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Review Article

Epithelial-Mesenchymal Transition and Tumour Progression: An Overview

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Abstract

Aim: The purpose of this review is to highlight the role of the epithelial-mesenchymal transition (EMT) in tumorigenesis and assess the value of manipulating this process in cancer therapy. ***Method***: A search was conducted for peer-reviewed articles in PubMed, Google Scholar, and ResearchGate employing keywords and phrases. ***Main Points***: The EMT program is a key driver of tumor initiation and progression. Inhibition of EMT in carcinoma cells can yield fruitful results in the treatment of cancer, particularly in overcoming resistance to chemotherapy and precision drugs. ***Conclusion***: Blocking the process of EMT in carcinoma cells can help overcome the resistance to chemotherapy and targeted precision medicine.

Keywords: epithelial-mesenchymal transition, EMT, mesenchymal-epithelial transition, MET, EMT and tumorigenesis, EMT and cancer drug resistance.

انتقال الخلايا من الشكل الظهاري الى شكل اللحمة المتوسطة وتطور الورم السرطاني : نظرة عامة

الخلاصة

الهدف: الغرض من هذه المراجعة هو تسليط الضوء على دور انتقال الخلايا من الشكل الظهاري الى شكل اللحمة المتوسطة في تكوين الاورام السرطانية وتقييم هذه العملية في معالجة السرطان. **الطريقة:** تم اجراء بحث عن مقالات تمت مراجعتها من قبل الأخصائين في ثلاث ادوات بحث رئيسية باستخدام كلمات وعبارات ملائمة. **النقاط الرئيسية:** برنامج انتقال الخلايا من شكل الى آخر هو المحرك الرئيسي لبدء الورم السرطاني وتطوره. يمكن ان يؤدي تثبيط هذا الانتقال الخلوي في خلايا السرطان الى نتائج مثمرة في علاج هذا المرض خاصةً في التغلب على مقاومة العلاج الكيمياوي والادوية الدقيقة. **الاستنتاج:** يمكن ان يساعد منع انتقال الخلايا من الشكل الظهاري الى شكل اللحمة المتوسطة في خلايا السرطان في التغلب على مقاومة العلاج الكيمياوي والطب الدقيق المستهدف.

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INTRODUCTION

Epithelial cells make up the lining of body organs such as the skin, the respiratory tract, the gastrointestinal tract, the genitourinary tract, and glands like the breast, the prostate, and the pancreas [1]. When these cells turn neoplastic, the result will be a type of tumor called carcinoma, which accounts for about 80% of all cancer cases and the majority of deaths from this disease [2,3]. Commonly occurring carcinomas include those of the lung, breast, colon, pancreas, bladder, kidney, ovary, prostate, and liver. The initial tumor in these organs continues to express the hallmarks of the epithelial state, including the tethering of cells to each other and to the basement membrane, employing adhesion

features such as tight junctions, gap junctions, adherence junctions, actins, desmosomes, and hemidesmosomes, as well as exhibiting a lack of motility (Figure 1) [2,4]. Cells derived from advanced carcinoma, on the other hand, exhibit mesenchymal characteristics, including the expression of biomarkers such as N-cadherin, Fibronectin, and Vimentin, as well as motility and invasiveness (Figure 1) [2].

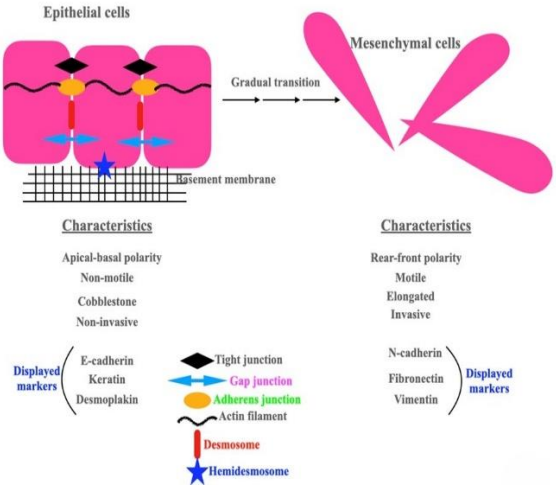

Figure 1: Main features of epithelial and mesenchymal cells.

