International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Cancer Metastasis in Correlation with Epithelial Mesenchymal Transition and Tumor Microenvironment: A Review Article

Nilanjan Sahu[1], Dr. Samarth Shukla[2]

[1]MBBS Student, Jawaharlal Nehru Medical College Datta Meghe Institute of Medical Sciences DMIMS (DU) Sawangi, Wardha. Pin-442001 Maharashtra, India
Email: *nilanjan2852001[at]gmail.com*

[2]Professor Department of Pathology, Jawaharlal Nehru Medical College, Acharya Vinoba Bhave Rural Hospital AVBRH, Datta Meghe Institute of Medical Sciences DMIMS (DU). Wardha, Maharashtra, India

Abstract: ***Human body is composed of distinct types of cells having different biological functions. In this review we would focus on two types of cells-Epithelial Cells and Mesenchymal cells. Epithelial cells are tightly adhered to the cell junctions by the intercellular adhesion complexes unlike the mesenchymal cells which are nonpolar and expresses greater mobility between the cells. The two cells are involved in the process known as Epithelial mesenchymal transition (EMT). According to initial studies this process was only noted in the embryonic stage but later this phenomenon was attributed to many other cellular processes that required greater mobility and increased migratory capacity of the cells. The term "Transition" in EMT signifies reversibility, EMT-MET. EMT is classified into three types according to the process involved-gastrulation and embryogenesis, regeneration and wound healing, and Carcinogenesis (metastasis, malignancy). Cancer Metastasis is an overly complex phenomenon which includes many predisposing factors. EMT is one of the major predisposing factors in cancer metastasis and is highly associated with tumor invasiveness, malignancy, and poor prognosis. EMT and tumor microenvironment both play a synergistic role and are linked to aggressive forms of cancer including its subtypes and various other types of cancer. The tumor microenvironment comprises of various cells of inflammatory and immune origin and extracellular matrix components-Tumor associated macrophages TAMs, Cancer associated fibroblasts CAFs, Myeloid derived suppressor cells (MDSCs), Tumor associated neutrophils TANs. EMT and TME mediated cancer metastasis include various distinct signaling pathways which express biomarkers such as CD 31, PECAM-1, FSP1-alpa SMA, TGF-beta, R-Smads. Inflammatory cells mediated metastatic progression includes-TNF, MPN-JAK2 mutation, CCL2-MCP1. At molecular level in ECM beta-Catenin mediated EMT results in poor prognosis. The other factors that regulate EMT and are highly correlated with TME include: Chronic inflammation and neoplastic clone emergence resulting in malignant transformation, Oncogenic viruses which cause mutation in P53 and Rb gene, Metabolic syndrome, Stem cell like characteristics in EMT cell progression, Snail pathway, paraneoplastic syndrome, Drug-chemical induced carcinogenesis, and epigenetic factors. Clinical approach with TME and EMT Biomark's open a wide arena of therapeutic expansion.***

Keywords: EMT, carcinogenesis, tumor microenvironment, poor prognosis, signaling pathway, biomarkers

1. Introduction & Background

Metastasis and tumor growth are the main reasons for patient death. Epithelial-mesenchymal transition may facilitate the progression of tumor metastasis, which develops from precursor lesions to the fully invasive, metastatic lesions and advances through histopathologically distinct phases (EMT) [1]. EMT supports the development of the body's framework and tissue healing. EMT is also a crucial stage in the tumor invasion process because epithelial cell layers lose their polarity and their ability to form cell-cell interactions before drastically modifying their cytoskeletons. EMT also affects cell-cell adhesion, apico-basal polarity loss, enhanced motility, matrix remodeling, and invasiveness, all of which contribute to the spread of malignancies [2]. Once tumor cells have relocated to the correct location, they re-express E-cadherin and other epithelial markers through a process described as "mesenchymal-to-epithelial transition" (MET). The tumor microenvironment is an essential component in fostering the growth and metastasis of cancer and includes the extracellular matrix (ECM), stromal components, immune and inflammatory cells, hypoxia, and soluble chemicals [3]. In the aggressive front of primary colorectal cancer and accompanying metastases, nuclear-catenin was found in dedifferentiated mesenchyme-like tumor cells in clinical practice. It was restricted to the cytoplasm and membrane. The tumor microenvironment may cause EMT in tumor cells, according to this study. EMT in tumor cells may be induced by a variety of stem cells found in the tumor microenvironment, including cancer stem cells (CSCs) and mesenchymal stem cells (MSCs), as well as hypoxia, which is present in a sizeable fraction of the tumor [4]. Several intriguing research have recently examined the origins of EMT and the underlying processes. This research describes the main EMT drivers and their interactions in the tumor microenvironment.

With new research on the role of EMT in tissue fibrosis and cancer metastasis, the field of EMT is now far broader in scope and understood than it was only a few years ago. There is a great deal of confusion regarding the nature of common signaling and transcriptional pathways indicative of EMT due to the research of many model systems involving a variety of epithelial cell types that are frequently explored in culture and outside of biological context [5]. This is especially true when attempting to compare mRNA pools

produced under different experimental circumstances. There are three sub-types of EMT.

Table 1: Different classification of Epithelial Mesenchymal transition (EMT) process

S. No	Epithelial Mesenchymal Transition Type	Remarks/ inference
1	Type 1 EMT	Primitive neuroepithelial cells produce migrating neural crest cells, while primitive epithelial cells change into motile mesenchymal cells as a result of gastrulation in type 1 EMT. In both cases, mesenchymal-epithelial transition re-induces some of the EMT-generated cells as secondary epithelial cells in mesodermal and endodermal organs (MET).
2	Type 2 EMT	Secondary epithelial or endothelial cells that are already present in the tissue change into fibroblasts in type 2 EMT. These fibroblasts are produced in mature tissues in response to ongoing inflammation.
3	Type 3 EMT	To move via the bloodstream and, in some cases, create secondary nodules in distant metastatic sites by MET, type 3 EMT entails epithelial carcinoma cells in primary nodules changing into metastatic tumor cells.

