

# EPITHELIAL- MESENCHYMAL TRANSITION (EMT)- ITS THERANOSTIC ROLE IN CANCER PROGRESSION AND METASTASIS

## ABSTRACT

Epithelial-mesenchymal transition (EMT) is a process by which a fully differentiated epithelial cell attains mesenchymal traits and capabilities such as motility and invasiveness. There are three types. Type 3 EMT is associated with tumour cells increasing their malignant potential and result in increased resistance to conventional chemo- and radiotherapy. Molecules involved are being used as biomarkers and therapeutic targets.

## DEFINING EMT

EMT refers to the biological process by which epithelial cells become mesenchymal cells. During this transition, epithelial cells undergo several biochemical and cytoskeletal modifications. Epithelial cells are polarised cells with distinct apical, lateral and basal plasma membrane domains. They are characterised by intercellular adhesion complexes. Upon undergoing EMT, the apico-basal polarity is lost, the intercellular adhesion complexes are disrupted, and the underlying basement membrane is degraded. The resulting mesenchymal cells are nonpolarised, lack intercellular junctions, and are able to migrate away through the extracellular matrix as individual cells from the epithelial layer in which they originated<sup>1,2</sup>.

EMT was first described by Elizabeth Hay<sup>3</sup>, who realised that the phenotypic conversion of epithelial cells was important during early embryo cell migration and gastrulation. She suggested that differentiated epithelial cells can “transform” into mesenchymal cells by undergoing phenotypic changes. Since then, studies have found that EMT is reversible, and mesenchymal cells can go back to epithelial cells through a reverse process of mesenchymal-epithelial transition (MET). For this reason, “transformation” has been amended to “transition”<sup>3,4</sup>.

Three subtypes of EMT are encountered. Type 1 EMT is associated with implantation, embryo formation, and organ

development. Type 2 EMT is associated with organ fibrosis, tissue regeneration, and wound healing<sup>5-7</sup>. Type 3 EMT occurs in epithelial cancer cells that have formed solid tumours. Here, EMT is linked in the switching of epithelial cells into metastatic cells which journey in the bloodstream and then form secondaries<sup>8</sup>.

## EMT AND ITS ROLE IN CANCER PROGRESSION AND METASTASIS

Several molecular processes are employed to initiate and complete an EMT. Some of these processes include changes in the expression of (a) specific cell-surface proteins, (b) cytoskeletal proteins, (c) extracellular proteins, (d) transcription factors, and (d) specific microRNAs.

### (A) EXPRESSION OF SPECIFIC CELL-SURFACE PROTEINS

**E-cadherin:** E-cadherin expression has been found to decrease during EMT<sup>9</sup>. Indeed, the induction of EMT in cancer is promoted by a loss of function of E-cadherin<sup>10</sup>. Cell lines that lack E-cadherin are more prone to increased tumourigenicity and metastasis when transferred into mice that are immunodeficient<sup>11</sup>. Mutations in the E-cadherin gene were identified in gastric cancers in patients under 35 years<sup>12</sup> and in the signet ring cell carcinoma of the stomach<sup>13</sup>. Bringuier et al showed that decreased E-cadherin correlates with a poor prognosis in bladder tumours<sup>14</sup>. A similar relationship was shown by Mattijssen et al in head and neck squamous-cell carcinoma<sup>15</sup>.

**Integrins:** Studies show that integrin signalling facilitates EMT<sup>16,17</sup>. However, some integrins are expressed on both epithelial and mesenchymal cells, and so they have limited use as biomarkers. Still, some can be used as EMT markers, e.g. (i)  $\beta 6$  integrin – in colon carcinoma, only cancer cells that have metastatic potential express high levels; normal epithelial cells and non-invasive tumour cells express low levels<sup>18</sup>; (ii)  $\alpha 5$  integrin increase expression in B16F10 melanoma cells<sup>19</sup>.