Moreover, epithelial cells show apical-basal polarity, while mesenchymal cells exhibit rear-front polarity. These features allow epithelial cells to adopt a more cobblestone appearance characterized by a lack of motility, while mesenchymal cells assume more elongated shapes, aiding their motility and invasiveness. In breast cancer, for instance, the acquisition of mesenchymal features has been associated with a more aggressive disease that is more likely to progress [5-8]. In cell lines derived from both human and mouse breast cancers, the depletion of factors that drive the process of EMT inhibits the metastatic dissemination of tumor cells from the primary site. Conversely, the forced expression of these factors was found to enhance the dissemination of cancer cells [9-14]. While epithelial cells are specialized and committed, mesenchymal cells, by contrast, are unspecialized and capable of differentiating into many different cell types. The gradual and reversible move from the epithelial to mesenchymal cell state is called epithelial-mesenchymal transition (EMT), and the process by which mesenchymal cells revert to epithelial cells is termed mesenchymal-epithelial transition (MET) [15-17]. Epithelial-mesenchymal transition plays a crucial function in embryogenesis, where it was originally identified and is now referred to as type I EMT. Later on, it was found that the EMT program has a role in wound healing, fibrosis, tumor progression, and metastasis [18-23]. Type II EMT describes alterations that take place during fibrosis and inflammation, and type III EMT refers to changes that occur in tumor cells [1]. The main focus of this review is type III EMT and its role in tumorigenesis. The ability of multiple EMT regulators to enhance tumor formation and progression presents compelling evidence of the involvement of this process in tumorigenesis [24,25]. However, there is some debate about whether EMT is actually required for neoplastic transformation or primary cancer metastasis [26]. This apparent skepticism arises from the rarity of observing morphological changes indicative of EMT in primary tumor sections and histological similarities between cells derived from distant metastases and their primary cancers. However, the presence of EMT markers at the tumor edge but not in the bulk of the tumor is reasonable evidence that EMT occurs during the development of cancer and that it regulates invasiveness and aggressiveness [25,27,28-30]. Furthermore, the similarity in cell morphology between metastasis-derived tumors and their primary sites may indicate that EMT (or partial EMT) must be followed by MET to aid the colonization of the secondary sites [25,31-33]. Therefore, tumor cells that have undergone EMT can move through the early stages of metastasis, i.e., intravasate the capillary blood vessels at the primary site and extravasate the circulation at the distant organ, but they must change back to the epithelial phenotype to seed a new tumor at the secondary site (see Figure 2). Unlike a binary switch in which cells reside in either epithelial or mesenchymal states, the EMT program allows the acquisition of certain mesenchymal traits while retaining epithelial characteristics. This leads to the graded appearance of cell phenotypes possessing mixed epithelial and mesenchymal attributes [3]. These intermediate cell states, between epithelial at one end of the spectrum and mesenchymal at the other end, are also called hybrid EMT, partial EMT, or incomplete EMT. Various reports point out that cells residing in partial EMT within a tumor exhibit plasticity and heterogeneity, which contribute to cancer progression, metastasis, and resistance to cancer therapies [15,34-37]. The EMT program plays an important role in the regulation of various aspects of tumor progression, including metastasis, metabolic reprogramming, stemness, inflammation, and resistance to cancer therapies [15,34,38-41]. The increased motility of cancer cells, brought about by the execution of EMT, enables the development of invasive phenotypes [38,42,43]. The EMT program also endows cancer cells with stemness, giving them the plasticity needed to transition between EMT and MET [38]. The implication of EMT in the aggressive behavior of neoplastic cells constitutes an opportunity for therapeutic intervention, as seen later in this review.

METHODS

Research articles and reviews published between January 2002 and September 2022 that were peer-assessed were examined in three major search tools: PubMed, Google Scholar and ResearchGate. Publications before January 2002 were also included if the initial reading of the literature suggested that these articles represent significant discovery and/or had a historical value. The keywords and key phrases employed were: epithelial-mesenchymal transition, EMT, mesenchymal-epithelial transition, MET, EMT AND tumorigenesis, EMT AND cancer drug resistance. The Boolean operator highlighted in capitals in the key phrases required both terms, on either side of it, to be in each article returned. From the large number of publications collected, only those relevant to the content of this review were included in the references list. The majority of studies collected were returned by the three search engines employed. Articles with inadequate data analysis or deemed insufficiently covering the subject were excluded.

Orchestrating the EMT program

The EMT program can manifest itself in epithelial cells regardless of whether they are cancerous or otherwise [3]. As already mentioned, EMT is a natural process of crucial importance for early fetal development, wound healing, and fibrosis. However, the EMT program is hijacked by carcinoma cells to get access to distant organs with improved oxygen and nutrient supplies, thus gaining a survival advantage. Following the activation of EMT, the epithelial cancer cells lose their polarity and adhesion and become motile and invasive mesenchymal cells [43,44]. This complex program provides neoplastic epithelial cells with the necessary properties for the dissemination, migration, and seeding of new tumors further away from the primary site [3,32]. The mechanistic elements of the EMT program can be placed in three categories: a) the inducers, which are the upstream growth factors and receptors that initially signal the epithelial-mesenchymal transition, b) the regulators, which are the transcription factors acting downstream of the inducers and upstream of the effectors, and c) the effectors, which are the proteins that execute the task such as eliciting a cellular shape or motility (Figure 2) [45].

Inducers of EMT

To maintain their growth, epithelial cells secrete growth factors such as epithelial growth factor (EGF), hepatocyte growth factor (HGF), fibroblast growth factor (FGF), and transforming growth factor beta (TGF-β) in an autocrine manner. Upon the binding of these growth factors to their respective receptors on the cell surface, a complex series of events is initiated, leading to the induction of EMT.

