Epithelial mesenchymal transition and Tissue Healing

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ABSTRACT

Epithelial mesenchymal transition (EMT) is the ability of the cells to lose the epithelial features with gaining of mesenchymal one. It is a dynamic and reversible process induced by damage, hypoxia or inflammation. The execution of EMT events could be complete or partial during tissue repair of different organs. During wound healing, EMT has important role for re-epithelialization, angiogenesis and for Langerhans cells immunologic role. On the other hand, sustained EMT is a key mechanism underlying the wound scarring, fibrotic pathology of multiple organs, cataract and endometriosis. Hence, the understanding of EMT regulation during wound healing and tissue repair has important clinical implications as chronic wounds represent major health care costs. EMT could yield adult cells with stem cell characteristics. Therefore, one could predict that, it contributes to the pool of different progenitor cells to maintain organs homeostasis. Further analyses are necessary to determine whether EMT in normal tissues leads to the production of normal stem cells.

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Epithelial mesenchymal transition (EMT) is cellular plasticity with ability of cells to alter their phenotypic and morphological characteristics leading to transdifferentiation of epithelial cells into motile mesenchymal cells.

The idea that epithelial cells can down regulate epithelial characteristics and acquire mesenchymal characteristics arose in the early 1980s from observations made by Elizabeth Hay 1980 who described epithelial to mesenchymal phenotype changes in the primitive streak of chick embryos.

Originally it was thought of EMT as a transformation, suggesting a unidirectional and committed switch. However, EMT is now considered a transition suggesting a transient and reversible process. Subsequently, the term “transformation” was replaced with “transition” reflecting in part the reversibility of the process and the fact that it is distinct from neoplastic transformation. The reverse process of EMT is termed mesenchymal-to-epithelial transition (MET).

1. The difference between epithelial cells and mesenchymal cells (Fig.1a&b):

Characteristics of Epithelial Cells:

• Typically a sheet 1 cell thick.
• Individual cells abutting each other.
• Regularly spaced cell junctions and adhesions between neighboring cells.
• Tight adhesion between cells resulting in inhibition of movement away from the monolayer.
• Enclose a 3-dimensional space within.
• Gives structural definition and rigidity.
• Epithelial sheet is polarized.
• Apical and basal surfaces often very different.
• Adheres to different substrates.
• Has different function.
• Movement of epithelial cells is done en block with the motive force usually generated within the sheet by the sum of the cells’ shape changes. e.g: during intestinal cell renewal.

Characteristics of Mesenchymal Cells:

• Lack regimented structure.
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- Weak adhesions which allow for ease of mobility.
- Forms irregular structures that are not uniform in composition or density.
- More extended and elongated in shape.
- Lacks rigid topological specialization (no compartments).
- Cells move as individuals, not en block, often leaving a trailing region behind.
- Migration mechanistically different and more dynamic.
- Filopodia and front end back end polarity (front-rear), migration capacity, cell-cell adhesion, and extracellular matrix interactions: At the front of the cell is the leading edge, Here, actin polymerization in the direction of migration allows cells to extend the leading edge of the cell and to attach to the surface. At the rear of the cell, adhesions are disassembled and bundles of actin microfilaments, called stress fibers, contract and pull the trailing edge forward to keep up with the rest of the cell. Without this front-rear polarity, cells would be unable to coordinate directed migration[6].

Through a bottle-shaped stage, which may have two functions: by constricting their apices cells may displace much of their intracellular contents basally and initiate movement out of the epithelium? Perhaps more important, apical constrictions reduce the amount of non-adhesive apical membrane and circumferential, apical junctions that must finally be broken upon ingestion. It also reduces the size of the hole left in the epithelium. It is generally thought that apical constriction is driven by an actin-myosin-based contraction, while the apical membrane is reduced by endocytosis. Changes in adhesion may also contribute to cell shape change on EMT[9].

These cell shape changes are preceded and/or accompanied by alterations in gene expression including up-regulation of hyperproliferation associated keratin 6 (K6) and K16, retraction of keratin filaments (which normally associates with desmosomes and hemidesmosomes) from the cell periphery, as well as major reorganization of the actin cytoskeletal network (which normally associated with adherens junctions)[9]. Mechanistically, the impact of endocytosis on these processes, which we did not cover in this review, can be rationalized within the same basic principle that posits that the endocytic machinery defines a vast program of intracellular communication that integrates different, apparently distinct, territories of cell regulation, as according to the concept of “endocytic matrix”[11].

II- The events of Change in cell shape (Fig.2):

Change in cell shape, generally by an apical actin-myosin contractile mechanism and/or changes in adhesion.

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Fig. 2: Cytoskeletal changes during EMT[12].

1. De-epithelialization:

It is the loss of the coherent contact between neighbors that characterizes a particular epithelium, and the eventual loss of an apical membrane domain. This involves a loss of the extensive circumferential apical junctions, specifically the circumapical tight and adherens junctions, in the case of epithelia that are physiologically and mechanically very impermeant and coherent. Cell–cell junctions associated with cytoskeletal filaments are the basis of the tensile strength and structural integrity of epithelial tissues. A typical epithelial cell contains several major classes of functionally diverse cell–cell junctions: adherens junctions, tight junctions, desmosomes, and gap junctions. Of these, only adherens junctions (AJ) and tight junctions (TJ) are known to play a necessary regulatory role in maintaining the epithelial program[10]. How these processes occur
is not understood. The evidence suggests that targeted endocytosis of epithelial junctions and adhesion molecules may be important and the apical membrane may eventually be completely eliminated by endocytosis.

Cell–cell adhesion is altered, characterized by reduced desmosomal adhesion between cells as well as the reduced presence of adherens junction components such as E-cadherin, leading to appearance of intercellular gaps. Failure to down-regulate desmosomal adhesion, as in mice where protein kinase C is deficient, is associated with delayed wound healing. Down-regulation of adherens junctions and tight junctions, but not desmosomes, has been shown to be mediated by ephrin-B-EphB signaling, as epidermal-specific knockout of both ephrin-B1 and ephrin-B2 results in impaired wound closure, characterized by persistent adherens junctions between cells in the migrating front.

