium-dependent interactions at their extracellular ends, whereas the intracytoplasmic region interacts with other components mainly  $\alpha$ -catenin/placoglobin

and  $\beta$ -Catenin, thus mediating the mechanical stability of the joints. The alteration of the components of the adhesion bonds, mainly E-cadherin and  $\beta$ -catenin, have been shown to play an important role in the induction of the EMT process, which makes us wonder how E-cadherin and  $\beta$ -catenin contribute to the maintenance of epithelial differentiation.<sup>1,4,6,7,11</sup>

Most of the knowledge about E-Cadherin/ $\beta$ -catenin function comes from murine models where it is observed that E-Cadherin expression is dynamically regulated during embryogenesis, is transiently lost in cells in migration but is expressed when Cell begins to differentiate into epithelial tissues. The physiological importance in organ development and tissue morphogenesis is now clear, knowing that in embryonic development E-Cadherin is a key player during compaction of the morula and is determinant for cellular epithelial differentiation, this is not just a Mediator of cell adhesion but also contributes to regulate diverse processes like proliferation, migration, apoptosis or maintenance of the epithelial polarity, so it must emit signals downstream and the main mediator of this seems to be  $\beta$ -catenin.<sup>11</sup>

### **E-cadherin/ $\beta$ -catenin /wnt signaling**

$\beta$ -catenin has a dual function in epithelial cells depending on its intracellular localization, at the adherent junctions acting on cell-cell adhesion with E-Cadherin, however it also acts as the main effector in the Wnt signaling cascade in the nucleus, In summary the free  $\beta$ -Catenin in the cytosol is rapidly degraded by proteasome unless the Wnt signaling pathway is activated. The binding of the WNT molecules to the frizzled receptors activates the signaling cascade and the LRP5/6 receptor co receptors are recruited to translate the signal to the cytoplasm, causing inhibition of GSK3 $\beta$ , whereby beta-catenin evades degradation, accumulating in the cytoplasm and finally translocate to the nucleus, acting as a transcriptional co-activator in a complex with the HMG-box proteins of the TCF/LEF family, molecules that interact with DNA in promoter regions of the target genes.<sup>6</sup>

In recent years it has become clear that E-Cadherin and the associated catenins especially  $\beta$ -catenin are not only static components of the adherent bonds, in this context E-Cadherin contributes to maintain cell differentiation in two ways, first establishing adhesion Cell-cell and basal apical polarization, and second acting as a negative regulator of the Wnt signaling pathway. Even though the loss of E-Cadherin is not necessarily associated with the nuclear localization of beta-catenin and its transcriptional activation on the target genes, but in addition an alteration of the components of the beta-catenin degradation complex must also exist to allow nuclear localization, where the most important is the beta-catenin interaction with GSK3 $\beta$ , APC and Axin, negative regulators of the Wnt pathway. The interaction of the previously mentioned events contributes to the control of the complex EMT morphogenetic process.<sup>5,6,10</sup>

### **E-cadherin in cancer**

Cell adhesion, cell polarity and epithelial differentiation are lost during tumor progression, whereas sustained expression of the CDH1 gene contributes to retaining the epithelial phenotype and it has been shown that E-Cadherin acts as an inhibitor of invasion in numerous cell lines and in human a reverse correlation between E-cadherin expression and survival in patients with neoplasias.<sup>3,7,12</sup> Genomic alterations of the CDH1 gene that cause the loss of E-cadherin function have been identified in a variety of tumors, for example in breast cancer somatic mutation was reported only in the lobular

histology of sporadic cancer, in addition somatic mutations were also identified in ovarian and endometrial carcinoma as well as in gastric cancer, and germinal mutations in patients with predisposition to gastric diffuse cancer. Another way to lose E-cadherin expression is by hypermethylation of the CpG islands of the CDH1 promoter. However, the most frequent mechanism seems to be the transcriptional inhibition of E-cadherin where the silencing of the expression of the CDH1 gene by specific transcriptional factors, through the interaction with the E-boxes located in the proximal promoter of the gene have been identified, everything described above represent the first step for local invasion and dissemination.<sup>3,11,13-15</sup>