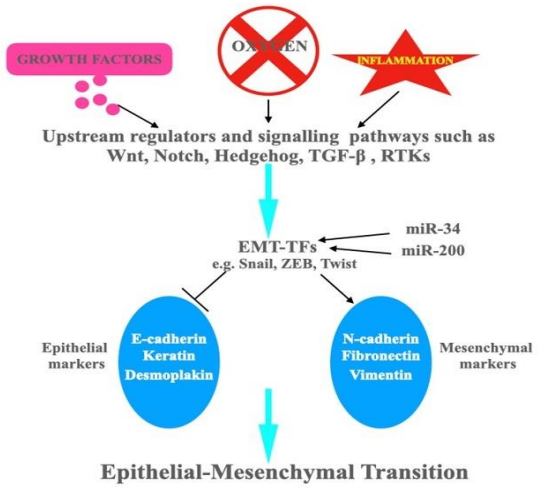

Figure 2: A simplified view of inducers, regulators and signalling pathways of epithelial-mesenchymal transition (EMT). TGF-β= Transforming growth factor-beta, RTKs= Receptor tyrosine kinases, EMT-TFs= Epithelial-mesenchymal transition transcription factors, miR-34= MicroRNA-34, miR-200= MicroRNA-200.

TGF-β is the most potent inducer of EMT that has been extensively studied among these growth factors [46]. The continued growth and enlargement of the primary tumor lead to the creation of a hypoxic zone in the center of the tumor, which in turn further stimulates the upregulation of the aforementioned factors and more angiogenic mediators [47]. Furthermore, the tumor can cause inflammation, which attracts immune cells and creates a tumor microenvironment in which cytokines like tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) are secreted. interleukin 6 (IL-6) and interleukin 1B (IL1B) [48]. These inflammatory cytokines can induce the expression of transcription factors (TFs) that repress the epithelial phenotype and promote mesenchymal characteristics (Figure 3).

Regulators of EMT

By default, the cell state of a growing carcinoma is epithelial. Many transcription factors stimulated by inducers act by repressing the genes responsible for characteristic features of the epithelial state (refer to Figure 2). For instance, SNAI1 (snail), SNAI2 (slug), and ZEB1 and ZEB2 are transcription factors that bind directly to the promoter of the gene encoding E-cadherin (epithelial cadherin, being a canonical biomarker protein of epithelial cells) to repress its transcription. Other transcription factors, such as Twist, can repress E-cadherin more directly [49]. Among the transcription factors (TFs) involved in EMT, SNAI1 (Snail) is a major driver, while ZEB1/2 and Twist are principally involved in retaining the mesenchymal phenotype [50]. Another potent regulator of EMT is hypoxia-inducible factor

1 alpha (HIF1α), which is stimulated in response to hypoxia [51].

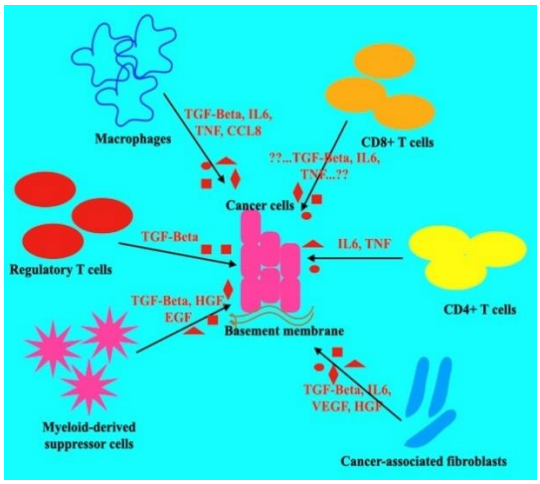

Figure 3: Major factors in the tumour microenvironment that impact on the epithelial-mesenchymal transition (EMT) of cancer cells. TGF-Beta= Transforming growth factor-beta, IL-6= Interleukin-6, TNF= Tumour necrosis factor, CCL8= Chemokine (C-C motif) ligand-8, HGF= Hepatocyte growth factor, EGF= Epidermal growth factor, VEGF= Vascular endothelial growth factor.

Furthermore, transcription factors such as SMAD and BMP can initiate and coordinate the EMT induced by TGF-β [51]. Other novel TFs, such as GATA, SOX, and FOX, were also found to play a role in regulating EMT [25,52,53]. Furthermore, microRNAs (miRNAs, miRs), while not transcription factors, can also function to silence gene expression of epithelial features and are thus considered regulators of EMT [54,55]. These small molecules, miRs, can bind and suppress TFs, deregulate the transcription of key EMT molecules such as E-cadherin, or affect epigenetic regulators of EMT [55,56]. MicroRNAs can promote EMT or its reversible counterpart, MET, depending on the target molecules they bind and suppress.