2. Basal lamina disruption:

The profound phenotypic transition in EMT involves the epithelial cells disrupting the basement membrane. Matrix metalloproteinases (MMPs) are known to cleave components of basement membranes, but MMP-basement membrane crosstalk during EMT in vivo is poorly understood. However, MMPs have been reported to play a role in EMT-related processes and a variety of basement membrane fragments have been shown to be released by specific MMPs in vitro and in vivo exhibiting distinct biological activities.

3. Ingression:

It is the withdrawal of the ingressing cell's apex from the epithelial layer and into the deep layer. It differs from de-epithelialization in that a cell could de-epithelialize and not move out of the sheet. Normal ingression is associated with de-epithelialization and adoption of basal mesenchymal characteristics, including an active motility and strong traction on deep tissues or structures, to pull the cell out of the epithelium. Front rear movement: (At the front of the cell is the leading edge, which is often defined by a flat ruffling of the cell membrane called the lamellipodium or thin protrusions called filopodia. Here, actin polymerization in the direction of migration allows cells to extend the leading edge of the cell and to attach to the surface. At the rear of the cell, adhesions are disassembled and bundles of actin microfilaments, called stress fibers, contract and pull the trailing edge forward to keep up with the rest of the cell. Without this front-rear polarity, cells would be unable to coordinate directed migration). A driving force behind this motility is the loss of the polarized cytoskeleton in epithelial cells, and the development of lamellipodia in the advancing edge of the transitioning mesenchymal cells.

4. Progression towards a mesenchymal phenotype (Fig.3):

Specification toward a mesenchymal phenotype initiates many important changes in gene expression and protein function that must all work in concert for a developmental EMT to occur correctly.

Fig. 3: progression towards mesenchymal phenotype during EMT.

1. loss of epithelial cell markers:

loss of epithelial cell markers: It is the first step of EMT during which there is decreased expression of E-cadherin which is responsible for maintaining the epithelial cells’ lateral contacts via adherens junctions, as well as cell adhesion and relative immobility in the tissue. E-cadherin downregulation is also mediated through upregulation of vimentin, an intermediate filament that decreases E-cadherin trafficking to the cell surface.

2. The cell then progresses towards a mesenchymal phenotype:

by gaining mesenchymal markers and capabilities. This change is orchestrated by temporally regulated expression of the following proteins. Vimentin (intermediate filament, in favor of the cytokeratin, that decreases E-cadherin trafficking to the cell surface. Neural cadherin (N-cadherin): adhesion molecules. Matrix metalloproteinases (MMPs): responsible for the metabolism of extracellular matrix (ECM) component of connective tissue. During this process, the continued enhanced expression of MMPs, released by a variety of cells (macrophages, keratinocytes, endothelial cells, and fibroblasts) plays crucial roles in degrading substrates of the provisional wound matrix. Integrin: Matrix receptors interact with extracellular matrix (ECM)
components are then upregulated to increase motility and Fibronectin: extracellular matrix (ECM) components\(^{24}\).

Cell–matrix adhesion alterations occur to facilitate migration. Specific changes include redistribution of α2β1, α3β1, and α6β4 integrins (receptors for collagen or laminin) on keratinocyte surface, activated expression of α5β1, αvβ6, α9β1, and αvβ5 integrin (receptors for fibronectin, tenascin, or vitronectin), as well as increased metalloproteinase activity that facilitates keratinocyte migration by promoting ECM remodeling and hemidesmosome breakdown\(^{25}\). Elevated expression of vimentin and fibroblast-specific protein 1 (FSP1) has been noted in the migrating epithelial tongues of acute wounds of thermal burn patients and in hypertrophic scars\(^{26}\). In a recent study, the spatiotemporal profile of keratinocyte migration and proliferation during wound healing in mouse tail was carefully dissected, again showing that these cellular events can be uncoupled\(^{27}\).

Gene expression analysis of the migrating leading edge revealed an enrichment of genes involved in cell migration (e.g., metalloproteinases) and cell adhesion (e.g., protocadherins, α5-integrin, desmosome, and gap junction proteins). Genes controlling cytoskeleton and actin remodeling (e.g., actin regulators, myosin, and tubulin) are also part of the leading edge molecular signature, consistent with epidermal migration being driven by actin-myosin filaments that generate traction forces and actin polymerization that generates protrusions\(^{28}\).

**III. Partial EMT:**

It is noteworthy that EMT process may not always be a complete. In some instances, cells may exist along a gradient where incomplete transition occurs, and both epithelial and mesenchymal characteristics are exhibited by the same cell\(^{29}\). There are several reports describing a partial or incomplete EMT phenotype of advanced carcinomas, displaying some mesenchymal features, but with a retention of well-differentiated epithelial-cell characteristics\(^{30}\). When the epithelial cell markers continue to be expressed, but the mesenchymal cells markers have been already obtained, such cells possibly represent the intermediate stage of EMT, or namely a partial EMT\(^{31}\).

The simple fact that these cells are at the edge of the sheet endows them with a distinct polarity: on one side they contact other cells, and on the other they find a free edge. This difference is reflected in their apico-basal polarity; whereas the leading edge cells maintain apico-basal polarity and junctions at the site of cell-cell contact, these are lost from the free edge resulting in a cell that is only partially apico-basal polarised. This can be seen by an absence of staining for certain polarity proteins, such as Discs large and Crumbs, or E-cadherin. Thus, using current terminology, cells at the leading edge would be described as ‘partially’ undergoing a ‘partial EMT, however, within our framework, epithelial leading cells can be considered to be acquiring certain mesenchymal features\(^{7}\).

Partial EMT is also possible, and this occurs when ≥1 of the key characteristics of complete EMT are not exhibited, such as loss of cell–cell contact. For example, during re-epithelialization of cutaneous wounds, keratinocytes undergo a series of changes reminiscent of EMT, including loss of polarity, rearrangement of the actin cytoskeleton, alterations in cell–cell contacts, and breakdown of basement membrane; however, these cells retain some intercellular junctions and migrate as a cohesive cell sheet\(^{24}\). However, angiogenic EC do not usually separate from their neighbors, suggesting that angiogenesis may involve a partial EndoMT\(^{32}\) (Fig. 4&5). Also, partial EMT was reported to taking place during wound healing and mammary tubulogenesis\(^{33}\).

**Fig. 4 : Partial EMT\(^{32}\).
### IV- EMT Importance:

It is integral in:

1. Embryonic development: Epithelium is the tissue phenotype of early embryos and a second tissue type, however, is produced by epithelial-mesenchymal transformation. Both types can form all the three germ layers, ectoderm, mesoderm and endoderm.