Table credits: Nilanjan Sahu (Author)

2. Review

In this section, an overview of Epithelial mesenchymal transition (EMT) is discussed with signaling pathways that leads to tumor metastasis and invasion. EMT is responsible for introduction of malignancy and poor prognosis. In this review, EMT is studied in correlation with tumor microenvironment (TME) and it has been established that TME also plays a key role in EMT progression and EMT is a reversible dynamic process which is associated with most aggressive phenotypes of cancer [6] [7]. Tumor budding model-EMT concept and tumor microenvironment is described as a concept relating many different cellular pathways and processes such as Chronic inflammation cell secreted chemokines mediated carcinogenesis and its role in EMT transition and metastasis. Tumor microenvironment and local extracellular matrix expresses epigenetic predisposition in neoplastic transformation [8]. Neo plasticity and increased migratory capacity of cells results in chronological changes such as-tumor cell growth, angiogenesis, Extracellular matrix destruction, Epithelial mesenchymal transition and reorganization leading to enrichment of tumor microenvironment [9].

Chronic Inflammation: Role in facilitating the emergence of neoplastic clone (malignant transformation)

It is possible to link inflammation to the idea of free energy. Like internal energy, enthalpy, and entropy, the free energy is a thermodynamic state function [10]. In order to preserve the normal cellular balance, including the rate of genetic mutations, biological living systems maintain a dynamic equilibrium in their system energies. In the cell system, the second rule of thermodynamics holds true [11]. Cellular chemokines released during inflammation and their active maintained state during chronic inflammation causes breakdown of this fine thermodynamic equilibrium. This is regarded as the hallmark of malignancy according to the universal concept of free energy [12]. Therefore, there are inflammatory and non-inflammatory pathways of Carcinogenesis. Normal balance of cellular energetics is controlled by a broad spectrum of fundamental biological components ranging from the epigenome to MicroRNA network. Metabolomics and inflammatory disorders contribute to autoimmune, collagen vascular disorders and cancer. According to the HLA system (Human leukocyte antigen system) differentiation between self and non-self-cells by the immune mediators is done on the degree of low free energy [13]. Inflammation in association with EMT has shown strong correlation with Hematologic malignancies through the MPN-JAK2 mutation (TNF mediated). Inflammation also facilitates malignant transformation in glioma. PAX-6 acts as a tumor suppressor gene in glioblastoma which mediates CCL-2 (MCP-1), chemokine attracting macrophages [14]. Different therapeutic approaches are being worked on such as the immune mediated strategies, checkpoint inhibitors-PD1 and CTLA-4 antagonists in metastatic prostate adenocarcinoma and CART treatment and acute leukemia [15].

Epithelial Mesenchymal Transition: signaling pathways and EMT cancer metastasis

Epithelial mesenchymal transition is a complex transformation program that enables the neoplastic cells in the tumor microenvironment to suppress their epithelial activity and start expressing mesenchymal features. Mesenchymal transition enhances cell mobility and the migratory capacitation, hallmark of cancer metastasis [16]. EMT provides new insights along with effect of Tumor mediated microenvironment. EMT increases the invasion frequency of in-situ carcinoma; this causes neoplastic cell mass migration from the site of primary focus to secondary tumor growth site through various intercellular pathways. Secondary tumor along with cancer stem cell characteristics, cell regeneration capacity in chronic inflammatory state, genetic mutations and metabolic disorders with other acquired immunological responses leads to poor prognosis and increased mortality [17]. EMT establishes the fact that the increased redifferentiation is the main cause of aggressive malignant phenotype. Through the process of EMT transformation during tumor growth, benign tumors that are non-invasive and non-metastatic are given the capacity to pierce the surrounding tissue and spread to secondary sites. Tumor pathology staging and grading provide clinical evidence in Favour of this hypothesis. The greatest case for EMT's role in oncogenesis is made by the ability of various EMT regulators to encourage tumor growth and/or metastasis. In human breast cancer, increased Snail 1 expression improves the malignant aggressiveness of the tumors, and upstream expression of Snail1 induces tumor relapse and mortality. Breast cancer proteomic analysis demonstrates that mesenchymal conversion, a hallmark of metastatic carcinoma, is present in circulating

mammary tumor cells or those found as micro-metastases [18].

It is possible to consider EMT or transdifferentiation (metaplasia) as a form of epithelial cell plasticity. Transdifferentiation, with or without cell division, is a process whereby one mature epithelial cell phenotypic changes into another mature epithelial cell. Whether transdifferentiation involves a transition state is unknown. Depending on the phenotypic of the output cells, three different forms of EMT are characterized. Following gastrulation or neural crest migration, type 1 EMT occurs when primitive epithelial cells change into mesenchymal cells, which form the diaspora of the basic body plan [19]. Either MET takes place in these mesenchymal cells to produce secondary epithelial cells, or they apoptosis. Type 2 EMT occurs when resident or inflammation-induced fibroblasts, the latter during prolonged damage, occupy interstitial spaces with secondary epithelial cells or endothelial cells. Epithelial tumor cells leave a primary tumor nodule, migrate to a new tissue site, and then reorganize into a secondary tumor nodule. This process is known as type 3 EMT.

