International Journal of Advance Research in Science and Engineering
Volume No.06, Issue No. 11, November 2017
www.ijarse.com
IJARSE
ISSN: 2319-8354

EPITHELIAL–MESENCHYMAL TRANSITION: ROLE OF TRANSCRIPTION FACTORS WITH EMPHASIS ON SIX1

Younus Ahmad Bhat[1], Asiya Batool[2], Sabreena Aashaq[3]

[123]*University of Kashmir, Srinagar,(India)*

ABSTRACT

Epithelial–mesenchymal transition (EMT) is a vital, multifunctional and tightly regulated cellular mechanism that has been long recognized as a core feature of normal development. Significant developmental processes including neural crest formation, gastrulation, and heart morphogenesis, reckon on the transition between epithelium and mesenchyme. More recent studies have envisaged that epithelial–mesenchymal transition occur during the progression of epithelial tumors, conferring cancer cells with the characteristics of increased motility and invasiveness. EMT is induced by multiple oncogenic pathways mediated by peptide growth factors, Src, Ras, Ets, Wnt/β-catenin, integrin and Notch signalling etc; but the molecular regulation of its signaling pathway is highly complex. We bring in light the different elements of EMT cascades that could be targeted. The review summarises the role of transcription factors in particular Six1 in promoting Epithelial–mesenchymal transition (EMT). We also discuss the role of Six1 in different cancers impinging the need to study EMT-Six1 axis in cancer progression.

Keywords: *Cadherin, Epithelium, Mesenchyme,Metastasis, Transition,*

I. INTRODUCTION

Epithelial-mesenchymal transition (EMT) is a significant developmental biological process that allows the polarised epithelial cells that are normally connected to basement membrane via adhesion undergo multiple biochemical changes, lose their characteristics of being non-motile and acquire characteristic properties of mesenchymal cellswhich includes enhanced migratory capacity, invasiveness, elevated resistance to apoptosis and augmented production of ECM components (1). This occurs during developmental processes like neural tube formation, and particularly when metastasis is initiated in cancer. The degradation of underlying basement membrane and generation of the mesenchymal cell ready to migrate away from the epithelial layer marks the completion of an EMT.

International Journal of Advance Research in Science and Engineering
Volume No.06, Issue No. 11, November 2017
www.ijarse.com
IJARSE
ISSN: 2319-8354

Epithelial to Mesenchymal Transition

	Epithelial cell	Metastable cell	Mesenchymal cell
Morphology			
Polarity	Apical- basal	Residual	Front-back
Junctions	Tight junctions Adherans Desmosomes	Residual	No junctions
Cytoskeleton	Cortical actin Cytokeratins	Cytokeratins Vimentin	Stress fibre Vimentin Smooth muscle action
Traits	Stationary	Mixed epithelial and mesenchymal markers	Increased scattering Migration Invasion Anoikis resistance

Table 1.Epithelial, metastable, and mesenchymal cell phenotypes.

A myriad of distinct molecular processes are engaged to initiate an EMT and enable it to reach completion. These processes include expression of specific cell-surface proteins, activation of transcriptionfactors, reorganization and expression of cytoskeletal proteins,production of ECM-degrading enzymes, and modification in the expression of specific microRNAs. In many cases, the engaged factors are also used as biomarkers to demonstrate the passage of a cell through an EMT (Figure 1).

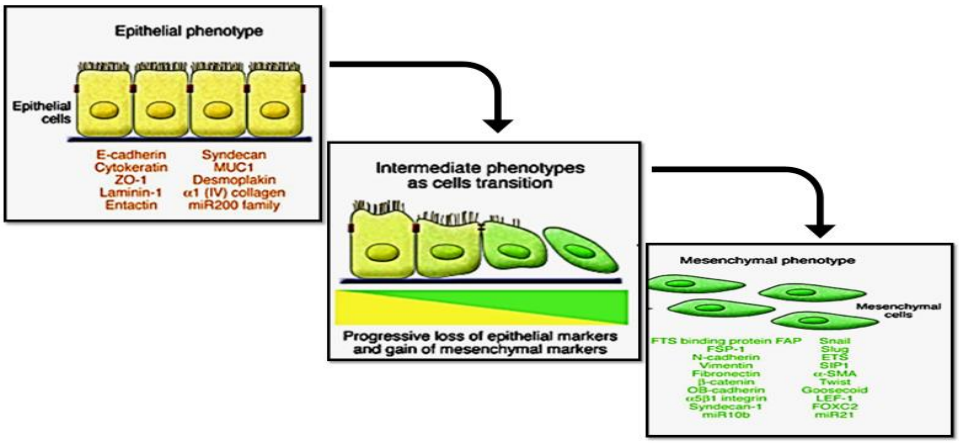

Figure 1.EMT. EMT involves a functional conversion of polarized non-mobile epithelial cells into mobile unpolarised mesenchymal cells. The epithelial and mesenchymal cell markers commonly used by EMT researchers are enlisted. Colocalization of these two sets of distinct markers defines an intermediate phenotype of EMT, depicting that cells have passed only partly through an EMT.