**Discoidin Domain Receptor 2 (DDR2):** DDR2 is a marker that reflects adjustment to the changed microenvironment of the extracellular matrix as a result of EMT. Expression of this collagen-specific receptor tyrosine kinase is associated with increased invasiveness<sup>20,21</sup>.

### (B) EXPRESSION OF CYTOSKELETAL PROTEINS

**FSP1:** Fibroblast-Specific Protein-1 is used as a biomarker for the detection of Type 3 EMT<sup>22,23</sup>. Indeed, as part of the molecular program of Type 3 EMT, metastatic cells express FSP1. Expression of FSP1 in tumour cells could determine the latency of tumour dispersion and it could be an appropriate therapeutic target to control metastatic progression<sup>22</sup>.

**Vimentin:** Vimentin is an intermediate filament expressed in mesenchymal cells. There is a positive relationship between vimentin expression and increased metastasis in infiltrating ductal breast carcinoma<sup>24</sup>. In their study, Raymond and Leong state that vimentin

expression could be a marker of aggressive behaviour and such carcinomas may profit from early adjuvant therapy. This is reiterated in a review by Kokkinos et al<sup>25</sup>.

**$\alpha$ -SMA:** Alpha-smooth muscle actin is used as a biomarker in breast cancers especially basal phenotype or basal-like breast cancers<sup>26</sup>, which are characterised by early recurrence and decreased overall survival<sup>27</sup>.

**$\beta$ -Catenin:** In normal epithelial cells and non-invasive cancer cells,  $\beta$ -Catenin is found located in the cell membranes. In cells that are undergoing EMT, however,  $\beta$ -Catenin is located either in the cytoplasm or in the nucleus<sup>28</sup>. Here, it plays a dual role – it links cadherins to the cytoskeleton, and, together with the T cell factor (TCF)/LEF, it serves as a co-transcriptional activator<sup>29</sup>. In fact, the resulting complex, the  $\beta$ -Catenin/TCF/LEF complex, directly controls gene expression that is associated with EMT, particularly Snail1<sup>30</sup>. For this reason,  $\beta$ -Catenin is used as a biomarker of EMT in various studies of cancer. Brabletz et al investigated the nuclear overexpression of  $\beta$ -Catenin in colorectal cancer, and found that the nuclear translocation of  $\beta$ -Catenin may play a direct role in the tumour invasion processes<sup>28</sup>.

### (C) EXPRESSION OF EXTRACELLULAR PROTEINS

**Laminins:** Certain laminins have become established biomarkers. For instance, the upregulation of laminin-5 is associated with invasive cancers, such as oral squamous carcinoma<sup>31</sup>, hepatocellular carcinoma<sup>32</sup>, and breast carcinomas of the ductal type<sup>33</sup>.

**Fibronectin:** Fibronectin is an integral constituent of the extracellular matrix associated with the desmoplastic stroma in tumours. However, its use as a biomarker is limited because it is also produced by other types of cells, such as epithelial cells<sup>34</sup>. Still, some studies have found that Type 3 EMT is associated with an increase of fibronectin expression *in vitro*<sup>35</sup>.

### (D) ACTIVATION OF TRANSCRIPTION FACTORS

**FTS-1:** Fibroblast transcription site-1 is a regulatory element that is present in the promoter region of various genes associated with EMT, including those that encode  $\alpha$ -SMA,  $\beta$ -Catenin, E-cadherin, FSP1, vimentin, Snail1 and Twist. In addition, FTS-1 forms a complex with CBF-A and KRAB-associated protein 1 (KAP-1)<sup>7</sup>; the resulting CBF-A/KAP-1/FTS-1 complex is a master regulator of EMT<sup>36</sup>.