Effectors of EMT

The transition from epithelial to mesenchymal cell states involves alterations in the levels and localization of several proteins that take part in establishing cell-cell and cell-extracellular matrix (ECM) adhesions and basement membrane adhesions. There are several such adhesion points, such as tight junctions, gap junctions, adherence junctions, desmosomes, and hemidesmosomes (refer to Figure 1) [57,58]. The cadherin switches, epithelial cadherin (E-cadherin) and neural cadherin (N-cadherin), are the canonical markers of the EMT program. The alterations in the cell-surface proteins as achieved by these switches, together with changes in the cytoskeleton proteins, shift the overall cell shape and behavior from stable, interconnected epithelial sheets to separate, spindle-shaped, motile mesenchymal cells. To escape the primary tumor and gain access to other sites, mesenchymal tumor cells must also be able to invade a capillary tube (blood or lymph, although most likely to be blood) by breaking through the anchoring ECM and basement membrane. To achieve this, the transitioned cells must upregulate many enzymes, including collagenases such as MMP2, MMP3, and MMP9 [59]. Loss of the epithelial cells' essential anchorage renders them susceptible to death by a form of apoptosis. For this reason, the transitioned mesenchymal-like cells must also be able to protect themselves from death, and this is achieved via several signaling mechanisms including PI3K/AKT, NF-kB, Wnt/β-catenin and p53/p63 pathways [60]. The overall orchestration of the EMT program increases drug resistance and blocks immune surveillance, which eventually promotes invasiveness and metastasis [61,62].

Varied Roles of EMT in the Progression of Cancer

Markers of EMT are frequently observed in high-grade tumors, often reflecting a poor prognosis and illustrating the crucial role of this program in tumorigenesis [63-65]. The contribution of EMT to the progression of cancer can be discussed under four hallmarks: a) the promotion of metastasis, b) metabolic reprogramming, c) the promotion of stemness, d) EMT and inflammation, and e) EMT and resistance to cancer treatments.

Promoting metastasis

The dissemination and subsequent seeding of cells from the primary tumor to distant organs is a complex multistep process known as the invasion-metastasis cascade (Figure 4) [3,66-68].

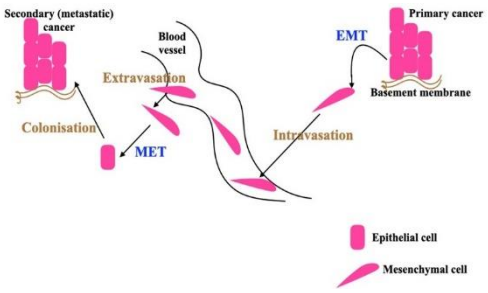

Figure 4: A schematic representation of the role of epithelial-mesenchymal transition (EMT) and mesenchymal-epithelial transition (MET) in the metastatic process.

Promoting this cascade is the canonical outcome of the activation of EMT. Many studies have shown that the over-expression of EMT-associated TFs, such as Snail and Twist, in benign cells can increase their invasiveness [9,69,70]. The EMT program may result in carcinoma cells exhibiting a range of characteristics, with cells exhibiting complete mesenchymal traits metastasizing under certain circumstances and cells with partial EMT spreading

under other circumstances [71-73]. More recent studies point to the involvement of partial (hybrid) EMT cell states in metastasis as they appear to possess a high potential for spreading [74-80]. The plasticity of carcinoma cells with properties in the middle of the epithelial-mesenchymal spectrum appears to be critical for the metastatic event [3]. The ability of epithelial cancer cells to lose their junctions and apical-basal polarity and to re-organize their cytoskeleton increases their motility, thus enabling the development of an invasive phenotype [42,43,81,82]. This in turn leads to the detachment of cancer cells from the ECM and their subsequent entry into the blood, resulting in the generation of circulating tumor cells (CTC) and the eventual metastasis of primary cancer (refer to Figure 4) [29]. The CTCs, following the extravasation into distant organs, remain dormant until the right conditions become available for the dormancy to be breached leading to the formation of secondary cancer [40]. The detachment of the primary tumor cells generally involves the collective migration of cohesive clumps of cells rather than the dispersal of individual cancer cells. Those cells residing in the center of the tumor mass continue to express epithelial biomarkers to sustain cohesiveness [83]. The CTCs often exhibit combinations of epithelial and mesenchymal properties, reinforcing the role of EMT in metastasis [29]. Although the dissemination of cancer cells to lymph nodes represents a standard clinical parameter that is often used for staging the disease, the transport of cancer cells by the blood is likely to be the main route of metastasis [3]. Traditional models of tumor progression assume that metastasis is a late event; however, more recent studies have shown that the dissemination of cells following the acquisition of mesenchymal traits can actually occur relatively early and might even be observed in some pre-neoplastic growths [84,85].