2. Wound healing.

3. Stem cell behavior.

4. Contributes pathologically to fibrosis and cancer progression.

#### Types of EMT (Fig.6):

EMT is often divided by biological context into three subtypes:

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The three types of EMT have a shared outcome: the production of motile cells with a mesenchymal phenotype from otherwise classically adherent epithelial cells with apical-basal polarity. However, in contrast to Types I and III, Type II EMT is induced exclusively by damage and inflammation. The type 2 EMTs associated with wound healing, tissue regeneration, and organ fibrosis are of a second type. In these, the program begins as part of a repair-associated event that normally generates fibroblasts and other related cells in order to reconstruct tissues following trauma and inflammatory injury. EMT has been proposed as the critical mechanism for the acquisition of malignant phenotypes by epithelial cancer cells.
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**EMT Role in physiological tissue healing**

Several studies support the importance of proper execution of EMT in achieving successful tissue repair following injury and EMT was reported to play a role in both physiologic and pathologic healing.

### I. EMT and wound healing:

Wound healing consists of several overlapping phases that involve an injury-induced inflammatory response which is associated with cellular proliferation, migration, and ECM remodeling. In a thermal burn wound, myofibroblast formation and keratinocyte reepithelialization both rely on EMT. (Fig. 7)

Of these processes, the one most reminiscent of EMT is the process of re-epithelialization, which has been termed “partial EMT”. As discussed above, a hallmark of EMT is cell-cell dissociation and acquisition of motility, and during re-epithelialization keratinocytes at the wound edge lose their intercellular adhesions and migrate across the wound. Specifically, these keratinocytes undergo changes in junctional complexes including reduction in desmosomes and adherens junctions, disruption of intermediate filaments, and cytoskeletal reorganization which results in the creation of intercellular gaps. These changes enable the keratinocytes to shift morphologically from cuboidal and stationary to flattened and migratory, with extended lamellipodia. Zebrafish keratocytes in explant culture, which serve as a well-studied model of epithelial wound healing, were reported to display evidence of EMT.

A recent study pioneered the use of intravital imaging of wound re-epithelialization in live mice to examine its spatiotemporal cellular dynamics (Park *et al.*, 2017). This work not only re-enforced the accepted notion of spatially separated migratory and proliferative zones, but also discovered the existence of a so-called mixed zone where migration and proliferation co-exist. More specifically, cells change their shape from being polarized cuboidal to being more flattened and elongated with extended cytoplasmic projections (Fig. 8).

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![Fig. 6: Types of EMT](image)

**Fig. 6:** Types of EMT.

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![Fig. 7: EMT in skin wound healing](image)

**Fig. 7:** EMT in skin wound healing.

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![Fig. 8: Partial EMT during cutaneous wound healing](image)

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There is also evidence that myofibroblasts, the key players in the remodeling and maturation phase of wound healing, are derived from resident epithelial cells that have transformed through EMT to synthesize ECM components and contract the wound bed, enabling approximation of the injured edges. EMT is a component of cutaneous wound healing, during which otherwise stationary keratinocytes - the resident skin epithelial cells - migrate across the wound bed to restore the epidermal barrier. For instance, during cutaneous wound healing, epidermal keratinocytes undergo EMT by losing their adherent epithelial phenotype to become motile cells with a mesenchymal phenotype which migrate across the wound bed.

Angiogenesis causes changes that allow for greater nutrient delivery over a long period of time. Capillaries are designed to provide maximum nutrient delivery efficiency, so an increase in the number of capillaries allows the network to deliver more nutrients in the same amount of time. A greater number of capillaries also allows for greater oxygen exchange in the network.

Langerhans cells in skin express a broad range of epithelial-like adhesive molecules that permit the functional integration into the keratinocyte layer. This includes tight junction proteins, such as claudin-1 and ZO-1/Met signaling in skin resident DCs including LCs appears to be a critical determinant for maintaining normal immune function and as an important constituent that interlaces tissue regenerative functions with the appropriate immune responses that must be accomplished.

II. Cardiac repair:

EMT is required to invade the regenerating myocardium and facilitate coronary neovascularization. In post-ischemic injury, EndMT involves both the endocardium (the inner endothelial layer of the heart) and the microvascular endothelium of the heart. It was reported that EMT and EndMT (endothelial mesenchymal transition) contribute to the pool of cardiovascular progenitor cells that maintain cardiac homeostasis.

Besides the connection of mesenchymal transition to the emergence of stem cells, both EMT and EndMT are recognized as important mechanisms in the generation of the myofibroblasts that take part in fibrosis. Therefore, it is possible that EMT and EndMT contribute to both cardiac regeneration and fibrosis after injury. In the last few years a number of studies have provided supportive evidence for this notion. For example, EndMT in the adult mouse heart gives rise to myofibroblasts that migrate and produce scar tissue in mouse models of pressure overload and chronic allograft rejection, recapitulating pathways that take place during formation of the atrioventricular cushions in the embryonic heart. Moreover, isolated adult epicardial cells can undergo EMT in culture, differentiating to smooth muscle and endothelial cells. Epicardial cells that express stem cell markers are induced after injury, migrate to the infarct, and contribute to both cardiac and vascular cell types that suggest a function of epicardial cells that echoes their role in the regeneration of the adult zebrafish heart after partial resection. Therefore, both endothelial and epicardial cells become activated after injury and give rise to cardiac, vascular and myofibroblast/smooth muscle cells; this seems similar to their capacity during development. Myocardial injury indicates that a subpopulation of epicardial cells undergo EMT to regenerate the damaged epithelial cover and help establish new vasculature.

III. Different organs repair:

There is additional evidence for EMT occurring during repair of organs other than the skin. During in vitro healing of a breast (mammary) epithelial cell line, time-lapse microscopy indicated that EMT-associated vimentin was expressed in a migration-dependent fashion, such that vimentin was exclusively induced in actively migrating cells at the leading wound edge, which was accompanied by actin filament reorganization. Vimentin expression subsequently disappeared once wound closure was achieved. During puberty, mammary gland tissue utilizes EMT to facilitate branching morphogenesis through which the developing gland migrates into and invades the fat pad.