### **Mechanisms responsible for the inactivation of E-cadherin during tumor progression**

#### **Mutation of the CDH1/E-cadherin gene**

Inactivating somatic mutations of the CDH1 / E-Cadherin gene cause the loss of expression or function of the same as the main mechanism of inactivation in Diffuse Type Gastric Carcinoma and Breast Lobular Carcinoma, they have also been observed in a certain type of Synovial sarcomas, and infrequent in other types of tumors. They usually occur in combination with the loss of heterozygosity of the normal allele.<sup>16</sup> Mutations in the germ line predispose to gastric carcinoma of the diffuse type and families with early onset of this cancer have been detected where the appearance of mutations in the early stages of these tumorigenesis allows proposing to CDH1 / E-Cadherin as a tumor suppressor gene in these types of cancer.<sup>10</sup>

#### **Epigenetic silencing**

It consists of the hypermethylation of the CpG islands of the gene promoter; this entails the recruitment of methylated DNA binding proteins and histone deacetylase activity enzymes, which together mediate chromatin compaction and gene silencing.<sup>17</sup> Initially described in prostate carcinoma and ductal breast carcinoma, non-mutated tumors in CDH1 responsible for their loss of expression where cells derived from such tumors were able to express an exogenous CDH1 promoter, with the recovery of E-Cadherin expression being observed. Dealing with demethylating agents. It is currently considered a general mechanism of inactivation of CDH1 in cancer.<sup>16,18</sup>

#### **Transcription repression**

#### **Defining EMT in cancer**

EMT is the process by which epithelial cells are transformed into mesenchymal cells, there are three distinct functional types: Type I - Embryological development, Type II - Inflammation and Fibrosis in adult tissues and Type III - cancer development. In type III the resulting abnormal mesenchymal cells are no longer under the influence of cellular control mechanisms and are highly mobile, leading to an invasive and aggressive phenotype, being the initial step of micro metastases.<sup>2,19</sup>

The EMT process begins with loss of apico-basal polarity and dissolution of the narrow junctions, allowing the intermixing of the basal and lateral components, allowing the degradation of the basement membrane and cell surface proteins such as E-cadherin and integrins are replaced by N-Cadherin and integrins that provide transient adhesiveness properties where the intermediate filaments of cytokeratin are replaced by vimentin with conformational changes acquiring the ability to move and invade the extracellular matrix.<sup>5</sup> Unlike the EMT process during development, oncogenic EMT occurs in the context of unpredictable genetic changes present in the tumor cell, as well as in an abnormal environment.<sup>20</sup>

The EMT process rarely occurs homogeneously throughout the entire tumor, with the exception of lobular breast carcinoma, however, based on the expression of EMT markers, recent evidence suggests that this occurs more localized in the invading tumor front, then with appropriate extracellular stimuli, including the activation of TGF- $\beta$  and Wnt pathway as a tumor attack front, in conjunction with expression of EMT regulators like Snail/Slug/Twist, Crypto-1 And Six1, the cell acquires the mesenchymal phenotype indistinguishable from fibroblasts, and the absence of pathological evidence of EMT at the secondary site suggest that the cell could traverse the inverse Mesenchymal Epithelium Transition (MET) process by demonstrating that it is an epithelial plasticity which is reversible and dependent on the local environment.<sup>2,14,21,22</sup>

Multiple observations in animal models suggest that the EMT process is necessary for metastases but not sufficient, and that non-EMT cells colonize distant tissues. Although the main role of EMT in tumor progression would be the induction of an invading phenotype, it also causes other alterations that would contribute to it, a recent work by Mani SA et al.,<sup>23</sup> identifies the correlation between EMT and the induction of a tumor initiating phenotype that has been related to stem cells, posing the doubt as to whether EMT is a transdifferentiation of epithelial cells to mesenchymal or a dedifferentiation to a more progenitor / stem cell phenotype and finally induction of EMT not only regulates intrinsic characteristics of the tumor cell, but also the interaction of this with the environment including the immune system allowing it to evade it.<sup>2,14</sup>

### EMT inducers

Signaling pathways that control EMT are complex and include cross-linking between several signaling pathways and transcription factors, as well as multiple cancer-related positive feedback loops, such as the TGF- $\beta$  and Wnt/ $\beta$ -Catenin pathway.<sup>2,24</sup> TGF- $\beta$  has been implicated as a key mediator of the process, whereas in the early stages of cancer progression, it is protective to control cell proliferation and differentiation. In later stages, TGF- $\beta$  plays a different role in inhibiting cell death. TGF- $\beta$  can induce EMT through multiple signaling mechanisms, including direct phosphorylation of SMAD transcription factors, also influencing the activity of other induction signals including Notch, Wnt and integrins, some of which may act in concert for Trigger MET programs.