International Journal of Advance Research in Science and Engineering
Volume No.06, Issue No. 11, November 2017
www.ijarse.com
IJARSE
ISSN: 2319-8354

II. EMT AND METASTASIS

The occurrence of invasion and metastasis is the major cause for most cancer-related deaths. During metastatic progression of carcinoma, polarized epithelial tumor cells gain invasive and migratory characteristics, leave the primary site, invade the basement membrane beneath, intravasate into blood or lymph vessels, transport through the circulation, extravasate from the circulation, disseminate into the secondary site, and grow at the metastatic foci. Epithelial carcinoma cells disseminate from primary tumor sites by using either collective cell migration (2) or single cell migration such as round shape, non-proteolytic amoeboid migration (3) and mesenchymal-type movement (4). This phenotypic conversion enables tumor cells dissociate from their original tissue and form metastasis in distant organ. This epithelial cell plasticity caused by breakdown of epithelial cell homeostasis leading to malignant cancer progression has been associated with the loss of epithelial traits and the acquisition of migratory phenotype. In carcinomas,cells awakening the event of mesenchymal transition become motile and increase invasive ability (4). Hence, the phenotypic transition from epithelial to mesenchymal-like cell state represents one important mechanism for epithelial plasticity and cancer metastasis.

Recent studies have implicated the role of Twist, the basic helix–loop–helix transcription factor in the early steps of metastasis (5,6). Twist has been previously known to be involved in neural crest cell migration and mesoderm differentiationand has recently been identified for genes associated with metastasis to the lungs of murine mammary tumor lines. Moreover, studies demonstrate that Twist overexpression results in EMT of human immortalized mammary epithelial cells, which is characterized by downregulation of E-cadherin, beta- and italic gamma-catenin, upregulation of fibronectin and vimentin, spindle-like morphological changes and increased migration.

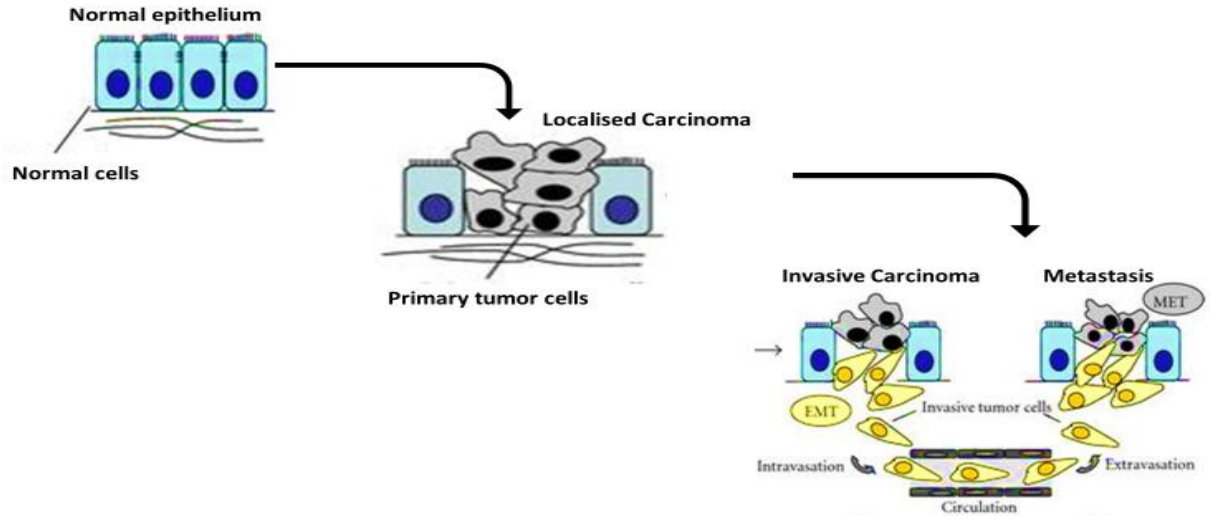

Figure 2. Stages from normal epithelium to cancer progression.

International Journal of Advance Research in Science and Engineering
Volume No.06, Issue No. 11, November 2017
www.ijarse.com
IJARSE
ISSN: 2319-8354

III. MOLECULAR BASIS OF EMT

Polarized cells are tethered together with wellformed junctional complexes, including tight junctions, adherens junctions, demosomes, and gap junctions. Disruption of adhesion complexes has been thought a preliminary step for losing cell contact in epithelial cells. When epithelial cancer cells gained invasive ability, they are usually associated with reduced expression of the cell-cell adhesion molecules and re-express markers of mesenchymal origin, frequently referred as EMT. The molecular changes in EMT event include (i) Loss of epithelial markers, such as E-cadherin and β-catenin; (ii) de novo expression of mesenchymal-related proteins N-cadherin and fibronectin as well as mesenchymal intermediate filament vimentin; (iii) cytoskeleton rearrangement mediated by Rho small GTPases; (iv) up-regulation and nuclear translocation of transcription factors which govern gene program. Changes in cell morphology and function during the EMT process are accompanied by changes in protein expression profiles, including the loss of the epithelial markers and the de novo expression of mesenchymal markers.

Epithelial cells sense environmental stimuli from extracellular matrix proteins and growth factors through integrins and growth factor receptors respectively. Previous studies have revealed that EMT can be triggered by interplay of extracellular signals, including extracellularmatrix components and soluble growth factors, such as transforming growth factor-beta (TGF-β) and fibroblast growth factor (FGF) families, epidermalgrowth factor (EGF), insulin-like growth factor (IGF-1) and scatter factor/ hepatocyte growth factor (SF/HGF) in cancer progression (7,8). Receptor-mediated signaling in response to these ligands transducer signaling activates intracellular modules and changes cytoskeleton reorganization. Finally, the signaling pathway leads to the activation of nuclear transcriptional regulators, which regulate the global cellular changes on the gene expression.