Similarly, in a murine model of lacrimal gland injury, inflammation induced by interleukin-1 (IL-1) injection triggered the generation and migration of cells with mesenchymal features to the site of injury, which subsequently reverted to an epithelial phenotype once repair was complete.

In addition, in each menstrual cycle the ovarian surface epithelium undergoes an EMT-like process during postovulatory wound healing. This EMT is induced by epidermal growth factor (EGF) and involves the activation of metalloproteases and of ILK and ERK kinases.

IV. EMT Confers Stem Cell Properties:

The cells that undergo EMT acquire stem cell-like properties. EMT induces stem cell properties, preventing apoptosis and senescence and contributing to immunosuppression. EMT also yields adult cells with stem cell characteristics. It is suggested that EMT causes cells into active and de-differentiated state and acquire stem cell-like characteristics.

Although further analyses are necessary to determine whether EMT in normal tissues leads to the production of...
normal stem cells, this intriguing concept is supported by studies on embryonic stem (ES) cells and mesenchymal stem cells. EMT is observed at the periphery of human ES cell clusters grown on matrigels[19]. These undifferentiated mesenchymal cells are characterized by a shift from E- to N-cadherin expression, the expression of Snail factors, vimentin, and metalloproteases. These cells also retain the expression of several totipotent transcription factors, including Oct-4 and Nanog, indicating that ES cells can adopt a mesenchymal phenotype without loosing their pluripotency[22].

Induction of an EMT in immortalized human mammary epithelial cells (HMLEs) results in the acquisition of mesenchymal traits, but in addition the expression of stem-cell markers, and an increased ability to form mammospheres, a property associated with mammary epithelial stem cells. Independent of this, stem cell-like cells isolated from HMLE cultures form mammospheres, differentiate into duct-like structures, and express markers similar to those of HMLEs that have undergone an EMT. Moreover, stem-like cells isolated either from mouse mammary glands, human reduction mammaplasty tissues, or mammary carcinomas express markers associated with cells that have undergone an EMT. Finally, transformed human mammary epithelial cells, that have undergone an EMT, forms mammospheres, soft agar colonies, and tumors more efficiently. These findings illustrate a direct link between the EMT and the gain of epithelial stem-cell properties[15].

Conversely, it is suggested that EMT causes cells into active and de-differentiated state and acquire stem cell-like characteristics[26]. EMT also contributes to tissue repair, but it can adversely cause organ fibrosis and promote carcinoma progression through a variety of mechanisms[28].

**Role of EMT in pathological healing**

1. Wound scaring:

   While EMT is necessary for proper re-epithelialization and extracellular matrix deposition, uncontrolled continued transition from epithelial cells to myofibroblasts may result in fibrosis. EMT in generating myofibroblasts from resident epithelial cells during the maturation phase of wound healing. Sustained EMT was reported to be a key mechanism underlying the fibrotic pathology of multiple organs including the skin. The role of EMT in pathophysiology of renal, pulmonary, cardiac and liver fibrosis, cutaneous scleroderma, and impaired wound healing are also discussed. In the setting of organ fibrosis, type 2 EMTs can continue to respond to ongoing inflammation, leading eventually to organ destruction. Tissue fibrosis is in essence an unabated form of wound healing due to persistent inflammation. However, EMT also plays a role in the development of scarring and fibrosis, as the matrix-producing myofibroblast arises from cells of epithelial lineage in response to injury but is pathologically sustained instead of undergoing MET or apoptosis. However, pathologically prolonged myofibroblast activity results in fibrogenesis[31].

   **EMT Characteristics in Hypertrophic Scars Are Associated with Persistent Inflammation and Fibrosis:**

   In normal wound healing, many myofibroblasts undergo apoptosis and disappear once re-epithelialization is complete[60]. An association was previously reported between EMT and inflammation and fibrogenesis in human hypertrophic scars. An active hypertrophic scar is denoted by the presence of infiltrating lymphocytes and numerous antigen-presenting cells[61]. Active scars are also characterized by their elevated appearance consisting of thick epithelium and condensed dermis with ample myofibroblasts and interstitial matrix fibers. Among the inflammatory group, TNF-α, MMP-9, and MMP-13 were significantly increased 2-, 14-, and 8-fold, respectively, in the scars compared normal skin. The classic fibrotic markers, type-I collagen and α-smooth muscle actin, were elevated 8- and 2-fold, respectively, in the scars versus normal skin. Finally, the EMT-related transcription factors slug and twist-1 were significantly expressed in the scars. However, E-cadherin, which is down-regulated in EMT of many epithelial cells cultured in vitro, is not significantly reduced at global level in the scar tissues, the fact that is explained by epithelial hypertrophy, which may compensate globally for the loss of E-cadherin in defined EMT locus. In contrast, gain of mesenchymal markers such as vimentin and N-cadherin was clearly increased in the scars. Together, these results demonstrate a clear association between unresolved inflammation and persistent EMT features in the developing scars, indicating a possible causal relationship. In this way, a link was demonstrated between unresolved inflammation and the development EMT characteristics during fibrogenesis in hypertrophic scar tissue in vivo[59].

   Scleroderma (Sc) is a systemic disorder characterized by autoimmunity, chronic inflammation, vasculopathy and extensive skin and organ fibrosis of unknown etiology[40]. In Sc, early vascular injury precedes fibrosis, and as with renal fibrosis, the persistently activated myofibroblast drives TGFβ-induced gene expression and increases pro-fibrotic cytokine and protease production. Although the origin of the myofibroblasts in Sc fibrotic skin is unknown, studies have once again indicated that the EMT process is one possible source[57].

2. Cardiac and other organs fibrosis (Fig. 9):

   Organ fibrosis, which occurs in a number of epithelial tissues, is mediated by inflammatory cells and fibroblasts that release a variety of inflammatory signals as well as
components of a complex ECM that includes collagens, laminins, elastin, and tenacins. More specifically, such EMTs are found to be associated with fibrosis occurring in kidney, liver, lung, and intestine\textsuperscript{[28,29]}. Some of the earliest proof of this came from the study of transgenic mice bearing germ-line reporter genes whose expression was driven by epithelial cell–specific promoters. The behavior of these reporters provided direct evidence for epithelial cells serving, via EMTs, as important precursors of the fibroblasts that arise during the course of organ fibrosis\textsuperscript{[30]}.