Epithelial cell plasticity

Early investigations of tissue regeneration in simple experimental models are largely responsible for the concept of epithelial cell plasticity. Many of the tissues used in these models contain an epithelial component [20]. Experimental research on epithelial cell plasticity in mammals primarily follows the paths of two overarching interests: metaplasia and EMT (Categories of epithelial cell plasticity). It is significant to remember that epithelial-mesenchymal transformation and epithelial-mesenchymal transdifferentiation will henceforth both be referred to as EMT [21]. This decision was made at the first conference of The EMT International Association (TEMTIA) in 2003 in Port Douglas, Australia. Metaplasia, which is seen in naturally occurring diseased tissue samples and experimental tissue remodeling, is the spontaneous transformation of one differentiated cell type into another. Cell biologists now refer to metaplasia as transdifferentiating. Mammalian cells have shown signs of transdifferentiation, though not as frequently as in amphibians. These cells include islet cells, hepatocytes, lactotrophs, pneumocytes, and intercalated tubular cells. EMT involves the development of motile cells from immobile parent epithelial cells. Early embryogenesis, when EMT is engaged in gastrulation and neural crest migration, is characterized by the discrete, age-or stage-dependent conversion of primitive epithelial cells into motile cells [22]. Later in organogenesis, secondary epithelial cells and endothelial cells (specialized squamous epithelial cells) undergo EMT to transform into fibroblasts as a result of the production of local developmental cytokines. In addition to closing the cranial plates, facial bones, and mid-line palate, the fibroblasts produced by this process also create endocardial cushions and occupy interstitial spaces with resident fibroblasts as part of the normal growth of connective tissues. In adulthood, the same mechanism that causes persistent tissue injury to produce a cytokine bath also causes an abundance of fibroblasts to form [23]. EMT is also observed in subsets of carcinoma cells that are undergoing phenotypic conversion for invasion and metastasis, indicating that epithelial carcinomas produce motile cancer cells by recycling a portion of the molecular EMT program that is typically used to build adult fibroblasts [24].

Possible Cancer Biomarkers Found Through Analysis of Epithelial-Mesenchymal Transition Metabolism May Be Useful in People with Different Genetic Backgrounds

The term "epithelial-to-mesenchymal transition" (EMT) refers to a variety of molecular and cellular changes that take place when epithelial cells switch from one differentiation pathway to another, giving rise to cells that resemble mesenchymal tissue and have recently acquired migratory and invasive properties. EMT causes drug resistance and metastases in cancer cells [25]. Additionally, variations in genetic origins even among patients with the same type of cancer affect therapy resistance. It is crucial to uncover critical metabolic components for this process since they can later be employed to treat cancer cells with various genetic backgrounds. Metabolic rewiring is necessary to cause EMT. The Krebs cycle, amino acid metabolism, and glutathione metabolism were found to be the most adversely affected pathways in studies on non-small cell lung cancer (NSCLC) cell lineages [26]. However, the transcriptional level or the metabolic responses in the cell lines were significantly altered by glutathione metabolism. One significant characteristic that is dysregulated during EMT is glutamate-cysteine ligase (GCL), a crucial enzyme in glutathione production. The idea that EMT results in the development of metastatic cancer cells is supported by the finding that the acquisition of mesenchymal markers by epithelial carcinoma cells, such as vimentin or S100A4 (also known as fibroblast-specific protein 1 [FSP1]), as well as nuclear overexpression of beta-catenin and loss of epithelial cell adhesion molecules like E-cadherin, are linked to increased metastatic potential [27]. While having some phenotypic parallels to other kinds of EMT, type 3 EMT also has the potential to cause the expression of more primitive markers that could be stem cell indicators. In the context of cancer, two recent intriguing remarks concerning fibroblasts that produce EMT can also be made. First, it appears that type 2 EMT producing fibroblasts occur before the full onset of carcinogenesis since fibroblasts nearby primary epithelial tumor nodules share some genetic abnormalities with the tumor cells. However, studies comparing cancer cell mutations to cancer-associated fibroblasts isolated from distant metastatic tumor tissue have been unable to show that these fibroblasts originated from cancer cells, indicating that once epithelial tumor cells appear, they are no longer a source of fibroblasts [28]. Instead, it is more likely that the fibroblasts linked with cancer in metastatic locales are either drawn to a secondary tumor nodule from the local population or come from endothelial sprouts that develop into neovascular arteries as the secondary nodules increase. Second, type 3 EMT implies a distinct process from type 2 EMT, which entails the conversion of mature epithelial or endothelial cells into fibroblasts. Type 3 EMT is a process that uses this mechanism to transition and get nodular tumor epithelial cells ready for motility, invasion, and metastasis. It is not a mechanism for producing fibroblasts. Here, we go through some ideas on the standards and typical biomarkers for EMT in all three subtypes. Unraveling this intriguing phenotypic

transition phenomenon has come a long way, especially when it comes to the production of fibroblasts. All three EMT subtypes have been demonstrated using a range of biomarkers. In this section, we look at a few of the more prevalent markers, some of which are acquired and some of which are attenuated throughout transition.