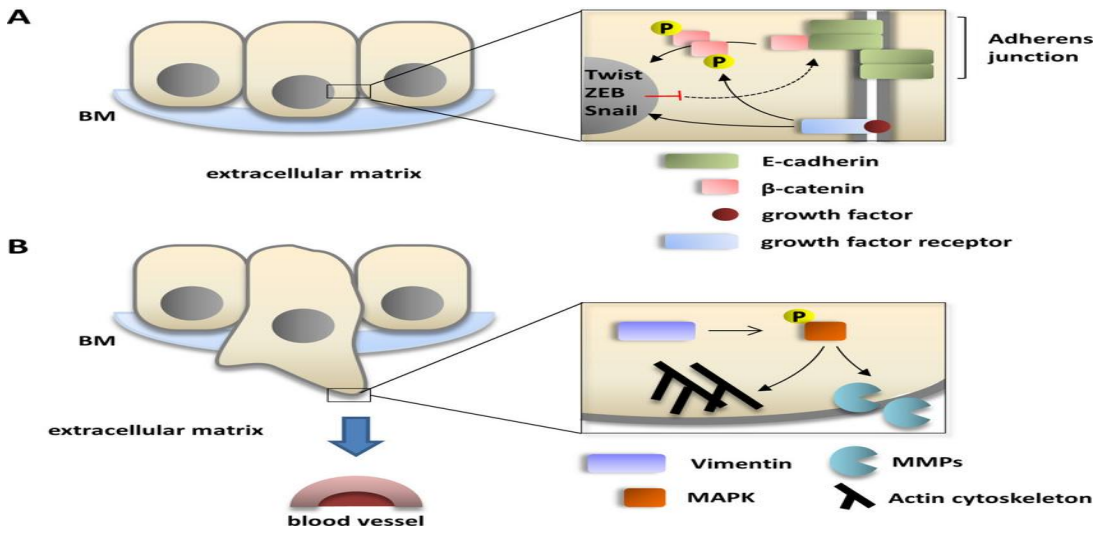

Figure 3: Basic molecular changes underlying EMT.

A, Signaling pathways activate EMT-promoting transcription factors (like Twist, ZEB, Snail, Six 1) to supress transcription of E-cadherin that results in the formation of adherens junction (AJ) complex in conjunction with β-catenin. Removal of E-cadherin from the adherens junction complex and associated phosphorylation via activated growth factor receptors leads to cytoplasmic accumulation and nuclear translocation of β-catenin, where it acts as a transcription factor for migration-associated genes. B, Extensive expression of Vimentin in migrating tumor cells shields phosphorylated MAPK from cytoplasmic phosphatases, thus ensuring signaling activity along the EGFR/MAPK axis. This supports pro-migratory effects on the cytoskeleton (for exampleRac-mediated actin polymerization) and secretion of lytic matrix metalloproteinases that cleave the surrounding extracellular matrix to allow for cell migration.(Adapted from Clinical significance of epithelial-mesenchymal transition Jul 2014)

In the mesenchymal trans-differentiation process of epithelial cells, downregulation of E-cadherin has been characterized as the major hallmark responsible for the loss of cell-cell contacts in the EMT events. E-cadherin, which is present in mature adherens junctions, is a pivotal molecule maintaining epithelial cell polarity. E-cadherin binds to β-catenin to form a protein complex which links to actin cytoskeleton. E-cadherin has anti-proliferation, anti-invasion, and anti-metastasis functions, and loss of E-cadherin contributes to metastatic dissemination in numerous cancer types (9). Mechanisms for E-cadherin loss in malignant cancers include genetic mutation, epigenetic silencing, transcription repression and proteolytic processing. It has been generally regarded that E-cadherin is not expressed in mesenchymal cell types due to the action of transcriptional repressors (10). Several transcription factors, which are majorly expressed in mesenchymal-like cells, have been implicated in the transcriptional repression of E-cadherin gene (CDH1) and EMT events. The zinc -finger protein family of Snail, Slug, and Smuc; two-handed zinc factors of family of ZEB1 and ZEB2 (also known as Smad-interacting protein-1, SIP-1); and basic helix-loop-helix proteins Twist1, Twist2 and E47 have been demonstrated to repress E-cadherin gene expression and regulate other gene function leading to EMT induction (11).

IV. ROLE OF TRANSCRIPTION FACTORS IN EMT

In the majority of human carcinoma cell populations, the loss of E-cadherin appears to be heterogeneous, with foci of E-cadherin-positive carcinoma cells scattered among E-cadherin negative areas. Moreover, E-cadherin is often detected in distant metastasis, which may derive from cells that have previously passed through an EMT (12,13). This suggests that after their initial dissemination, some metastatic cells may revert to an epithelial state, and it indicates that other, nongenetic mechanisms controlling transcription of the E-cadherin gene or posttranslational modification of the E-cadherin protein may be responsible for the lack of expression of this protein in some tumor cells.Indeed, many EMT-inducing transcription factors, including Snail, Slug,Six1, dEF1, SIP1, Twist1, FOXC2 and Goosecoidhave been associated with tumor invasion and metastasis. Expression of almost all of these has been detected in certain invasive human and mouse tumor cell lines. Expression of Twist1 has been shown to be essential for a mouse breast carcinoma cell line to metastasize from the mammary

gland to the lung (5). And overexpression of FOXC2 or Goosecoid increases the ability of weakly metastatic human carcinoma cells to disseminate (14,15). The involvement of several EMT-inducing transcription factors has also been reported in human carcinomas. For example, when gene expression profiling was performed for ‘‘poor prognosis’’ signatures, overexpression of Twist1 was associated with distant metastasis and poor survivalin N-Myc-amplified neuroblastomas (16) and in melanomas (17). A recent review has presented an excellent survey on the published reports of their expression in human cancers (18). Moreover, overexpressionof Six 1 homeoprotein transcription factor has been reported in various tumors including cervical cancer. It has been reported to promote cancer metastasis and EMT through enhancing TGF-βsignaling(19)