The Wnt pathway can lead to EMT through the inhibition of GSK3- $\beta$ -mediated phosphorylation and the associated degradation of  $\beta$ -Catenin in the cytoplasm, translocating to the nucleus where it serves as a subunit of transcription factor aiding gene expression related to the process. Notch can also induce EMT through the activation of the NF- $\kappa$ B pathway or by modulating the activity of the TGF- $\beta$  pathway.<sup>3,24,14</sup> Hypoxia is one of the physiological mechanisms that can induce EMT in tumors through regulation in more of HIF $\alpha$ , HGF, SNAIL1 and TWIST, activation of the Notch or NF $\kappa$ B pathways or induction of DNA hypomethylation. The vast majority of signaling pathways that trigger EMT converge in the induction of E-cadherin repressors.<sup>14,15</sup> Transcriptional Control: individual members of a group of 6 to 8 transcription factors have been shown to be able to orchestrate the program during embryologic development and in cancer, these include direct transcriptional repressors of E-Cadherin- SNAIL1, SLUG (SNAIL2), ZEB2 And E47- and others such as TWIST1 and ZEB2 that act less directly on E-Cadherin, emerging data suggest extensive cross-linkages between them allowing them to form a signaling network responsible for establishing and maintaining the mesenchymal cellular phenotype, and some of these factors

including TWIST1 play a role in overcoming cellular senescence and generating stem cells.<sup>3,14,25</sup>

Non-coding RNAs as EMT regulators and metastases: miRNAs have been recognized as important players in the regulation of gene and protein expression, where recent studies have described the role in miR200 family activation of the program and MiR205<sup>25,26</sup> as it maintains E-cadherin expression and epithelial phenotype, repressing ZEB1 and ZEB2.<sup>3,13</sup>

### EMT generates cells with stem cell properties

Sendurai A Mani<sup>23</sup> and colleagues performed a complex mammary cell study to determine if the cells that initiated the EMT and stem cells had similar traits, thereby inducing EMT in mammary epithelial cells immortalized by Twist's ectopic expression. And Snail as expected the resulting cells acquired a mesenchymal fibroblast-like appearance with suppression of mRNA expression encoding epithelial markers and a regulation in mRNA encoding for epithelial markers and then by flow cytometry analyzed cell-based expression of CD44 and CD24, two surface markers whose low CD44 / low CD24 expression is associated with both mammary normal epithelial stem cells and mammary tumor stem cells, demonstrating that the induction of EMT in transformed mammary epithelial cells led to cells with an antigenic phenotype High CD44 / CD24 cells, and that such cells are enriched with tumor-initiating cells and also that the number of tumor-initiating cells are increased to at least twice the magnitude in transformed cells which were forced to constitutively express transcription factors as Twist and Snail.<sup>23,26-28,14</sup>

### Clinical relevance of EMT and MET

EMT has been implicated in two of the most important processes responsible for cancer-related mortality: progression and metastasis as well as therapeutic resistance, both processes related to a third, the generation of cells with trunk characteristics the latter have been enriched in persistent residual tumors after therapeutic treatment in some breast tumors after neoadjuvant treatment where there was a significant increase in CD44 + CD24 cells expressing EMT associated genes in post-treatment biopsies.<sup>2</sup>

Several of the important drivers like SNAIL 1 and 2 have been shown to be significantly correlated with relapse and overall survival in patients with breast, colorectal and ovary cancer leading to poor clinical outcomes. Several studies demonstrated profiles associated with certain clinical-pathological parameters such as histological grade and tumor subtype in the case of metaplastic tumors or basal type of breast, usually groups with poor prognosis. In addition, EMT suppression may increase sensitivity to anti-EGFR treatments in cell line models (hepatocellular and pancreatic carcinoma) as well as in patients with lung cancer. Therefore the identification of characteristics of this type in tumor samples could provide a tool to better stratify patients and predict results.<sup>14</sup>

The transition between the epithelial and mesenchymal state highlights the plasticity of the epithelial cell and contributes to tumor progression as to intratumoral heterogeneity, a process triggered by various stimuli including signaling by growth factors, stroma-tumor cell interaction and hypoxia. There is a wide interaction between the inducing signals and transcription factors that can lead to this reprogramming.<sup>2,24</sup> It has also been shown to transform tumor cells into stem cells and micro RNA have been identified as a new class of regulators partly due to their interaction on transcription factors.<sup>14,24</sup>