**Snail:** Several studies<sup>37-9</sup> have shown that the transcription factor Snail mediates EMT. Indeed, all known events occurring appear to be associated with the activation of Snail. Amongst others, Snail activation brings about the suppression of E-cadherin expression and the increased expression of mesenchymal cell markers such as fibronectin<sup>40</sup>. A correlation between Snail activation and EMT was established in human colorectal cancer cells<sup>37</sup>, in oral squamous carcinoma cells<sup>38</sup>, and in thyroid cancer cells<sup>39</sup>.

Studies, such as those by Medici et al<sup>41</sup>, have also demonstrated that Snail-mediated EMTs are promoted by the transforming growth factor- $\beta$  (TGF- $\beta$ ). Separate *in vitro* studies have shown that TGF- $\beta$  can induce EMT in certain types of cancers such as breast<sup>42</sup>, ovarian<sup>43</sup> and skin cancers<sup>44</sup>.

**Twist:** Twist is a protein that is upregulated during cancer metastasis. Specifically, Twist acts independently of Snail to repress E-cadherin<sup>45</sup> and to upregulate fibronectin and N-cadherin<sup>35</sup>.

**FOXC2:** Forkhead boxC2 is another transcription factor. Overexpression of any inducer of EMT (e.g. Snail, TGF- $\beta$ , Twist) increases expression of FOXC2. Overexpression of FOXC2 itself induces EMT, suggesting a significant role for FOXC2 in Type 3 EMT, especially when ductal breast cancers and metastatic breast cancer cells are involved<sup>46</sup>.

### (E) CHANGES IN THE EXPRESSION OF SPECIFIC MICRORNAS

The fact that EMT is reversible, suggests that EMT can also have an epigenetic background. Non-coding microRNAs are the ones most documented and form components of the cellular signalling circuitry choreographing the EMT program<sup>7</sup>. miR200 was found to inhibit the transcriptional repressors of E-cadherin expression, ZEB1 (zinc finger E-box binding homeobox 1) and ZEB2, and thus helps in preserving the epithelial cell phenotype<sup>47,48</sup>. miR200 have been found to be downregulated in ductal and metaplastic breast cancers, where a loss of miR200 is correlated with increased vimentin expression and a decrease in E-cadherin levels in cancer cells<sup>47</sup>.

In addition there is also miR21 and miR-10b; TGF- $\beta$ 1-induced EMT involving keratinocytes is associated with miR21<sup>49</sup> and Twist-induced EMT, involving breast cancer cells, is associated with miR-10b<sup>50</sup>.

### THERANOSTIC IMPLICATIONS

Many of the aforementioned molecules are being used as biomarkers to assess the presence or severity of cancer and its response to medical treatment. They are also being used to optimize therapy for individual patients. Furthermore, clinical studies based on EMT mechanisms have started.

One study by Chang et al<sup>51</sup> showed that radio-resistance in prostate cancer is associated with EMT events and the PI3K/Akt/mTOR signaling pathway. The *in vitro* tests carried out showed that the combination of BEZ235 (PI3K/mTOR inhibitor) with radiotherapy is a promising approach to overcome radio-resistance in the treatment of prostate cancer. This modality leads to reduced expression of EMT markers and PI3K/Akt/mTOR signaling pathway proteins. Such findings have laid down a platform for *in vivo* animal studies and clinical trials.

Another showcase is the work by Cufi et al<sup>52</sup> which showed that silibinin (from thistle extract) reversed levels of certain microRNAs associated with EMT and repressed mesenchymal-related markers in NSCLC (non-small cell lung cancer) refractory to erlotinib treatment. Silibinin was also found to activate a mesenchymal-to-epithelial transition (MET) and prevent the extremely migratogenic phenotype. Now clinical trials are on the way to assess how silibinin might be useful to prevent or reverse NSCLC progression after treatment with erlotinib.

### CONCLUSION

Even though some insight into the mechanisms involved in EMT in cancer progression and metastasis has been achieved, more research is warranted so that the knowledge gained can be translated into coherent, clinically effective systems. ❄️