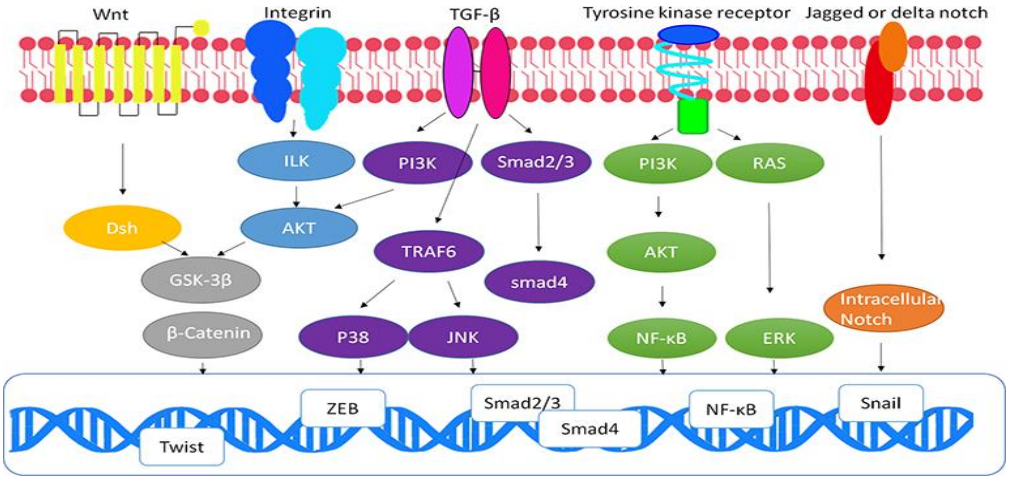

Figure 4.The Molecular Network of Known Signaling Pathways and Transcription Factors that Regulate the Epithelial Mesenchymal Transition Program in Carcinoma Cells.(adapted from Epithelial-Mesenchymal Transition and its Regulation in Tumor Metastasis By Tao Sun, Yuan Qin and Wei-long Zhong DOI: 10.5772/64497).

V. SIX 1(SINE OCULIS HOMEOBOX 1)

Six1 (Sine Oculis homeobox 1) is a transcription factor that plays an important role in the survival and proliferation of precursor cells during normal development in various tissues including, amongst others, the inner ear, the kidney and muscle. It is also demonstrated to play a role in the proliferation of cancer cells and in cancer metastasis. It is explicitly known to activate several target genes, including c-MYC, cyclin A1, , GDNF, and SLC12A2. The members of the Six family of genes are the homologs of the Drosophila sine oculis (So), optix and Dsix4 genes. In Drosophila, loss of So leads to the elimination of the compound eyes and Optix induces the eye formation when expressed in nonretinal tissues, while Dsix4 plays an important role in several mesoderm derivatives, including somatic muscles, somatic cells of the gonad, and fat tissue(20). In vertebrates, the Six family is classified into three subgroups, Six1/Six2 (So), Six3/Six6 (Optix), and Six4/Six5 (Dsix4), which are characterized by a Six-type homeodomain (HD, 60 amino acids) and Sixdomain (SD, 110–115 amino acids)(20). So far, Six family genes have been identified in various species from lower invertebrates to higher

mammal(19). Among these, Six homeobox 1 (Six1), the most extensively investigated, is involved in the development of many tissues and organs, such as muscle (21–23) kidney(24–26) the auditory system(27–29) sensory organs(31–35) and craniofacial structures(25,36,37)**.** The protein coded by six1 gene is a homeobox protein that is similar to the Drosophila 'sine oculis' gene product. This gene is found in a cluster of related genes on chromosome 14(14q23.1) in humans and is thought to be involved in limb development. Defects in this gene are a cause of autosomal dominant deafness type 23 (DFNA23) and branchiotic syndrome type 3 (BOS3).So this gene is also known as BOS3 or DFNA23 gene.In addition, much attention has been paid to the role of Six1 in tumorigenesis. Numerous studies have documented that Six1 participates in the occurrence of various human cancers including breast cancer(38–42) ovarian cancer(43) cervical cancer(44,45), hepatocellular carcinoma(46,47) rhabdomyosarcoma(48) background Wilms tumors(49) and colorectal cancer(50).

The chromosomal locations of several Six genes have been identified in Drosophila, mouse and human species. For example, so is mapped very close to another Six gene, optix/D-Six3, on Drosophila chromosome 2, and the orthologous Six2 and Six3 genes are also found very near each other on mouse chromosome 17. (51,52). Most importantly, it has been shown that SIX4, SIX1 and SIX6 (or OPTX2), each of which belongs to a different subfamilyare very closely linked on human chromosome14 .