The discovery that it generates stem cell properties promises to solve a major problem in cancer biology, several types of tumors that leave the primary site seem to rely on this process to facilitate the execution of most steps in the cascade of invasion and metastasis, however the last step called colonization which represents the passage from micro to macro metastases has represented a conceptual dilemma. If most of the cells that are able to spread do not have the capacity for self-renewal their possibility of colonizing distant sites would be limited, this problem could be partially explained by the findings that the program allows cells to disseminate but also gives them Self renewal that could generate epithelial-type differentiated structures where this reversal of differentiation through the Mesenchymal Epithelium Transition (MET) process would be important for the formation of macro metastases and the formation of a secondary tumor mass.<sup>14,22</sup>

Preventing or reversing the process may reduce recurrences, metastases or resistance to antineoplastic treatments, although several pathways lead to the process, the TGF- $\beta$  pathway is the most important driving force, therefore the prevention or reversal of its effect could have clinical relevant consequences. Metformin, the most widely used oral hypoglycemic agent, has been shown within its actions to repress major transcription factors (TGF- $\beta$  ZEB, TWIST and SLUG) with a regulation in more than E-Cadherin, the use of this in breast cancer retrospective studies were associated with a higher rate of complete pathologic response compared to non-metformin, and non-diabetic diabetics, then broad use and low toxicity would make metformin an attractive option in potential prevention / reversal of EMT.<sup>2,14,22,24</sup>

E-Cadherin and related markers may confer specific prognostic information where SMAD4, SNAI1, SLUG, and TWIST have been correlated with survival and risk of metastases in certain studies of patients with colorectal cancer although their incorporation as clinically useful biomarkers requires greater investigation.<sup>29</sup>

Regulating the activity of E-Cadherin repressors appears to be an obvious strategy to combat tumor progression; however these inducers of the complete program are transcription factors, being very difficult to block targets.

Antibodies or small molecules directed against EGFR, IGFR, PDGFR, cMET, TGF $\beta$ R and endothelin A, have been effective in preclinical and clinical models, although originally developed as inhibitors of cell proliferation and angiogenesis, these molecules are likely to interfere with EMT.<sup>14</sup>

Other signaling pathways are attempting to be blocked to interfere with EMT, including the use of antibodies against TGF $\beta$ , which are found in Phase I studies for renal cell carcinoma and pulmonary fibrosis, are also being studied in the Hedgehog inhibitors, the propeptide Of lysyl oxidase to reverse TEM produced through HER2. Strategies for blocking IGF/IGFR were recently reviewed. Another possible approach to overcoming refractoriness is to directly attack the stem cells, recently Sialomycin was identified for its selective cytotoxicity between breast cancer cells enriched in stem cells.<sup>14,15</sup>

However, the complexity of the signaling pathways that regulate the induction of EMT and its reversibility represents significant challenges. Moreover, it is still not clear which tumors should be treated and at which point in their evolution. If systemic dissemination occurs early in tumor development, as suggested by some studies, then at the time of diagnosis it would be too late to successfully block EMT inducing events, on the other hand an important point would be how to measure the efficacy of an anti EMT therapy in development, without waiting decades to determine the difference between distant

dissemination incidents.<sup>14,15</sup> Future studies are needed to identify additional molecular events that contribute to tumor colonization, as a result of the knowledge that some patients may develop metastases from dormant tumor cells years after resection of the primary tumor. In the study by Tsai et al.,<sup>30</sup> suggest the possibility that cells in dormancy could be in EMT.

## Conclusion

The initiation of the EMT program not only promotes the change in cellular characteristics to acquire motility and invasiveness, but also that it develops new interactions with the extracellular environment, so studying this process under physiological conditions is critical to elucidate its role in the process of invasion and metastasis.<sup>1,6</sup>

The cooperation between different transcription factors is the milestone of EMT, and since this process is controlled by a network of transcriptional regulators coupled to post-transcriptional and post-translational modifications that amplify their initial signals, to define the regulatory genes that operate these networks during Embryogenesis will be instrumental in understanding those of regulating EMT in cancer.<sup>22</sup>

A network of regulatory genes could help design genetic signatures for human tumors and pave the way for designing or improving specific therapies.<sup>15,31-36</sup>

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## Conflicts of interest

Author declares there are no conflicts of interest.

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