Table 2: Clinical markers of EMT

Sr. No	Biomarker type (Both Acquired and Attenuated markers)	Remarks
1.	Cell-surface proteins N-cadherin, E-cadherin OB-cadherin, α5β1 integrin αVβ6 integrin , Syndecan-1 ZO-1	The classic epithelial cell EMT marker is a shift in E-cadherin expression. In tissue fibrosis, cancer metastasis, and embryonic development, E-cadherin expression is downregulated during EMT in epithelial cells. Additionally, EMT is promoted by E-cadherin function loss. In fact, EMT during embryonic development and cancer growth have both been closely watched to see how the cadherin switch from E-cadherin to N-cadherin, which is expressed in mesenchymal cells, fibroblasts, cancer cells, and neural tissue, is progressing. Additionally, an E-cadherin-OB-cadherin flip is intriguing for type 2 EMT linked to fibrogenesis because OB-cadherin is a more conclusive marker for active fibroblasts.
2.	Cytoskeletal markers FSP1, Cytokeratin, α-SMA, Vimentin β-Catenin	A member of the S100 protein family that binds Ca2+ is FSP1. It is a characteristic fibroblast marker for fibrogenesis and EMT in cancer. More than one-third of all FSP1+ fibroblasts in fibrotic kidneys and livers are EMT generated, according to lineage tagging in transgenic reporter mice. Most epithelial cells undergoing type 2 EMT in tissue fibrosis express FSP1 early in transition to fibroblasts. Additionally, metastatic cells frequently express FSP1 as a component of type 3 EMT in cancer model organisms. EMT is facilitated in adult epithelial cells and cancer cells by ectopic expression of FSP1. A cytoplasmic plaque protein called beta-catenin binds cadherins to the cytoskeleton and functions as a co-transcriptional activator with T cell factor (TCF) /LEF in the process of EMT. The majority of the processes that regulate the amount of-catenin in the cytoplasm include either recruiting it to cadherin-binding partners or ubiquitinating it and then degrading it. Snail1 is one of the EMT-related genes whose expression is directly regulated by the-catenin/TCF/LEF complex. In numerous research investigations on embryonic development, cancer, and fibrosis, beta-catenin has been employed as a marker of EMT.
3.	ECM proteins α1 (I) collagen, α1 (IV) collagen, α1 (III) collagen, Laminin Fibronectin, Laminin 5	Despite being a crucial component of the desmoplastic stroma in tumors and the fibrotic ECM linked to tissue fibrosis, fibronectin's utility as a type 2 and type 3 EMT biomarker is constrained in part due to the fact that it is produced by a variety of cell types, including fibroblasts, mononuclear cells, and epithelial cells. But greater fibronectin expression in vitro is related to both type 2 and type 3 EMT. Laminin is the best-established biomarker of EMT among the major components of the basement membrane that are downregulated throughout the process, including type IV collagens, nidogen, and sulfated proteoglycans. Laminin 5 has been linked to type 3 EMT in hepatocellular carcinoma, oral squamous carcinoma, and breast carcinomas of the ductal type. It is found in the discontinuous laminin patterns connected to invasive malignancies. Laminin 5 is likewise a component of the fibrotic ECM linked to idiopathic pulmonary fibrosis. Laminin5 and EMT in the embryo have not yet been linked in studies.
4.	Transcription factors Snail1 (Snail), Snail2 (Slug), ZEB1, CBF-A/KAP-1 complex, Twist LEF-1, Ets-2, Goosecoid.	Several EMT-associated genes, including those that code for FSP1, Twist, Snail1, high mobility group AT-hook 2 (HMGA2), LEF1, Ets-1, E-cadherin,-catenin, ZO-1,-SMA, and vimentin, contain the cis-acting regulatory element known as fibroblast transcription site-1 (FTS-1). To control gene activity, CBF-A, KRAB-associated protein 1 (KAP-1), and FTS-1 create a complex. Kidney tubular epithelial cells undergo type 2 EMT when the CBF-A/ KAP-1/FTS-1 complex forms. Controlling the subcellular location of a snail controls its transcriptional activity. Snail is exported from the nucleus into the cytoplasm as a result of phosphorylation, which renders it inactive as a transcription factor. Snail activation causes type 2 EMT and renal fibrosis in tamoxifen-inducible Snail1-transgenic mice, and elevated Snail1 expression is linked to fibrosis in human renal biopsies.
5.	Epigenetic EMT and microRNAs miR10b, Mir-200 family, miR-21	MicroRNAs of the miR-200 family were shown to be considerably downregulated in TGF-1-induced EMT and cancer cell lines that demonstrated an EMT phenotype in two recent microRNA array investigations. Both results show that miR-200 microRNAs specifically target zinc finger E-box binding homeobox 1 (ZEB1) and ZEB2 as E-cadherin transcriptional repressors. Moreover, ductal and metaplastic breast tumors exhibit downregulation of the microRNA miR-200. In contrast, Twist-induced EMT involving breast cancer cells is linked to miR-10b induction while TGF-1-induced EMT involving keratinocytes is linked to miR-21 induction. With future research, it is certain that the precise part microRNAs play in controlling all EMT subtypes will become clear.

Table credits: Nilanjan Sahu (Author)

Tumor Microenvironment (TME) and Cancer Metastasis in correlation with EMT pathway.

TME is composed of numerous cells of diverse origins-inflammatory, immune, hypoxia induced, stromal, extracellular components including ECM, other additional soluble factors and is one of the major pre-requisites for cancer metastasis and progression [29]. Substantial studies have shown in colorectal carcinoma that the primary areas of metastasis have expressed increased concentration of nuclear b-catenin and local and adjacent epithelial cells dedifferentiate into mesenchymal cells. The studies suggest EMT occurs in tumor mediated microenvironment at a more aggressive rate. An enormous number of inflammatory cells infiltrate the TME. Hypoxia exists in large area of tumor. In addition, cells present in the TME are of stem cell origin such as the cancer stem cell (CSCs), mesenchymal stem cell (MSCs) and all of these induce EMT in tumor cells [30].