VI. ROLE OF SIX1 IN EMT

Six1 is a homeoprotein that is essential for the development of a number of organs and is upregulated in proliferating precursor populations relative to differentiated adult tissues (53). Six1 was first reported to be upregulated in breast cancer cells in 1998 (53). In the decade since, subsequent studies have shown that it may play a critical role in breast cancer development. Six1 shows elevated expression in human breast cancer via gene amplification (53,54), and ectopic overexpression of Six1 in mammary cells is sufficient to induce malignant transformation and chromosomal instability (55). Mechanistic insights come from the observation that overexpression of Six1 leads to abrogation of cell cycle checkpoints (53). The current studies by McCoy et al. (56) and Micalizzi et al. (57) significantly extrapolate these previous investigations by using mouse models to define how Six1 induces EMT to stimulate tumor development and metastasis. McCoy et al. (56) present evidence revealing how Six1 may function to promotetumors at the earliest stages of development through the use of new transgenic mice that inducibly express Six1 in mouse mammary epithelial cells. Expression of Six1 resulted in alveolar expansion and epithelial hyperplasia within the first few weeks. Sustained expression of Six1 led to the formation of aggressive tumors characterized by highly divergent epithelial differentiation that included regions displaying evidence of EMT. Six1 expression was also shown to increase the fraction of epithelial cells expressing mammary stem/progenitor characteristics: isolated primary epithelial cells from Six1-expressing mice showed increased expression of the stem/progenitor cell–associated cell surface markers CD24 and CD29, as well as greatly increased growth as mammospheres, an assay that reflects cell self-renewal. Thus, Six1-induced EMT was associated with increases in the population of stem/progenitor cells and spontaneous tumor growth. Micalizzi et al. (57) used a parallel approach to define how Six1-induced EMT facilitates tumor

metastasis using xenograft assays. Overexpression of Six1 in cultured human mammary cells resulted in rapid morphological EMT, and Six1-overexpressing cells showed a greatly increased propensity for spontaneous metastasis in orthotopic xenografts, as well as substantially greater metastatic capability following intracardiac injection. Inappropriate activation of developmental pathways is a well-recognized tumor-promoting mechanism. The ectopic expression of the homeoprotein Six1, normally a developmentally restricted transcriptional regulator, augments TGF-βsignaling in human breast cancer cells and induces an epithelial-mesenchymal transition (EMT) that is in part dependent on its ability to increase TGF-βsignaling. TGF-βsignaling and EMT have been implicated in metastatic dissemination of carcinoma. In addition, like its induction of EMT, Six1-induced experimental metastasis is dependent on its ability to activate TGF-βsignaling. Importantly, in human breast cancers Six1 correlated with nuclear Smad3 and thus increased TGF-βsignaling.

The epithelial mesenchymal transition (EMT) is regulated at all levels og gene expression may it be transcription, post-transcription, translation or post translational levels. Transforming growth factor-β (TGFβ) induces EMT by acting at some of these levels via SMAD-mediated and non-SMAD mediated signaling. TGFβ –SMAD signaling triggers the expression of EMT transcription factors, and also increases the expression of microRNAs (miRNAs) that repress the expression of epithelial proteins, and target mesenchymal components, thus promoting EMT. TGFβ signaling also downregulate the expression of the epithelial splicing regulatory proteins (ESRPs), which results in the differential splicing programme following EMT. TGFβ can also induce non-SMAD signaling pathways that contribute to EMT. It activates PI3K–AKT-mammalian TOR complex 1 (mTORC1) signalling, which increases translation and cell size; active AKT also derepresses the translation of specific mRNAs by phosphorylating heterogeneous nuclear ribonucleoprotein E1 (hnRNPE1). TGFβ also increases cell junction dissolution and induces cytoskeletal changes by regulating RHO-GTPases. TGFβ results in TβRII association with a TβRI-partitioning defective 6 (PAR6) complex at tight junctions, that enables TβRII to phosphorylate PAR6; this triggers the recruitment of E3 ubiquitin ligase SMAD ubiquitylation regulatory factor 1 (SMURF1), RHOA ubiquitylation and degradation, and the loss of tight junctions. TGFβ also enhances RHOA activity; this promotes actin reorganization by leading to the activation of diaphanous (DIA1) and also RHO-associated kinase (ROCK), which phosphorylates myosin light chain (MLC) to activate LIM kinase (LIMK) and thus inhibit cofilin. RAC and CDC42 also participate in cytoskeletal changes through p21 activated kinase 1 (PAK1) and direct the formation of lamellipodia and filopodia. 4E–BP1, eukaryotic translation initiation factor 4E–binding protein1; S6K1, ribosomal S6 kinase 1.

VII. SIX1 AND HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma (HCC) accounts for 90% of all liver cancers and causes more than 500,000 deaths in the world annually(58). In 2006, Ng et al. first reported that Six is closely associated with tumour recurrence and metastasis of HCC, overexpression of Six1 mRNA was observed in about 85% of liver tumour tissues, while Six1 mRNA was absent in 91.7% of liver notumour tissues. Similarly, Six1 protein was overexpressed in about 60% of tumour tissues and not detected in non-tumour tissues(59). Further analysis indicates that elevated Six1

protein is significantly associated with advanced tumor stage and poor overall survival after surgical resection of HCC in human patients(59). Subsequently, it has been demonstrated that Six1 markedly increases the tumorigenicity and metastatic capacity of HCC using short hairpin RNA (shRNA) interference technique in vitro and in vivo, and identified 52 possible target genes by cDNA microarry; however,whether these targets are involved in the development and metastasis of HCC requires confirmation(60).