Reprogramming metabolism

During EMT, cancer cells reprogram their metabolism of glucose, amino acids, and lipids to meet the higher demands of these nutrients and the increasingly aggressive behaviors of cells during the transition. A key study has shown that mesenchymal-like cancer cells share common metabolic gene signatures as well as exhibit TFs affecting the EMT process [65]. Although cancer cells have been known to utilize glycolysis rather than oxidative phosphorylation in what is termed "the Warburg effect," it has also been reported that metastatic cancer cells prefer oxidative phosphorylation to glycolysis to achieve more efficient ATP production during EMT [65,86-88]. Several studies indicated the presence of crosstalk between various players in the tricarboxylic acid (TCA) cycle and EMT factors [89-94]. A shift in lipid metabolism towards fatty acid oxidation is closely related to metastasis and consequently with EMT, as seen in metastasis to the lymph nodes [17,95]. Cancer cells usually exhibit increased fatty acid synthesis, mostly from carbohydrates in the form of Acetyl-CoA, and the fatty acids will ultimately be esterified to form triglycerides [96]. Additionally, the requirements for morphological changes during EMT demand alterations in the lipid composition of cell membranes, which are achieved through metabolic reprogramming of lipid utilization. Lipid metabolism also regulates the death of EMT cells through a process known as "ferroptosis," induced by lipid peroxidation [17,97]. Cancer cells consume amino acids quickly, with glutamine being the second-highest nutrient in demand after glucose [98]. Asparagine is a non-essential amino acid in humans, and its high levels have been associated with EMT. Increasing the intake of asparagine in the diet led to an increased incidence of metastasis, and conversely, reducing the intake of this amino acid lowered the metastasis potential without affecting the growth of the primary tumor [99]. Asparagine can become an essential amino acid when the tumor microenvironment is deprived of glutamine to maintain protein synthesis and cell proliferation [100]. Proteins that are upregulated during EMT have about 20% higher asparagine content [99]. Cancer cells can also become dependent on cystine, and that reliance may be associated with EMT [101]. The oxidation of two cystine molecules and their link by a disulfide bond produces a cystine molecule, the main circulating form of cystine that cells can take up. The upregulation of miR-200c, which inhibits EMT in breast cancer cells that are addicted to cystine, resulted in these cells being less vulnerable to cystine deprivation, an indication that cystine can become an essential amino acid during EMT [102].

Promoting Stemness

According to the cancer stem cell theory, most cancer cells lack the tumor-initiating capability, and only a very small fraction of the neoplastic cell population can lead to the spread of primary cancer [1,103]. This subpopulation of cells is called cancer stem cells (CSC), or tumor-initiating cells, and is responsible for the initiation and maintenance of neoplasia. Those CSCs that manage to disseminate from the mass of the tumor and spread are called metastasized cancer stem cells (MCSCs). The differences between CSCs and non-CSCs within a tumor are likely to be driven by epigenetic factors and orchestrated by EMT [104,105]. Forced induction of the EMT program in certain carcinoma tissues increases their ability to initiate tumors and confers resistance to therapies, which is also a distinguishing feature of CSCs [106-110]. The increased activity of EMT at the leading edge of tumors suggests the existence of this motile subset of cancer stem cells, the MCSCs [17]. Following the

initial discovery of the connection between EMT and stemness in breast cancer cells, several studies have reported a similar link between EMT and the entrance of cells into a stem cell-like state in multiple cancer types, including pancreatic, prostate, colorectal, and ovarian [107,111-113]. Several types of carcinoma cells have been found to possess tumor-initiating capability following the induction of EMT [106,107]. The stemness state of cancer cells appears to be critical for the progression of these cells through the various stages of metastasis. Indeed, the importance of cancer stem cells is such that some investigators believe that the failure of conventional therapies to eliminate cancer is due to these treatments leaving CSCs behind despite effectively attacking the bulk of the tumor [114-116]. Moreover, accumulating evidence points to the higher resistance of CSCs to conventional cancer therapies when compared to their non-CSCs counterparts [117,118]. Cancer stem cells, and stem cells in general, exhibit two remarkable features: a) the ability to differentiate into daughter cells with specific functions, and b) the ability to self-renew, thus maintaining the stem cells' reservoir [119]. The acquisition of stemness, in the context of EMT, is associated with several EMT-TFs, and ZEB1 was the first one to be investigated [120]. Lowering the ZEB1 level causes pancreatic cell lines to lose the ability to form tumors, confirming the need for this transcription factor to achieve stemness [121]. Unlike "ordinary" tumor cells, CSCs initiate and maintain tumor growth, and their subgroup of MCSCs can escape the boundaries of the primary tumor upon receiving the necessary signals [122-125]. Carcinoma CSCs express phenotypic traits of both epithelial and mesenchymal cells, allowing them to move between these two cell states during EMT. The possession of these features enables the involvement of CSCs not only in tumor initiation and maintenance but also in drug resistance and cancer relapse following therapy [44].