Such cells continued to exhibit epithelial-specific morphology and molecular markers, such as cytokeratin and E-cadherin, but showed concomitant expression of the FSP1 mesenchymal marker and α-SMA. Such cells are likely to represent the intermediate stages of EMT, when epithelial markers continue to be expressed but new mesenchymal markers have already been acquired. The behavior of these cells provided one of the first indications that epithelial cells under inflammatory stresses can advance to various extents through an EMT, creating the notion of “partial EMTs”\textsuperscript{[38]}.

Recent experiments in mice have demonstrated that endothelial cells associated with the microvasculature can also contribute to the formation of mesenchymal cells during the course of fibrosis, doing so via an analogous process known as EndMT\textsuperscript{[63]}.

In cardiac fibrosis associated with post-ischemic injury of the heart, EndMT, which involves both the endocardium (the inner endothelial layer of the heart) and the microvascular endothelium of the heart, has been demonstrated to play a key role in contributing to the emergence of newly formed fibroblasts\textsuperscript{[29]}. In tissue culture models of EndMT, TGF-β1 induces EndMT of capillary endothelial cells and the loss of endothelial markers, such as CD31 and integrin αVβ3, as well as the acquisition of fibroblast- and myofibroblast-specific markers such as FSP1, α-SMA, DDR2, collagen I, and vimentin. While not yet documented, it is plausible that many of the molecular regulators of EMTs also play critical roles in orchestrating EndMTs\textsuperscript{[38]}.

Type 2 EMTs are associated with organ fibrosis and regeneration occurring in the liver, lung, kidney and intestine. FSP1, α-SMA and collagen I are the characterized markers of the mesenchymal products generated by the EMTs during the development of organ fibrosis\textsuperscript{[64]}. The aforementioned markers, along with vimentin, desmin and discoidin domain receptor 2 (DDR2), have been used to distinguish the epithelial cells that are undergoing EMTs in response to ongoing inflammation. With the development of EMTs, these cells continue to exhibit epithelial-specific morphology and molecular markers, such as E-cadherin and cytokeratin, but showed concomitant expression of FSP1 and α-SMA\textsuperscript{[31]}.

Another source of myofibroblasts during fibrosis has been hypothesized to originate from endothelial cells by a process of endothelial-mesenchymal transition (EndoMT).

This event has been characterized in animal models of fibrosis; however, similar phenomena in human fibrosis patients have not yet been well characterized\textsuperscript{[66]}.

Different sources of fibroblasts in organ fibrosis. Four possible mechanisms are depicted. One study suggests that about 12% of fibroblasts are from bone marrow, about 30% can arise via local EMT involving tubular epithelial cells under inflammatory stress, and about 35% are from EndMT. The remaining percentage likely emerge via proliferation of the resident fibroblasts and other still unidentified sources (Fig.8)\textsuperscript{[67]}.

\(\approx30\% \) to 50% of fibroblasts coexpressed the EC marker CD31 along with markers of myofibroblasts and fibroblasts, including fibroblast-specific protein-1 and α-smooth muscle actin. Lineage tracing experiments confirmed the EC origin of these cells\textsuperscript{16}. More recent work has suggested that only \(\approx10\%\) of the myofibroblasts present in kidney fibrosis derive from an EndoMT, whereas the remainder come from proliferation of local fibroblasts and differentiation from bone marrow cells\textsuperscript{17}. Other studies, however, have suggested that these fibroblasts may be derived from pericytes\textsuperscript{18}. It should be borne in mind, however, that these are all mouse studies and strain-specific differences are always a possibility. Other fibrotic diseases where EndoMT has been implicated as a source of fibroblasts/stromal cells include intestinal fibrosis\textsuperscript{19} and scleroderma\textsuperscript{[32]}.

In fibrotic tissues, myofibroblasts accumulate and secrete an excessive amount of collagen that is deposited as fibers, thereby compromising organ function and leading to its failure. Fibrosis had been thought to originate through
the pathological activation of interstitial fibroblasts that convert to myofibroblasts to form the fibrotic collagen network. However, elegant cell tracing studies have shown that a significant portion of these myofibroblasts arise from the conversion of epithelial cells through an EMT process[80]. Initially demonstrated in differentiated cells of renal tubules and ducts, it is now clear that lens epithelium, endothelium, hepatocytes, and cardiomyocytes can all undergo EMT and contribute significantly to tissue fibrosis. Indeed, lineage-tracing in transgenic mice also indicates that hepatocytes undergo EMT during CCL4-induced liver fibrosis[79], as do the alveolar epithelial cells during the pulmonary fibrosis induced by TGFβ[80]. Interestingly, hepatocytes derived from cirrhotic livers also display characteristics of EMT, which has implications for the progression to hepatocellular carcinoma[80].

EMT involving transformation of endothelial cells into mesenchymal cells is evident during cardiac and renal fibrosis (EndMT)[80]. Mesothelial cells are also converted to mesenchyme in patients that receive ambulatory peritoneal dialysis and that develop peritoneal fibrosis[80].

Type 2 epithelial to mesenchymal transition (EMT) is known as one of the key mechanisms of intestinal fibrosis. Activated myofibroblasts, which are key effector cells in intestinal fibrosis and produce excessive amounts of collagen-rich extracellular matrix, can be derived from epithelial cells by EMT[71].

3. EMT and cataract:

Furthermore, the EMT undergone by lens epithelial cells contributes to capsular opacification after cataract surgery[72]. There is compelling evidence implicating epithelial-mesenchymal transition (EMT) in the pathogenesis of anterior subcapsular cataract (ASC) and PCO. During EMT, polarized lens epithelial cells (LECs) relinquish their cobblestone morphology and transdifferentiate into spindle-shaped myofibroblastic cells that elongate and migrate across the lens capsule. Transforming growth factor-β (TGFβ) has been studied extensively as a key inducer of this lens EMT in vitro[71].

4. Endometriosis:

Estrogen induces an epithelial-mesenchymal transition (EMT) during implantation of the ectopic endometrium, which consists of stromal and epithelial cells. EMT is a process whereby epithelial cells convert to a mesenchymal phenotype, and EMT may be essential for the migration, invasion, and relocalization of epithelial cells[76]. EMT can be induced by a number of signals, including the acute stress response[70]. Stimulating signals such as hypoxia and estrogen activate the origination and migration of endometriotic lesions. Basic pathways of EMT, such as TGF-β and Wnt, could provide potential therapeutic approach to inhibit endometriotic cell motility and invasiveness[76].