The link between EMT and Cancer stem cell (CSCs) phenotypes (MSCs) in TME

Existence of CSCs in the tumor microenvironment or tumor initiating cells with the ability to self-multiply to an undefined potential give rise to heterogenous differentiated tumor cells. The researchers have identified CSCs in several

solid tumors of brain, colon, and breast [31]. Cells that undergo the EMT process tend to resist the toxic injuries and chemoradiation therapy. It has been studied that CSCs are more resistant to conventional therapies than other differentiated type of cells. So, the CSCs go through the endothelial mesenchymal transition along with other local tumor cells. EMT is believed to provide CSCs with invasive and metastatic abilities necessary for aggressive malignancy [32]. This characteristic is shown both in-vivo and in-vitro. In chemo-resistant pancreatic carcinoma it is evident that there is increased number of CSCs. EMT is also related to the tumor cell's ability to self-renew, thus there is increased frequency of secondary tumors at distant sites through metastatic spread. All the underlying factors are responsible for poor prognosis [33]. Furthermore, under the influence of induced hypoxic conditions the tumor cell acquires the properties of CSCs. A specific phenotype of stem cell origin known as the mesenchymal stem cell (MSCs) from the bone marrow are expressed and recruited in number of injury sites in pathological conditions such as inflammation, tissue repair and neoplastic transformation. Recent evidence suggests that MSCs participate in tumor metastasis. Some of the TME mediated EMT cancer metastasis cells are studied below:

Cancer associated fibroblasts, CAFs

CAFs are regarded as the primary source of EMT inducing signaling pathways through specific markers such as IL-6, TNF-alfa, and TGF-beta. Increased CAFs in tumor microenvironment is related to increased chemoresistance in non-small cell lung carcinoma (NSCLC), ovarian cancer aggressive metastasis and gastric squamous cell carcinoma. CAFs are associated with many other types of cancers such as Prostate Cancer (TGF-beta mediated EMT) [34]. The main initiator cells are the CAFs and the mutation that causes progression of malignancy is DNA methylation by DNMTA3A. The EMT pathway mediators such as CDH1 and GRHL2 play the role of epithelial silencing genes. In colorectal carcinoma it has been noted that CAFs and EMT together signifies poor prognosis and increased metastasis. TGF-beta regulated Wnt pathway leads to CAFs diversity in TME and Extracellular matrix crosstalk that initiates mutations [35]. Recent advances in EMT and TME have explored many therapeutic approaches such as anti-CSFR1 immunotherapy in TME immunosuppressive cancer.

Tumor associated macrophages, TAMs

Macrophages in TME play contrasting role. Macrophages are cells of immunogenic responses but in the presence of EMT chemical mediators M1 macrophages transform into pro-tumorigenic M2 phenotype. Studies have shown extracellular matrix crosslinking and TGF-beta mediated metastasis express clinically important tumor markers such as CD4+ T cell and IL-4. Signaling pathways include macrophage derived WNT7B-Epithelial growth factor. In teratocarcinomas NMuMG are seen intramurally in TME [36]. In colorectal carcinomas, EMT is accelerated by TNF-alfa secreting macrophages which induces p38 MAPK signaling in tumor organoids. Oher type of cancers in which TAMs play a significant role includes-Renal Cell carcinoma, NSCLC and Breast carcinoma. Specific markers IL-6, IL-35 promote EMT mediated by prostaglandins/ beta-catenin (JAK2-STAT6, GATA3 signaling mechanism) [37].

Myeloid-derived suppressor cells, MDSCs

These are diverse cells that were produced by neutrophils and monocytes. They are primarily related to cancer patients' T-cell-mediated immunosuppression. TGF-beta and HGF expression is elevated by MDSCs. Toll-like receptors are the mechanism by which High Motility Group Protein B1 (HMGB1) functions (TLRs) [38]. The aforementioned components and additional processes, including as SNA1, MMP7, and NFKB1, all contribute to the development of EMT (NF-kB). Monocytic CXCR-2-expressing (mMDSC) cells that are unique to the main tumor site encourage the induction of EMT. Breast cancer cells secrete CXCL1 and CXCL2 to attract MDSCs. Granulocytic gMDSCs are discovered at the metastatic location in lung malignancies. TGF-beta, VEGF, and IL-10 are secreted by them as they become more active and expand to enhance operational stress. These indicators play an important role in encouraging lung metastasis [39].

Tumor associated neutrophils, TANs

TANs are attributed for promoting metastasis at the pre-metastatic niche in lung carcinoma and hepatocellular carcinoma by T-cell mediated immunosuppression. TANs promote EMT at the primary tumor site. TANs can polarize between extreme states. N1-N2 transition takes place. There is change in the functional capacity of the TANs after EMT process: N1, antitumor and pro-immunogenic functions to N2, pro-tumorigenic and pro-metastatic functions. N1 is mediated by IFN-beta and N2 is mediated by TGF-beta, hence TGF-beta is an EMT mechanism induced metastasis causing essential biomarker. Neutrophils in stroma of Gastric carcinoma express IL-17a, CXCL-5, both promoting EMT by upregulation of VIM and ZEB1 and causing CDH1 repression [40].

Complexity of EMT signaling pathways in tumor microenvironment

In order to change into mesenchymal cells, epithelial cells must lose many of their epithelial features. This process is known as EMT. In a complicated tumor microenvironment, EMT is induced. As a result, the following distinct signaling mechanisms have been briefly discussed: NF-kB, Wnt, Notch, TGF-beta, etc. The development and spread of tumors are significantly influenced by TGF-beta signaling pathways. Through both a Smad-dependent and Smad-independent transcriptional mechanism, TGF-beta causes the EMT phenomenon [41]. TGF-beta ligand binding via Smad-dependent pathways causes the tight complex formation between TGF-beta type 1 and type 2 receptors. Smad2 and Smad 3 are phosphorylated as a result (receptor related Smad proteins-rSmad). After forming heteromeric complexes with Smad4, phosphorylated Smads move into the nucleus to regulate the transcription of their target genes, such as Snail, ZEB, and Slug. The EMT pathway is used to regulate the transcription of the regulatory genes. TGF-beta directly stimulates a variety of non-Smad signaling pathways in addition to the Smad signaling pathways. Significantly, it has recently been shown that Ras/Erk, c-Jun N-terminal kinase (JNK), Par6, phosphattidylinsitol-3 (PI3) kinase, and Cdc42 GTPases are essential for TGF-beta-induced EMT [42]. As a result, since elevated TGF-b expression is a significant modulator of the tumor EMT process, targeted therapy targeting TGF-b signaling seems to