EMT and Inflammation

Virtually all tumors are characterized by chronic inflammation driven by the production of cytokines and chemokines by cancer cells as well as by other cells of the tumor microenvironment (TME) [126,127]. Additionally, the secretion of these soluble factors (cytokines and chemokines) drives the recruitment of more immune cells from the bone marrow, which can further contribute to the inflammatory process. This chronic inflammation response favors immunosuppression, survival, and proliferation of cancer cells [128]. The relationship between EMT signaling pathways and inflammation can be categorized in terms of responses from cells and soluble mediators. The cellular part of the inflammatory response comprises resident cells in TME such as endothelial cells, cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), and dendritic cells (DCs), with TAMs being the most abundant of these cells. Although macrophages function to eliminate tumor cells when they get the appropriate signals, the situation is different in the immunosuppressive TME, where the macrophages are primed to support tumors [129]. The tumor microenvironment also recruits bone marrow-derived cells such as neutrophils, macrophages, and myeloid-derived suppressive cells (MDSCs). The soluble factors arm of the inflammatory response consists mostly of cytokines and chemokines, which have been shown to accelerate the progression of different cancer types. Moreover, cancer cells driven by EMT have also been shown to increase the secretion of pro-inflammatory soluble mediators, thus establishing a loop to maintain inflammation and the EMT phenotype [129]. As there are many hybrid phenotypes between the purely epithelial and mesenchymal states, the effects of specific mediators can vary depending on the particular cell state [129]. The cooperation between EMT and inflammation to foster aggressiveness and spread of the primary tumor has been validated in mouse models, and biomarkers of these two processes are correlated with poor outcomes in patients with multiple cancer types [130-135].

EMT and Resistance to Cancer Therapy

Following the initiation of cancer treatment, one of the major obstacles to complete remission is the development of resistance. This treatment resistance is not only against traditional chemotherapy and radiotherapy but also toward the more modern immunotherapies and targeted approaches [136-139]. In recent years, the epithelial-mesenchymal transition has emerged as a major contributor to the development of resistance to cancer therapy [140-142]. The mechanism by which EMT contributes to drug resistance may include: a) altered influx transporters leading to a decrease in drug uptake; b) increased drug efflux due to over-expression of efflux transporters; c) altered expression of anti-apoptotic proteins; d) slow cell proliferation; and e) avoidance of the immune response through changes in the expression of proteins involved in immunosuppression or immunoevasion [4,15,137,139,142-144]. Fischer *et al*. were able to demonstrate that the inhibition of EMT and the consequent abolition of ZEB1 and ZEB2 by the over-expression of miR-200a re-established the effectiveness of (and by implication ended resistance to) cyclophosphamide [145]. Moreover, the deletion of the transcription factors Snail or Twist in mouse models of pancreatic cancer led to an elevated sensitivity to Gemcitabine therapy [146]. The research investigations summarized below provide further supporting evidence of the involvement of EMT in giving rise to resistance to cancer treatments. The EMT program enhanced the

resistance of breast cancer cells to being lysed by cytotoxic T cells, and the activation of the transcription factor ZEB1 in lung cancer cells was linked to higher expression of the immunosuppressive molecule PDL1 [2,147,148]. Breast cancer patients that are resistant to chemotherapy exhibit a close association with increased expression of genes that are usually expressed by stromal cells and appear to be activated by the induction of EMT [149]. In ovarian and lung cancers, the EMT program reduces the dependence of tumor cells on EGFR and switches signaling to other pathways, thus giving rise to resistance against therapies targeting EGFR [150,151]. Other investigations confirmed the involvement of EMT in the resistance to cancer treatments through *in vitro* studies [152,153]. One of the main mechanisms of drug resistance is elevated drug efflux caused by many cell membrane transporter proteins, particularly the ATP-binding cassette (ABC) proteins [154,155]. The over-expression of EMT-TFs like Snail, Twist, and FOXC2 enhanced the expression of ABC proteins in breast cancer cells and led to a 10-fold higher resistance to doxorubicin [156]. Apoptosis is another mechanism behind the EMT-driven drug resistance to cancer therapy, as reported by several studies [157-159]. Many cancer drugs, such as EGFR-Tyrosine Kinase Inhibitors such as Erlotinib and Gefitinib, bind to and inhibit the EGFR's ATP-binding site, causing apoptosis. Growing evidence suggests that EMT-TFs can inhibit this apoptosis, thus conferring resistance to the TKI's [157-159]. The tumor microenvironment (TME) can also mediate the emergence of resistance to cancer treatment. Cytokines secreted by cancer-associated fibroblasts (CAFs) and hypoxia through the effect of HIF1alpha can both facilitate tumor cells to undergo EMT and acquire resistance to treatment [160-162]. Drug resistance (also referred to as chemoresistance) to cancer treatment can be broadly divided into two types: a) inherited (primary) resistance, which exists in the tumor before the initiation of the treatment, and b) acquired (secondary) resistance, which develops during the treatment. Additionally, cancer heterogeneity is reflected as differences between patients with the same tumor (inter-individual) or differences in the composition of the same tumor within a patient (intra-tumour) [4]. Inter-individual heterogeneity is often correlated with inherited resistance, while intra-tumor differences usually correspond to acquired resistance and are associated in most cases with EMT-induced drug resistance [139].