MET (Reversed EMT) in healing

EMT is now considered a transition suggesting a transient and reversible process[51]. The reverse process of EMT is termed mesenchymal-to-epithelial transition (MET). Conceivably, the adhesive and cytoskeletal changes that occur in the leading edge must be kept in check so that migrating epithelial cells are able to eventually resume their full epithelial state to execute a terminal differentiation program to regenerate a stratified epithelium (Fig. 10)[77].

Epithelial-to-mesenchymal transition (EMT) is a reversible process that controls the phenotypic changes due to cell mobility[78]. Experimental evidence has shown that the removal of EMT inducers results in the reversal of EMT and EMT-associated phenotypes[51]. Reverse epithelial-mesenchymal transition was reported to contribute to the regain of drug sensitivity in lung cancer cells[79].

Type 1 EMTs can generate mesenchymal cells (primary mesenchyme) that have the potential to subsequently undergo a MET to generate secondary epithelia[78].

The undergoing of MET or apoptosis are important during wound healing to prevent the development of scarring and fibrosis in response to injury as the persistence of inflammation and EMT leads to the development of active scars which is characterized by their elevated appearance consisting of thick epithelium and condensed dermis with ample myofibroblasts and interstitial matrix fibers[80]. Indeed, E-cadherin returns to normal levels soon after the two migrating fronts mee[77]. Moreover, the expression of genes within the leading edge signature decreases as wound re-epithelialization progresses, and disappears upon fusion of the two edges whereas proliferation is resumed at the wound center[77]. Furthermore, loss of a desmosomal component Perp leads to impaired re-epithelialization due to enhanced keratinocyte migration while proliferation is unaffected[80]. These findings implicate the transient and reversible nature of the molecular/cellular events that occur during wound reepithelialization. However, a systematic comparison between the reverse events in the neoeipidermis and MET has not yet been performed[81]. However, the depletion of vimentin delays wound healing by mesenchymal fibroblasts trans-differentiating into epithelia through MET[81].

MET was reported to have a role in endometrial regeneration and have important implications for proliferative diseases of the endometrium[81,82].

MET is involved in stem cell inactivation, cell polarization and differentiation. This process is associated with a reduction of vimentin and accumulation of CK8/18 in stem cells. Furthermore, the variation of vimentin in stem cells is an indicator of cell proliferative activity[81].
Systemic administration of recombinant BMP-7 to mice with severe fibrosis resulted in reversal of EMT and repair of damaged epithelial structures, with repopulation of healthy epithelial cells, all presumably mediated via a MET[62].

Metastasizing cancer cells must shed their mesenchymal phenotype via a MET during the course of secondary tumor formation this behavior with the proposed role of EMT as a facilitator of metastatic dissemination requires the additional notion that metastasizing cancer cells must shed their mesenchymal phenotype via a MET during the course of secondary tumor formation[63]. These considerations indicate that induction of an EMT is likely to be a centrally important mechanism for the progression of carcinomas to a metastatic stage and implicates MET during the subsequent colonization process[60].

**Molecular mechanisms of EMT**

A better understanding of epithelial plasticity regulation during wound healing has important clinical implications. Managing chronic wounds represents major health care costs, and our ability to manipulate such plasticity holds promise in improving wound repair in human patients. Insights from wound studies are likely also applicable to cancer research, where EMT has been considered a major contributing factor to metastasis and/or chemoresistance, and to tissue fibrosis, which is shown to be associated with enhanced/prolonged EMT[3].

**Growth factors: (Fig. 11)**

Several growth factors were reported to be common in both wound healing and EMT, such as fibroblast growth factor (FGF), hepatocyte growth factor (HGF), epidermal growth factor (EGF) and transforming growth factor-beta (TGFβ) and keratinocyte growth factor (KGF)[35and39].

FGF, is increased in the acute wound and plays a role in granulation tissue formation, epithelialization, and tissue remodeling[83]. In vitro studies have shown that activation of the FGF receptor increases keratinocyte and fibroblast motility[84], and stimulates fibroblasts to produce collagenase[85]. The FGF family is also induced during EMT, with the role to ensure that epithelial cells adopt a mesenchymal phenotype through classic effects such as down-regulation of E-cadherin and catenins and induction of mesenchymal MMPs[86].

EGF signaling leads to the activation of a number of converging signaling pathways promoting keratinocyte migration and proliferation[87]. EGF also aids to accomplish EMT by down-regulating E-cadherin via E-cadherin internalization.

HGF signaling is an additional example of the wound
Epithelial mesenchymal transition and tissue healing

healing – EMT crosstalk. HGF, mainly produced by fibroblasts, exerts its function by binding to its tyrosine kinase receptor c-Met (mesenchymal epithelial transition factor, or HGFR), which is expressed on the surface of keratinocytes[88]. Both HGF and c-Met are upregulated during wound healing and promote granulation tissue formation and neoangiogenesis[89].

TGFβ progresses via two pathways, SMAD-dependent and SMAD-independent. In SMAD-dependent pathways, the TGFβ cell surface receptors (known as TGFβ receptors type II) are activated by ligand and phosphorylate the transmembrane kinases (TGFβ receptor type I), which then forms a SMAD complex; this complex can enter the nucleus, subsequently activating or inhibiting transcription factors important for either wound healing or EMT. In wound healing, TGFβ1 play important roles in inflammation, angiogenesis, re-epithelialization, and connective tissue regeneration[90].

Other developmental signaling pathways such as Wnt, Hh, and Notch have also been implicated in wound healing[91]. A functional involvement of Wnt signaling has been shown for hair follicle regeneration in large wounds[92].

Osteopontin (OPN) is a secreted glycoprotein also known as secreted phosphoprotein that has recently been implicated in all three processes that involve EMT: embryogenesis, wound healing/fibrosis, and tumorigenesis. OPN is known to bind to multiple integrin receptors. With regard to wound healing, OPN is upregulated during the inflammatory phase and plays several key roles in wound healing. TGF-β is a key cytokine involved in many elements of wound healing, including the induction of EMT via many pathways. OPN appears to play an important role in TGF-β-dependent processes and likely is involved in TGF-β-mediated EMT[90].