VIII. SIX1 AND OVARIAN AND CERVICAL CANCER

Ovarian carcinoma and cervical carcinoma are the most prevalent cancers affecting women worldwide. Six1 makes an important contribution to ovarian and cervical cancer, and is considered a potential biomarker because of its abnormally high expression in ovarian and cervical cancer(61,62). When compared to normal ovary, Six1 is overexpressed in 50% of early-stage (stage I) and 63% of late-stage (stages II, III, and IV) ovarian carcinomas. Further analysis showed that Six1 can promote ovarian carcinoma cell proliferation and prevent tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated ovarian carcinoma cell apoptosis(61). While the cell apoptosis pathway mediated by TRAIL depends on the interaction of the death receptors DR4/DR5 and the Fas-associated death domain adaptor molecule (FADD). FADD recruits the initiator caspases 8 and 10 to the death receptors DR4/DR5 to form the death-inducing signaling complex, (63,64) which can further activate the terminal executioner caspases 3, 6, and 7, thereby causeing cell death(65). Epithelial-mesenchymal transition (EMT) is involved in the metastasis of primary tumors, thus the regulatory program of EMT is considered a molecular mechanism for cervical cancer metastasis. In colorectal cancer (CRC), EMT seems to depend on the activation of ZEB1, which is regulated by Six1(66). Moreover, Six1 induces mammary tumors through EMTsince the sarcomatoidtumors display full oncogenic EMT, while 80% of nonsarcomatoidtumors display partial EMT(66). The underlying molecular mechanism is partly dependent on increased TGF-b signalling (67), which is mediated by Eya2 in human mammary carcinoma cells(68). In addition, the lateststudy suggests that SIX1 is a most likely candidate marker for background Wilms tumors (WT), the most common renal neoplasm in children. WT consist of three tissue elements: blastema, epithelium and stroma, while SIX1 gene is abnormally predominant expressed in blastema cells(69). In addition, Six1 also participates in the pancreatic tumorigenesis; it is overexpressed in pancreatic cancer and its expression level correlates with the process of tumorigenesis, and further analysis indicates that Six1 contributes to pancreatic cancer through the upregulation of cyclin D1(70).

IX. CONCLUDING REMARKS

Epithelial and mesenchymal cells represent two of the main cell types in mammals with distinct characteristics. In recent years, accumulating support the fact that EMT is a critical process not only in development but also in tumorigenesis. The acquisition of EMT properties during tumor progression are described well in the review. We discuss the molecular basis of EMT and in particular the role of various transcription factors in inducing

EMT. We lay emphasis on Six1- EMT nexus for activation of oncogenic signaling and disruption of tumor suppressor networks.

REFERENCES

[1] Kalluri, R., and Neilson, E.G. 2003. Epithelialmesenchymal transition and its implications for fibrosis. J. Clin. Invest. 112:1776–1784.

[2] Friedl P, Gilmour D. Collective cell migration inmorphogenesis, regeneration and cancer. NatRevMol Cell Biol 2009; 10: 445-457.

[3] Guck J, Lautenschlager F, Paschke S and Beil M. Critical review: cellular mechanobiology and amoeboid migration. IntegrBiol (Camb) 2010; 2: 575-583.

[4] Thiery JP, Acloque H, Huang RY and Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell 2009; 139: 871-890.

[5] Yang J, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C, Savagner P, Gitelman I, Richardson A and Weinberg RA. (2004). Cell, 117, 927–939. | Article | PubMed

[6] Yang Z, Rayala S, Nguyen D, Vadlamudi RK, Chen S and Kumar R. (2005). Cancer Res., 65, 3179–3184. | PubMed | ISI | ChemPort

[7] Huber MA, Kraut N and Beug H. Molecular requirements for epithelial-mesenchymal transition during tumor progression. CurrOpin Cell Biol 2005; 17: 548-558.

[8] Mimeault M, Batra SK. Interplay of distinct growth factors during epithelial mesenchymal transition of cancer progenitor cells and molecular targeting as novel cancer therapies. Ann Oncol 2007; 18: 1605-1619.

[9] Van Roy F, Berx G. The cell-cell adhesion molecule E-cadherin. Cell Mol Life Sci 2008; 65: 3756-3788.

[10] Hennig G, Behrens J, Truss M, Frisch S, Reichmann E and Birchmeier W. Progression ofcarcinoma cells is associated with alterations in chromatin structure and factor binding at the Ecadherin promoter in vivo. Oncogene 1995; 11: 475-484

[11] Peinado H, Olmeda D and Cano A. Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? Nat Rev Cancer 2007; 7: 415-428.

[12] Bukholm, I.K., Nesland, J.M., Karesen, R., Jacobsen, U., and Borresen-Dale,A.L. (1998). E-cadherin and a-, b-, and g-catenin protein expression in relationto metastasis in human breast carcinoma. J. Pathol. 185, 262–266.

[13] Bukholm, I.K., Nesland, J.M., and Borresen-Dale, A.L. (2000). Re-expressionof E-cadherin, a-catenin and b-catenin, but not of g-catenin, in metastatic tissuefrom breast cancer patients. J. Pathol. 190, 15–19.

[14] Hartwell, K.A., Muir, B., Reinhardt, F., Carpenter, A.E., Sgroi, D.C., and Weinberg, R.A. (2006). The Spemann organizer gene, Goosecoid, promotes tumormetastasis. Proc. Natl. Acad. Sci. USA 103, 18969–18974.

[15] Mani, S.A., Yang, J., Brooks, M., Schwaninger, G., Zhou, A., Miura, N., Kutok,J.L., Hartwell, K., Richardson, A.L., and Weinberg, R.A. (2007). Mesenchyme Forkhead 1 (FOXC2) plays a key role in metastasis and is associated with aggressive basal-like breast cancers. Proc. Natl. Acad. Sci. USA 104, 10069–10074.

[16] Valsesia-Wittmann, S., Magdeleine, M., Dupasquier, S., Garin, E., Jallas, A.C.,Combaret, V., Krause, A., Leissner, P., and Puisieux, A. (2004). Oncogeniccooperation between H-Twist and N-Myc overrides failsafe programs in cancercells. Cancer Cell 6, 625–630.

[17] Hoek, K., Rimm, D.L., Williams, K.R., Zhao, H., Ariyan, S., Lin, A., Kluger, H.M., Berger, A.J., Cheng, E., Trombetta, E.S., et al. (2004). Expression profiling reveals novel pathways in the transformation of melanocytes to melanomas.Cancer Res. 64, 5270–5282.