hold promise. For instance, the TGF-b receptor 1 kinase inhibitor (LY2109761) inhibits Smad-2, which reduces HCC cell invasion and migration while increasing the expression of the cell adhesion protein E-cadherin in the membranes of HCC cells. For the NF-kB pathway to be activated, there needs to be an increase in the expression of inflammatory cytokines such TNF-alfa, IL-6, LPS, and reactive oxygen species in the tumor's microenvironment [43]. In addition, this pathway can directly stimulate the expression of Snail and ZEB factors, two powerful EMT inducers. It has been observed that the NF-kB pathway increases the expression of the mesenchymal-specific gene vimentin while suppressing the expression of the epithelial gene E-cadherin. In order to suppress E-cadherin following the loss of epithelial phenotype, Snail is thought to be the key transcription factor. Additionally, it increases the transcription factors ZEB1 and ZEB2, which inhibits the production of E-cadherin during EMT. NF-kB transcription factor has been identified as yet another important regulator of TGF-b-induced EMT in recent research. Pancreatic cancer cells, which lack functional SMAD4 and are not susceptible to TGF-b, can be promoted to undergo EMT by NF-kB. It's interesting to note that TNF-a, through NF-kB, was still able to induce an EMT-like phenotype in these TGF-b-unresponsive cells. TGF-b and NF-collaboration kB's is therefore essential for EMT and is crucial for the invasion and metastasis of cancer [44]. Additionally emerging as significant regulators of EMT in cancer cell lines and the preservation of stemness features in stem cells are the Wnt/b-catenin and Notch pathways. B-catenin signaling is also crucial to preserve the stemness qualities of CSCs in skin cancer. Translocation of b-catenin to the nucleus may result in the loss of E-cadherin to promote EMT. The EMT program is activated by transforming growth factor (TGF)-b, canonical and noncanonical Wnt signaling, which then work in an autocrine manner to sustain the resultant mesenchymal state. EMT transcription factors can be blocked, and epithelial differentiation promoted by inhibiting Wnt signaling. Recent research suggests that Snail2, one of the transcription factors involved in EMT, is a Snail2 target of Notch signaling [45]. In embryonal brain tumors, pharmacological inhibitors of c-secretase that block the Notch pathway may lead to a reduction in CD133 stem-like cells. In carcinogenesis, EMT and cancer stem-like cell properties are influenced by both of the two signaling pathways.

Targeted therapeutic expansion using EMT signaling pathways in correlation with tumor microenvironment

A significant clinical hurdle in the treatment of cancer is the spread of malignant cells to distant tissues. By promoting an invasive phenotype, epithelial-mesenchymal transition (EMT) has become a significant regulator of metastasis in several malignancies. EMT is believed to produce cancer stem cells, promote metastasis, and contribute to treatment resistance. In order to prevent the spread of tumors in individuals who are at high risk for developing metastatic lesions or to eliminate existing metastatic cancer cells in patients with more advanced disease, the EMT pathway could be targeted therapeutically. It has long been hypothesized that EMT contributes to cancer treatment resistance [46]. When researchers discovered striking parallels in the gene expression profiles and marker expression between cells undergoing EMT and CSCs, the situation became more evident. Because CSCs are resistant to the majority of existing chemotherapeutic treatments, they are very challenging to remove. Scientists have effectively created assay tools to investigate EMT phenotype and drug screening based on our improved understanding of molecular switches and important signaling pathways in EMT [47]. Finding small compounds and miRNAs that inhibit EMT phenotype and subsequently treatment resistance in cancer has received a lot of attention. As a result, a number of small compounds have been identified as EMT inhibitors that can improve the chemo-sensitivity of cancer cells that are resistant to chemotherapy. Many of them were tested on humans in conjunction with common chemotherapies or specific therapies, such as gemcitabine and mocetinostat for metastatic leiomyosarcoma, metformin and cisplatin/radiation for non-small cell lung cancer, bufalin and gemcitabine for pancreatic cancer, palbociclib and tamoxifen for HR (+) /HER2 (') advanced breast cancer. Even though some of these medications have been shown to improve the effectiveness of chemotherapy, EMT inhibitor toxicity is still a problem. Only a few small molecule TGF inhibitors, such galunisertib, were able to reach clinical studies because small molecule TGF inhibitors shown high cardiotoxicities in animal preclinical models [48]. Additionally, the safety of using EMT inhibitors over an extended period of time is not yet known. It has been established that EMT-MET is crucial for the spread of cancer cells and distant metastases. The most recent research indicates that EMT might not be necessary for cancer cell spread despite its function in chemoresistance. This result, however, is hotly contested because it should be taken into account that the EMT phenotype is heterogeneous and changeable. Only a portion of EMT populations may be under the control of Fsp1. Another two study teams independently showed that at least two different EMT phenotypes-one with stemness phenotype (Twist1-type), and the other with non-stemness phenotype-are implicated in cancer metastasis (Prrx1 type). An EMT-TF Twist1 is able to dedifferentiate epithelial type cancer cells into non-proliferative, migratory mesenchymal cells in the stemness phenotype (possessing stemness properties) [49]. When Twist1 is downregulated, EMT cells begin to colonize and proliferate at the metastatic site and reversibly dedifferentiate into epithelial cells. In contrast, a recently found EMT-TF called Prrx1 stimulates cells to undergo EMT that lacks stemness features. For MET, the acquisition of stemness features, and the colonization of cancer cells at the site of metastasis, Prrx1 must be downregulated. It should be noted that for cancer metastasis in both situations, reversible cell transitions between EMT (for dissemination) and MET (for colonization) are essential. Drug resistance and metastasis are both impacted by the heterogeneity and adaptability of the EMT phenotype. EMT inhibitors would have to be divided into different categories, such as stable EMT inhibitors and plasticity inhibitors [50]. According to this perspective, plasticity inhibitors may hold a lot of promise for the treatment of cancer because they have the ability to stop both drug resistance and cancer spread. EMT is a crucial cancer cell phenotype that promotes treatment resistance, to sum up. As effective "partners" for chemotherapy or targeted treatment medications, inhibitors

of this cellular mechanism can greatly enhance the clinical outcomes of current cancer therapeutics.