The intracellular calcium signal was reported to play an important role in the induction of EMT in human breast cancer cells. Manipulation of calcium-signaling pathways controlling EMT induction in cancer cells may therefore be an important therapeutic strategy for preventing metastases[93].

After the first 2 to 3 days of the treatment of TNF-α or TGF-β, epidermal keratinocytes isolated from adult human skin, cells displayed spindle-shaped body and “long-armed” intercellular connections, demonstrating morphological plasticity of epithelia cells under stress. this transition of cell morphology from polygonal to spindle shapes is reversible. In the prolonged exposure to the cytokines for 4 to 5 days, the epithelial cells were scattered from patched clones and stably express mesenchymal-markers. Under TNF-α or TGF-β, most of the epithelial cells expressed vimentin, an intermediate filamentous cytoskeleton involved in migration of mesenchymal cells[94].

Transcription factors: (Fig.12)

This switch in cell differentiation and behavior during EMT, is mediated by key transcription factors, including SNAIL, zinc-finger E-box-binding (ZEB) and basic helix-loop-helix transcription factors, the functions of which are finely regulated at the transcriptional, translational and post-translational levels[1].

The EMT-promoting transcription factors includes Snail (SNAI1), Slug (SNAI2), Zeb1, Zeb2, and Twist, and EMT-inhibiting transcription factors, such as Grhl2, Ovol1, and Ovol2[9,95]. Wound healing in mouse embryos is distinctly different from that in adult animals, particularly in

Fig. 11: Growth factors for EMT (Stone et al., 2016).
that embryonic wounds heal perfectly without scarring\textsuperscript{[96]}. A possible explanation for regeneration in embryos vs. scar formation in adults is the absence of inflammation during embryonic wound healing in mice\textsuperscript{[96]}.

To begin with, HGF can regulate master EMT transcription factor SNAIL1 (which decreases E-cadherin) and Slug (which decreases desmoplakins) aiding in the breakdown of intercellular adhesions\textsuperscript{[97]}. To start with, the EMT transcription factor Slug has been implicated in the process of re-epithelialization in numerous studies. Healing of excisional wounds is impaired in Slug knockout mice almost twofold in comparison to wild-type controls\textsuperscript{[98]}. In ex vivo skin explants from Slug null mice, epithelial cell outgrowth is also severely impaired, again indicating compromised motility\textsuperscript{[99]}. Indeed, Slug expression is elevated in wild-type keratinocytes at the edges of murine wounds in vivo\textsuperscript{[100]} and its expression specifically increases in the actively migrating mouse keratinocytes\textsuperscript{[101]}.

TGF\(\beta\) and SMAD complexes induce SNAIL1 expression, and themselves are potent downregulators of E-cadherin, occludin, and other epithelial phenotypic markers, while promoting mesenchymal markers such as vimentin and N-cadherin\textsuperscript{[102]}. Mechanistically, Slug regulates keratinocyte motility during re-epithelialization by repressing E-cadherin, leading to decreased cell-cell adhesion\textsuperscript{[103]}.

The epidermal growth factor receptor (EGFR) signaling pathway that is integral to re-epithelialization in physiologic wound healing may be the master regulator of EMT/Slug-mediated effects, since EGFR ligands stimulate the expression of Slug as well as subsequent migration in keratinocytes in a process that is mediated by ErkS\textsuperscript{[99]}.

Foxn1, a potent mammalian wound healing factor, also appears to be involved in EMT-driven re-epithelialization during repair, as evidenced by studies in Foxn1 transgenic mice. In these mice, the induction of EMT post-wounding was demonstrated though the upregulation of EMT transcriptional regulator Snail1, increased MMP9 expression, presence of vimentin+/E-cadherin+ cells, and migratory keratinocytes at the wound edge expressed Foxn1 which co-localized with Snail\textsuperscript{[104]}.

Similarly, antimicrobial peptides shown to enhance wound healing concurrently induce Slug at the edge of wounded HaCaTs\textsuperscript{[105]}. These data reinforced the idea that biological responses to TGF-\(\beta\) result from extensive cross talk of different signaling pathways. They further argue that harnessing those networks, such as the endocytic matrix that integrates multiple cellular functions is instrumental to achieve plasticity of cell identity, as typified by EMT\textsuperscript{[106]}. Moreover, Slug is the only well-known EMT-inducing transcription factor for which a function in wound re-epithelialization has been shown\textsuperscript{[3,29]}.

Wnt signaling is a central regulatory knot that controls two of the landmark events in the biology of stem cells, EMT and cell cycle regulation. Interestingly, Wnt signaling also regulates fibrosis, controlling the generation of mesenchymal cells by EMT as well as their proliferation and motility. Collectively, these data suggest that proper modulation of Wnt signaling is key in balancing cardiac fibrosis and regeneration after injury\textsuperscript{[49]}.

Snail is associated with the activation of immunosuppressive cytokines. EMT process in general, can accelerate cancer metastasis not only by increasing invasion, but also by acting on multiple immunosuppression and immunoresistance mechanisms, reflecting an alteration in the response of the host to the tumor. Thus, therapies directed to interfere with EMT might not only be anti-invasive but also able to restore immunocompetence in patients\textsuperscript{[32]}. 
Fig. 12: EMT molecular mechanisms (https://www.regmednet.com/users/19900-bio-techne/posts/13565-getting-to-know-emt-re-epithelialization-during-wound-healing)
EMT markers:

In the prolonged exposure to the cytokines for 4 to 5 days, under TNF-α or TGF-β, most of the epithelial cells expressed mesenchymal-markers vimentin, an intermediate filamentous cytoskeleton involved in migration of mesenchymal cells. Vimentin fibers were observed in the perinuclear regions, whereas cortactin, an organizer of filamentous actin, was found in the outreaching lamellapodia. These features are prominent characteristics of mesenchymal cells, demonstrating plasticity of the epithelial cells under the injury signals. Induction of vimentin protein was also measured by Western blot analysis, which is consistent with its mRNA levels. Expression of MMPs is another benchmark of EMT to facilitate cell migration. Expression of multiple MMPs by the keratinocytes under the injury signals, such as TNF-α and IL-1, in the keratinocytes may weaken the intercellular adhesions, and provoke ECM degradation to facilitate cell migration.[107]

Using the association of vimentin and β-catenin was suggest for the diagnosis of EMT in renal pathology because it is both sensitive and prognostic, thus satisfying the properties required for a screening test[108].