Targeting EMT for Cancer Treatment

The key role of EMT in the pathogenesis of cancer makes it an attractive therapeutic target despite the complexity of the network, pathways, and number of proteins involved in the overall control of this process [163]. The hijacking of EMT by cancer cells enables them to disarm the normal antitumor body defenses, resist apoptosis, resist therapy, and spread throughout the body [2,164]. To translate the targeting of EMT into clinical intervention, several approaches have been suggested: a) preventing the induction of the EMT program; b) identifying and selectively targeting cells in the EMT-like state; and c) reversing the process of EMT by forcing more mesenchymal carcinoma cells to revert to an epithelial state via the activation of the MET program [4,144,165]. The identification and selective elimination of cells that have already undergone EMT are difficult, and such cells tend to be highly resistant to chemical treatment [144]. The remaining two strategies, preventing the induction of EMT or reversing EMT, would in principle inhibit tumor malignancy, although neither on its own would eliminate the cancer cells. For this reason, drugs targeting the EMT process are envisaged as being employed in conjunction with chemotherapy or targeted cancer treatments. For the remaining part of this review, we will be focusing on drugs targeting the EMT to overcome resistance to therapies. The induction of the EMT program can be blocked by interfering with the signaling pathways that are critical to its activation and its subsequent maintenance. The cytokine TGF-β (transforming growth factor beta) and its associated signaling pathways are well characterized in the context of EMT induction. Inhibition of this pathway is a tempting therapeutic target. However, the TGF-β signaling pathway is also implicated in dampening tumor formation at the initiation stage through its anti-proliferative effects [166,167]. Thus, caution must be exercised in the selection of this pathway as a therapeutic target. Nevertheless, Morris *et al*. have demonstrated that an anti-TGF-β monoclonal antibody called Fresolimumab is safe and has preliminary evidence of anti-tumor activity in patients with advanced malignant melanoma [168]. Selected examples of other potential targets besides TGF-β, for the prevention of EMT induction are given in Table 1. Targeting components of the tumor microenvironment (TME) could also, in principle, lead to blocking the induction of the EMT program. Targets can include inflammatory cytokines, hypoxia-inducing factors, and specific cells residing in the TME such as CAFs and TAMs [169,170]. The selective targeting of carcinoma cells that have already undergone EMT could include blocking the function of EMT-associated molecules such as vimentin and N-cadherin. The anti-angiogenic agent Withaferin A was found to inhibit the spread of breast cancer cells *in vitro* and their metastasis *in vivo* through the disassembly of Vimentin structures [171]. An anti-N-cadherin monoclonal antibody was also found to inhibit prostate cancer cell growth and metastasis [172].

Table 1: Representative molecules targeting the epithelial-mesenchymal transition (EMT)

Drugs	Targets	Function	Reference
Metformin	ZEB1, Slug, Twist and Vimentin	Inhibition of EMT	186-188
Curcumin	BMI1, SUZ12 and EZH2	Inhibition of EMT and reversal of 5-fluorouracil resistance	189
Tanomastat	MMP	Inhibition of EMT	190
Disulfiram	ERK/NF-kappa B/Snail pathway	Inhibition of EMT	191
Fresolimumab	TGF-β	Inhibition of EMT	168
Catumaxomab	EpCAM	Inhibition of EMT	192
Denosumab	RANKL	Inhibition of EMT	193
YC-1	HIF1α	Lowering ABCB1 expression	194,195
Resveratrol	PTEN/Akt, miR-200c, TGF-β and p38-MAPK	Inhibition of EMT	196,197
Salinomycin	WNT pathway	Inhibition of EMT	198
Zidovudine	Akt-GSK3beta/Snail pathway	Inhibition of EMT and reversal of Gemcitabine resistance	199
Moscatilin	Slug Snail and Vimentin	Inhibition of EMT	200
Evodiamine	WNT pathway	Inhibition of EMT and reversal of Oxaliplatin resistance	201

ZEB1=zinc finger E-box binding homeobox 1, Slug=sometimes referred to as SNAI2 which is a zinc finger protein encoded by *SNAI2* gene, Twist=Twist-related protein , BMI1=proto-oncogene, polycomb ring finger protein 1, SUZ12=polycomb repressive complex 2 subunit, EZH2=enhancer of zeste homolog 2, MMP=matrix metalloproteinase, ERK=extracellular signal-regulated kinase, NF-kappa B=nuclear factor kappa light chain enhancer of activated B cells, TGF-β=transforming growth factor beta, EpCAM=epithelial cellular adhesion molecule, RANKL=receptor activator of NF-kappa B ligands, HIF1α=hypoxia-inducible factor 1 alpha, ABCB1=ATP binding cassette subfamily B member 1, PTEN/Akt=phosphatase and tensin, Akt=also known as PKB (protein kinase B), miR-microRNA, p38=mitogen-activated protein kinase, MAPK=mitogen-activated protein kinase, GSK3beta=glycogen synthase kinase 3 beta, Snail=sometimes referred to as SNAI1 which is a zinc finger protein encoded by *SNAI1* gene and WNT="Wingless/Integrated" signaling pathway.