[18] Peinado, H., Olmeda, D., and Cano, A. (2007). Snail, Zeb and bHLH factors intumour progression: an alliance against the epithelial phenotype? Nat. Rev.Cancer 7, 415–428.18. christen et al.2009

[19] Kumar JP. The sine oculis homeobox (SIX) family of transcription factors as regulators of development and disease. Cell Mol Life Sci 2009;66:565–83.

[20] Kawakami K, Sato S, Ozaki H, et al. Six family genes–structure and function as transcription factors and their roles in development. Bioessays2000;22:616–26.

[21] Laclef C, Hamard G, Demignon J, et al. Altered myogenesis in Six1-deficient mice. Development 2003;130: 2239–52.

[22] Grifone R, Demignon J, Houbron C, et al. Six1 and Six4 homeoproteins are required for Pax3 and Mrf expression during myogenesis in the mouse embryo. Development 2005;132:2235–49.

[23] Richard AF, Demignon J, Sakakibara I, et al. Genesis of muscle fiber-type diversity during mouse embryogenesis relies on Six1 and Six4 gene expression. Dev Biol2011;359:303–20.

[24] Xu PX, Zheng W, Huang L, et al. Six1 is required for the early organogenesis of mammalian kidney. Development 2003;130:3085–94.

[25] Laclef C, Souil E, Demignon J, et al. Thymus, kidney and craniofacial abnormalities in Six 1 deficient mice. Mech Dev 2003;120:669–79.

[26] Nie XG, Xu JS, El-Hashash A, et al. Six1 regulates Grem1 expression in the metanephric mesenchyme to initiate branching morphogenesis. Dev Biol2011;352:141–51.

[27] Zheng W, Huang L, Wei ZB, et al. The role of Six1 in mammalian auditory system development. Development 2003;130:3989–4000.

[28] Ozaki H, Nakamura K, Funahashi J, et al. Six1 controls patterning of the mouse otic vesicle. Development 2004;131:551–62.

[29] Bricaud O, Collazo A. The transcription factor six1 inhibits neuronal and promotes hair cell fate in the developing zebrafish (Danio rerio) inner ear. J Neurosci2006;26:10438–51.

[30] Bricaud O, Collazo A. Balancing cell numbers during organogenesis: Six1a differentially affects neurons and sensory hair cells in the inner ear. Dev Biol2011;357:191–201.

[31] Konishi Y, Ikeda K, Iwakura Y, et al. Six1 and Six4 promote survival of sensory neurons during early trigeminal gangliogenesis. Brain Res 2006; 1116:93–102.

[32] Bessarab DA, Chong SW, Korzh V. Expression of zebrafish six1 during sensory organ development and myogenesis. Dev Dynam 2004;230: 781–6.

[33] Christophorou NAD, Bailey AP, Hanson S, et al. Activation of Six1 target genes is required for sensory placode formation. Dev Biol 2009;336: 327–36.

[34] Anderson AM, Weasner BM, Weasner BP, et al. Dual transcriptional activities of SIX proteins define their roles in normal and ectopic eye development. Development 2012;139:991–1000.

[35] Zou D, Silvius D, Fritzsch B, et al. Eya1 and Six1 are essential for early steps of sensory neurogenesis in mammalian cranial placodes. Development 2004;131:5561–72.

[36] Guo CS, Sun Y, Zhou B, et al. A Tbx1-Six1/ Eya1-Fgf8 genetic pathway controls mammalian cardiovascular and craniofacial morphogenesis. J Clin Invest 2011;121:1585–95.

[37] Moody SA, Mhaske P, Pignoni F, et al. Identification of novel candidate Six1-interacting proteins proteins with potential roles in cranial placode development. Dev Biol2010;344:4556.

[38] Ford HL, Kabingu EN, Bump EA, et al. Abrogation of the G2 cell cycle checkpoint associated with overexpression of HSIX1: a possible mechanismof breast carcinogenesis. Proc Natl Acad Sci USA 1998;95:12608–13.

[39] Reichenberger KJ, Coletta RD, Schulte AP, et al. Gene amplification is a mechanism of Six1 overexpression in breast cancer. Cancer Res 2005;65: 2668–75.

[40] Coletta RD, Christensen KL, Micalizzi DS, et al. Six1 overexpression in mammary cells induces genomic instability and is sufficient for malignant transformation. Cancer Res 2008;68:2204–13.

[41] Micalizzi DS, Wang CA, Farabaugh SM, et al. Homeoprotein Six1 Increases TGF-beta type I receptor and converts TGF-beta signaling from suppressive to supportive for tumor growth. Cancer Res 2010;70:10371–80.

[42] Wang CA, Jedlicka P, Patrick AN, et al. SIX1 induces lymphangiogenesis and metastasis via upregulation of VEGF-C in mouse models of breast cancer. J Clin Invest 2012;122:1895–906.

[43] Behbakht K, Qamar L, Aldridge CS, et al. Six1 overexpression in ovarian carcinoma causes resistance to TRAIL-mediated apoptosis and is associated with poor survival. Cancer Res 2007; 67:3036–42.

[44] Tan J, Zhang CX, Qian JZ. Expression and significance of Six1 and Ezrin in cervical cancer tissue. TumorBiol2011;32:1241–7.

[45] Wan F, Miao X, Quraishi I, et al. Gene expression changes during HPV-mediated carcinogenesis: a comparison between an in vitro cell model and cervical cancer. Int J Cancer 2008;123:32–40.