3. Conclusion

Cancer cells with metastases are fatal. Treatment of these diverse and resistant neoplasms depends on understanding the molecular pathways that support the shift from benign to malignant. A conserved cellular function called the epithelial-mesenchymal transition (EMT) modifies the shape, adhesion, and mobility of cells. The transition to a more mesenchymal-like phenotype may not be required for extravasation or colonization into that environment, but it can increase tumor cell migration to other organs and intravasation of nearby blood arteries. On the other hand, lymphatic dispersion might not need EMT. MicroRNA and exosomes are used by tumor cells to encourage EMT/MET and set up the pre-metastatic niche. A significant number of research over the last few decades have demonstrated the link between EMT and the spread and progression of cancer. EMT can be caused by a number of tumor microenvironment-related causes. Through intricate pathways, inflammation, hypoxia, and stem cells in the tumor environment are intricately related to EMT. The current comprehension of conventional signal pathways combined with novel EMT ideas may hasten the advancement of cancer research.

References

[1] Kalluri R, Weinberg RA: The basics of epithelial-mesenchymal transition. J Clin Invest.2009, 119: 1420-8. . 10.1172/JCI39104. Erratum in: J
[2] Jing Y, Han Z, Zhang S, Liu Y, Wei L: Epithelial-Mesenchymal Transition in tumor microenvironment. Cell Biosci.2011, 31: 29.
[3] Ribatti D, Tamma R, Annese T: Epithelial-Mesenchymal Transition in Cancer: A Historical Overview. Transl Oncol.2020, 13: 100773.
[4] Arneth B: Tumor Microenvironment. Medicina (Kaunas.2019, 30: 15.
[5] Aggarwal V, Montoya CA, Donnenberg VS, Sant S: Interplay between tumor microenvironment and partial EMT as the driver of tumor progression. iScience.2021, 28: 102113.
[6] Jo M, Lester RD, Montel V, Eastman B, Takimoto S, Gonias SL: Reversibility of epithelial-mesenchymal transition (EMT) induced in breast cancer cells by activation of urokinase receptor-dependent cell signaling. J Biol Chem.2009, 21: 22825-33.
[7] Plygawko, Andrew & Kan, Shohei & Campbell, Kyra. (2020: Epithelial-mesenchymal plasticity: emerging parallels between tissue morphogenesis and cancer metastasis. Philosophical Transactions of the Royal Society B: Biological Sciences.375.20200087, 10.1098/rstb.2020.0087
[8] Li H, Xu F, Li S, Zhong A, Meng X, Lai M: The tumor microenvironment: An irreplaceable element of tumor budding and epithelial-mesenchymal transition-mediated cancer metastasis. Cell Adh Migr.2016, 3: 434-46.
[9] Martin TA, Ye L, Sanders AJ, et al.: Cancer Invasion and Metastasis: Molecular and Cellular Perspective. In: Madame Curie Bioscience Database [Internet]. Austin (TX): Landes Bioscience; 2000-2013. Available from: https: //www.ncbi. nlm. nih. gov/books/NBK164700/.
[10] Greten FR, Grivennikov SI: Inflammation and Cancer: Triggers, Mechanisms, and Consequences. Immunity.2019, 16: 27-41.
[11] Singh N, Baby D, Rajguru JP, Patil PB, Thakkannavar SS, Pujari VB: Inflammation and cancer. Ann Afr Med.2019, 18: 121-126.
[12] Murata M: Inflammation and cancer. Environ Health Prev Med.2018, 20: 50.
[13] Ohnishi S, Ma N, Thanan R, Pinlaor S, Hammam O, Murata M, Kawanishi S: DNA damage in inflammation-related carcinogenesis and cancer stem cells. Oxid Med Cell Longev.20132013, 387014.
[14] Afrasiabi K, Zhou YH, Fleischman A: Chronic inflammation: is it the driver or is it paving the road for malignant transformation?. Genes Cancer.2015, 6: 214-9.
[15] Buchbinder EI, Desai A: CTLA-4 and PD-1 Pathways: Similarities, Differences, and Implications of Their Inhibition. Am J Clin Oncol.2016, 39: 98-106.
[16] Mittal, Vivek. (2018: Epithelial Mesenchymal Transition in Tumor Metastasis. Annual Review of Pathology: Mechanisms of Disease.13: 395-412. 10.1146/annurev-pathol-020117-043854
[17] Stuelten, C., Parent, C. & Montell, D: Cell motility in cancer invasion and metastasis: insights from simple model organisms. Nat Rev Cancer.18: 296-312.
[18] Ramos FS, Wons L, Cavalli IJ, Ribeiro EM (2017: Pressurized Intra Peritoneal Aerosol Chemotherapy (PIPAC) in patients suffering from peritoneal carcinomatosis of biliary carcinoma. Integr Cancer Sci Therap.4: 10.15761/ICST.1000243
[19] Pich-Bavastro, C., Di Domizio, J.: Integrated multi-omics reveals cellular and molecular interactions governing the invasive niche of basal cell carcinoma. Nat Commun.13: 4897.
[20] Lu W, Kang Y: Epithelial-Mesenchymal Plasticity in Cancer Progression and Metastasis. Dev Cell.2019, 6: 361-374.
[21] Schirge, S., Burtscher, I: Epithelial cell plasticity drives endoderm formation during gastrulation. Nat Cell Biol.23: 692-703.
[22] Nakaya Y, Sheng G: Epithelial to mesenchymal transition during gastrulation: an embryological view. Dev Growth Differ.2008, 50: 755-66.
[23] Montell DJ: EMT, One of Many Morphological Transitions in Cellular Phase Space. Methods Mol Biol.20212179, 13-18.
[24] Jormay Lim, Jean Paul Thiery: Epithelial-mesenchymal transitions: insights from development. Development 1 October 2012; 139 (.19: 3471-3486.
[25] Singh A, Settleman J: EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. Oncogene.2010, 26: 4741-51.
[26] Bachhawat AK, Yadav S: The glutathione cycle: Glutathione metabolism beyond the γ-glutamyl cycle. IUBMB Life.2018, 70: 585-592.
[27] J Clin Invest.2009: 1429-1437.
[28] Brabletz T, Kalluri R, Nieto MA, Weinberg RA: EMT in cancer. Nat Rev Cancer.2018, 18: 128-134.