6- Methods to study EMT

Cell Characterization (Fig. 13): A critical step to studying EMT is being able to clearly distinguish epithelial and mesenchymal phenotypes. Antibodies are readily available against cell surface markers and transcription factors specific to epithelial and mesenchymal phenotypes. A great reference for these markers and corresponding antibodies can be found at Novus Biologicals website. To make it more simple, one-step simultaneous immunofluorescence staining of human epithelial and mesenchymal markers can be performed with the aptly named Human EMT 3-Color Immunocytochemistry Kit from R&D Systems, which features a combination of fluorophore-conjugated antibodies against E-Cadherin (epithelial marker), Snail and Vimentin (mesenchymal markers)[1].

Fibroblast-specific protein 1 (FSP1; also known as S100A4 and MTS-1), an S100 class of cytoskeletal protein, α-SMA, and collagen I have provided reliable markers to characterize the mesenchymal products generated by the EMTs that occur during the development of fibrosis in various organs[48,49,51]. These markers, along with discoidin domain receptor tyrosine kinase 2 (DDR2), vimentin, and desmin, have been used to identify epithelial cells of the kidney, liver, lung, and intestine that are in the midst of undergoing an EMT associated with chronic inflammation[52].

Functional Assays (Fig. 14): A key aspect of EMT as it relates to wound healing is the migration of mesenchymal-like cells into the injury site of injury. Chemotactic assays are a standardized and effective way to monitor the migration of mesenchymal stem cells after EMT induction. These assays enable researchers to assess the efficiency of EMT induction as well as investigate the mechanisms underlying re-epithelialization through migration[109].

Therapeutic application of EMT

EMT induction:

Nevertheless, assessing the presence of the classic EMT biomarkers in non-healing tissues and organs in vivo will be critical to define the role for EMT in initiating and sustaining a poor healing response, and may represent a way forward to potential targeting of EMT as a novel and global therapeutic approach for difficult-to-treat wounds[35].

Several diseases, such as ischemic chronic wounds, are the result of failure or insufficient blood vessel formation and may be treated by a local expansion of blood vessels, thus bringing new nutrients to the site, facilitating repair. Other diseases, such as age-related macular degeneration, may be created by a local expansion of blood vessels, interfering with normal physiological processes[101].

Under physiological and pathological conditions, EMT can be induced by extrinsic factors, such as growth factors and cytokines, or intrinsic regulators, such as Twist1,
Snail and Slug, and a significant proportion of cancer cells exhibit intermediate states of EMT or hybrid EMT\(^{[111]}\).

Lipocalin 2 (LCN2) which is an estrogen-dependent, acute-phase protein is an induced stressor that promotes the epithelial–mesenchymal transition (EMT)\(^{[122]}\).

Cell culture models of EMT are employed to understand the biology of EMT and for identifying and evaluating novel prognostic markers and therapeutics for fibrotic diseases, wound healing, and metastasis. The ability to induce EMT in vitro typically requires TGF-beta stimulation or labor-intensive genetic modification. While TGF-beta stimulation is the most common method used to induce EMT, it is not always sufficient. To more reliably induce EMT, specialized cocktails of recombinant proteins and neutralizing antibodies can be utilized. One such cocktail is the StemXVivo® EMT Induction Media Supplement from R&D Systems. This supplement, which contains an optimized cocktail of proteins and antibodies, is designed for straightforward and quick induction of EMT. (https://www.rndsystems.com/products/epithelial-mesenchymal-transition-products).

**EMT inhibition:**

Because of its potent role of EMT in pathogenesis of fibrotic diseases, detection of EMT in biopsy specimens could be useful diagnostically, and anti-EMT therapy has emerged as a target for drug development\(^{[108]}\). Inhibition of the TGF-β receptor kinase using its specific inhibitor SB431542 blocks TGF-β-induced EMT in pancreatic cancer cells\(^{[113]}\). It was reported that the fibrosis response can be reversed in vivo by inhibition of Snail1 expression in mouse models of kidney injury\(^{[114]}\).

Cells undergoing EMT show a feature similar to cancer stem cells (CSCs), such as an increase in drug efflux pumps and prevent apoptosis and senescence. Therefore, targeting EMT has been considered a novel opportunity to overcome cancer drug resistance\(^{[115,116]}\). The therapies directed to interfere with EMT might be reported to not only be anti-invasive but also able to restore immunocompetence in patients\(^{[12]}\).

While TGFβ induces aberrant lens cell behavior, other growth factors found in the ocular media, such as bone morphogenetic protein-7 (BMP-7) regulate normal cellular processes in the lens. There is a growing body of literature supporting the role of BMP-7 in antagonizing TGFβ-driven EMT\(^{[111]}\). BMP-7 functions as an endogenous inhibitor of TGF-β-induced EMT. Among other effects, it reverses the TGF-β–induced loss of the key epithelial protein, E-cadherin. Systemic administration of recombinant BMP-7 to mice with severe fibrosis resulted in reversal of EMT and repair of damaged epithelial structures, with repopulation of healthy epithelial cells, all presumably mediated via a MET\(^{[64]}\).

Inhibition of TGFβ signaling is a promising strategy to treat the disease. Accordingly, systemic injection of the endogenous TGFβ antagonist BMP7 can revert renal fibrosis in mice\(^{[116]}\). Paricalcitol, a synthetic vitamin D analog that suppresses the expression of TGFβ and of its type I receptor, also attenuates ureteral obstruction-induced renal fibrosis\(^{[117]}\). Inhibition of Snail may perhaps be a more specific alternative to treat kidney disease that would preserve the beneficial effects of TGFβ antagonists\(^{[12]}\). Basic pathways of EMT, such as TGF-β and Wnt, could provide potential therapeutic approach to inhibit endometriotic cell motility and invasiveness\(^{[70]}\). Manipulation of calcium-signaling pathways controlling EMT induction in cancer cells may therefore be an important therapeutic strategy for preventing metastases\(^{[83]}\). MicroRNAs (miRNAs) are non-coding small RNA molecules that post-transcriptionally regulate protein expression via binding to the target genes leading to the regulation of EMT either as an inhibitor or as a promoter\(^{[118]}\).

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