[46] Ng KT, Man K, Sun CK, et al. Clinicopathological significance of homeoprotein Six1 in hepatocellular carcinoma. Br J Cancer 2006;95:1050–5.

[47] Ng KTP, Lee TKW, Cheng QA, et al. Suppression of tumorigenesis and metastasis of hepatocellular carcinoma by shRNA interference targeting on homeoprotein Six1. Int J Cancer2010;127:859–72.

[48] Yu Y, Khan J, Khanna C, et al. Expression profiling identifies the cytoskeletal organizer ezrin and the developmental homeoprotein Six-1 as key metastatic regulators. Nat Med 2004;10: 175–81.

[49] Sehic D, Karlsson J, Sandstedt B, et al. SIX1 protein expression selectively identifies blastemal elements in Wilms tumor. Pediatr Blood Cancer 2012;59:62–8.

[50] Ono H, Imoto I, Kozaki K, et al. SIX1 promotes epithelial-mesenchymal transition in colorectal cancer through ZEB1 activation. Oncogene 2012; 31:4923–34.

[51] Oliver G, Mailhos A, Wehr R, Copeland NG, Jenkins NA, Gruss P. Six3, amurine homologue of the sine oculis gene, demarcates the most anterior border of the developing neural plate and is expressed during eye development. Development 1995;121:4045±4055.

[52] Oliver G, Wehr R, Jenkins NA, Copeland NG, Cheyette BN, Hartenstein V, Zipursky SL, Gruss P. Homeobox genes and connective tissue patterning. Development 1995;121:693±705.

[53] Ford, H.L., Kabingu, E.N., Bump, E.A., Mutter, G.L., and Pardee, A.B. 1998. Abrogation of the G2 cell cycle checkpoint associated with overexpression of HSIX1: a possible mechanism of breast carcinogenesis. Proc. Natl. Acad. Sci. U. S. A. 95:12608–12613.176.

[54] Reichenberger, K.J., Coletta, R.D., Schulte, A.P., Varella-Garcia, M., and Ford, H.L. 2005. Gene amplification is a mechanism of Six1 overexpression in breast cancer. Cancer Res. 65:2668–2675.

[55] Coletta, R.D., et al. 2008. Six1 overexpression in mammary cells induces genomic instability and is sufficient for malignant transformation. CancerRes.68:2204–2213.

[56] McCoy, E.L., et al. 2009. Six1 expands the mouse mammary epithelial stem/progenitor cell pool and induces mammary tumors that undergo epithelial-mesenchymal transition. J. Clin. Invest. 119:2663–2677.

[57] Micalizzi, D.S., et al. 2009. The Six1 homeoprotein induces human mammary carcinoma cells to undergo epithelial-mesenchymal transition and metastasis in mice through increasing TGF-b signaling. J. Clin. Invest. 119:2678–2690.

[58] Jelic S, Sotiropoulos GC, Grp EGW. Hepatocellular carcinoma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 2010;21:v59–v64.

[59] Ng KT, Man K, Sun CK, et al. Clinicopathological significance of homeoprotein Six1 in hepatocellular carcinoma. Br J Cancer 2006;95:1050–5.

[60] Ng KTP, Lee TKW, Cheng QA, et al. Suppression of tumorigenesis and metastasis of hepatocellular carcinoma by shRNA interference targeting on homeoprotein Six1. Int J Cancer 2010;127:859–72.

[61] Behbakht K, Qamar L, Aldridge CS, et al. Six1 overexpression in ovarian carcinoma causes resistance to TRAIL-mediated apoptosis and is associated with poor survival. Cancer Res 2007; 67:3036–42.

[62] Wan F, Miao X, Quraishi I, et al. Gene expression changes during HPV-mediated carcinogenesis: a comparison between an in vitro cell model and cervical cancer. Int J Cancer 2008;123:32–40.

[63] Thomas LR, Henson A, Reed JC, et al. Direct binding of Fas-associated death domain (FADD) to the tumor necrosis factor-related apoptosisinducing ligand receptor DR5 is regulated by the death effector domain of FADD. J Biol Chem2004;279:32780–5.

[64] Thomas LR, Johnson RL, Reed JC, et al. The Cterminal tails of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and Fas receptors have opposing functions in Fasassociated death domain (FADD) recruitment and can regulate agonist-specific mechanisms of receptor activation. J BiolChem2004;279:52479–86.

[65] Rowinsky EK. Targeted induction of apoptosis in cancer management: the emerging role of tumor necrosis factor-related apoptosis-inducing ligand receptor activating agents. J ClinOncol2005;23:9394–407.

[66] Ono H, Imoto I, Kozaki K, et al. SIX1 promotes epithelial-mesenchymal transition in colorectal cancer through ZEB1 activation. Oncogene 2012; 31:4923–34.

[67] Jones AM, Mitter R, Poulsom R, et al. mRNA expression profiling of phyllodes tumours of the breast: identification of genes important in the development of borderline and malignant phyllodes tumours. J Pathol2008;216:408–17.

[68] Micalizzi DS, Christensen KL, Jedlicka P, et al. The Six1 homeoprotein induces human mammary carcinoma cells to undergo epithelialmesenchymal transition and metastasis in mice through increasing TGF-beta signaling. J ClinInvest2009;119:2678–90.

[69] Sehic D, Karlsson J, Sandstedt B, et al. SIX1 protein expression selectively identifies blastemal elements in Wilms tumor. Pediatr Blood Cancer 2012;59:62–8. 163.

[70] Li Z, Tian T, Lv F, et al. Six1 promotes proliferation of pancreatic cancer cells via upregulation of cyclin D1 expression. PLoS One 2013;8: e59203.