[29] Sohal, Sukhwinder & Soltani, Amir & Weston, Steve & Wood-Baker, Richard & Walters, Haydn. (2013: Intermediate filament vimentin and potential role in epithelial mesenchymal transition.
[30] Bu Y, Cao D: The origin of cancer stem cells. Front Biosci (Schol Ed.2012, 1: 819-30.
[31] Ravindran S, Rasool S, Maccalli C: The Cross Talk between Cancer Stem Cells/Cancer Initiating Cells and Tumor Microenvironment: The Missing Piece of the Puzzle for the Efficient Targeting of these Cells with Immunotherapy. Cancer Microenviron.2019, 12: 133-148.
[32] Roato I, Ferracini R: Cancer Stem Cells, Bone and Tumor Microenvironment: Key Players in Bone Metastases. Cancers (Basel.2018, 20: 56.
[33] Huang T, Song X, Xu D: Stem cell programs in cancer initiation, progression, and therapy resistance. Theranostics.2020, 9: 8721-8743. 10.7150/thno.41648
[34] Joshi RS, Kanugula SS, Sudhir S, Pereira MP, Jain S, Aghi MK: The Role of Cancer-Associated Fibroblasts in Tumor Progression. Cancers (Basel.2021, 19: 1399. 10.3390/cancers13061399
[35] Działo E, Tkacz K, Błyszczuk P: Crosstalk between the TGF-β and WNT signalling pathways during cardiac fibrogenesis. Acta Biochim Pol.2018: 341-349.
[36] Bonde AK, Tischler V, Kumar S, Soltermann A, Schwendener RA: Intratumoral macrophages contribute to epithelial-mesenchymal transition in solid tumors. BMC Cancer.2012, 24: 35. 10.1186/1471-2407-12-35.
[37] Cinier J, Hubert M, Besson L: Recruitment and Expansion of Tregs Cells in the Tumor Environment-How to Target Them?. Cancers (Basel.2021, 13: 1850. 10.3390/cancers13081850
[38] https: //doi. org/10.1016/B978-1-4160-4252-5.50162-3.
[39] Liu, W., Zheng, Y.: CXCL1 derived from tumor-associated macrophages promotes breast cancer metastasis via activating NF-κB/SOX4 signaling. Cell Death Dis 9.880:
[40] Cui D, Zhao Y, Xu J: Activated CXCL5-CXCR2 axis promotes the migration, invasion and EMT of papillary thyroid carcinoma cells via modulation of β-catenin pathway. Biochimie.2018, 148: 1-11. 10.1016/j. biochi.2018.02.009
[41] Kim, B. N., Ahn, D. H., Kang, N: TGF-β induced EMT and stemness characteristics are associated with epigenetic regulation in lung cancer. Sci Rep.10: 10597.
[42] Xu, J., Lamouille, S. & Derynck, R: TGF-β-induced epithelial to mesenchymal transition. Cell Res.19: 156-172.
[43] Morrison CD, Parvani JG, Schiemann WP: The relevance of the TGF-β Paradox to EMT-MET programs. Cancer Lett.2013, 28: 30-40.
[44] Giuliani C, Napolitano G, Bucci I, Montani V, Monaco F: Il fattore di trascrizione NF-kB: ruolo nella patogenesi delle malattie infiammatorie, autoimmuni, neoplastiche e implicazioni terapeutiche [Nf-kB transcription factor: role in the pathogenesis of inflammatory, autoimmune, and neoplastic diseases and therapy implications]. Clin Ter.2001, 152: 249-53.
[45] Wang Y, Shi J, Chai K, Ying X, Zhou BP: The Role of Snail in EMT and Tumorigenesis. Curr Cancer Drug Targets.2013, 13: 963-972. 10.2174/15680096113136660102
[46] Housman G, Byler S, Heerboth S, Lapinska K, Longacre M, Snyder N, Sarkar S: Drug resistance in cancer: an overview. Cancers (Basel.2014, 5: 1769-92. 10.3390/cancers6031769
[47] Safa AR: Resistance to drugs and cell death in cancer stem cells (CSCs) . J Transl Sci.2020, 6: 341.
[48] Huang CY, Chung CL, Hu TH, Chen JJ, Liu PF, Chen CL: Recent progress in TGF-β inhibitors for cancer therapy. Biomed Pharmacother.2021, 134: 111046. 10.1016/j. biopha.2020.111046
[49] Imani S, Hosseinifard H, Cheng J, Wei C, Fu J: Prognostic Value of EMT-inducing Transcription Factors (EMT-TFs) in Metastatic Breast Cancer: A Systematic Review and Meta-analysis. Sci Rep.2016236, 28587: 10.
[50] Du B, Shim JS: Targeting Epithelial-Mesenchymal Transition (EMT) to Overcome Drug Resistance in Cancer. Molecules (Basel, Switzerland.2016, 21: 965. 10.3390/molecules21070965