The fact that these mesenchymal biomarkers, vimentin and N-cadherin, are also widely expressed in non-cancerous mesenchymal cells. The resulting off-target effects upon blocking them must be considered during their clinical use. Signaling pathways employed by cells that have undergone EMT could also be considered as a further approach to yielding anticancer therapies. One such investigation identified AXL, a receptor tyrosine kinase activated by a ligand known as GAS6, as a signaling protein whose expression and function are tightly linked with the EMT signature. A small molecule-specific inhibitor of AXL called BGB324 entered clinical trials in 2013, and a subsequent study demonstrated its safety and assessed its value alone and in combination with Erlotinib in NSCLC patients [173,174]. Another study has identified salinomycin (refer to Table 1) as having a cytotoxic effect on cells that have undergone EMT [175]. Reversing the EMT process (i.e., inducing MET) is based on the assumption that certain cancer cells with an active EMT program will regain their epithelial characteristics, thereby losing their enhanced tumorigenic activity, metastasis capability, and resistance to various therapeutic agents [4]. Huang and Huang have described how reversing the EMT program could provide a platform for new drug discovery for more effective treatment of cancer [176]. Screening for agents that reactivate the E-cadherin gene (*CDH1*) in mesenchymal cells has identified two possible compounds: cholera toxin and forskolin. Treatment of mesenchymal cells with either of these two agents resulted in the induction of MET and a reduction in the invasiveness of cells, together with sensitizing the cells to therapeutic agents such as Paclitaxel, Doxorubicin, protease inhibitors, and EGFR inhibitors [4,177]. Given that reversion of mesenchymal cells to epithelial cells is a required final step in the metastasis process, the therapeutic activation of the MET program might adversely promote metastatic colony formation [4]. Hence, the timing of any MET induction, as a therapeutic strategy, must be carefully considered. During the last two decades, micro-RNAs (miRs) have been the subject of increasing interest as targets for EMT-driven resistance to cancer treatment [155]. These are single-stranded non-coding RNA molecules that regulate the expression of a wide variety of genes [178-180]. One family of these micro-RNAs, the miR-200 family, inhibits the EMT program by targeting the transcription factors ZEB1 and ZEB2 and enhancing the sensitivity to Nintedanib in NSCLC [181-183]. However, ZEB1 and ZEB2 themselves can inhibit the expression of miR-200, thus creating a negative feedback loop that promotes EMT and the consequent tumorigenicity of cancer cells [184,185]. More examples of the functions of selected miRs are shown in Table 2.

Conclusion

The literature survey carried out for this review points overwhelmingly to the involvement of epithelial-mesenchymal transition (EMT) in the initiation, metastasis, and development of resistance to cancer treatment. During this transition, hybrid cell states can exist, sharing characteristics of both epithelial and mesenchymal cells and providing the necessary plasticity for invasiveness and resistance.

Table 2: Representative microRNAs (miRNAs) associated with epithelial-mesenchymal transition (EMT)

miRNA type	Target	Function	Reference
miR-200	ZEB1 and ZEB2	Inhibition of EMT and reversal of Nintedanib resistance	183
miR-186	Twist1	Inhibition of EMT and reversal of Cisplatin resistance	202
miR-203	Slug	Inhibition of EMT and reversal of Imatinib resistance	203
miR-15b	PEBP4	Induction of EMT and causing Cisplatin resistance	204
miR-375	MTDH	Inhibition of EMT and reversal of Tamoxifen resistance	205

miR=microRNA, ZEB=zinc finger E-box binding homeobox, Twist1=Twist-related protein 1, Slug=sometimes referred to as SNAI2 which is a zinc finger protein encoded by *SNAI2* gene, PEBP4=phosphatidylethanolamine binding protein 4, MTDH=metadherin encoded by *MTDH* gene.

The EMT program is reversible, and indeed, the process by which mesenchymal cells transit back to the epithelial state (termed mesenchymal-epithelial transition, or MET) is seen as a pre-requisite for the successful seeding of new colonies at distant sites following metastasis of the primary tumor. Deeper understanding and investigation of the different molecules involved in orchestrating the EMT program can yield medicinal substances that may prove useful in conjunction with chemotherapy or with targeted precision medicines to reduce the frequently encountered resistance to these cancer treatments.

Conflict of interests

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