CRITICAL REVIEWS IN ORAL BIOLOGY & MEDICINE

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J Dent Res 92(2):114-121, 2013

Biomarkers of Epithelial-Mesenchymal Transition in Squamous Cell Carcinoma

ABSTRACT

An understanding of the process by which tumor cells destroy the basement membrane of the surface epithelium, invade, and metastasize is essential to the development of novel treatment of head and neck squamous cell carcinoma (HNSCC). In recent years, there has been increased interest in the role of epithelial-mesenchymal transition (EMT) in invasion. EMT is a process that describes the development of motile, mesenchymal-like cells from non-motile parent epithelial cells. There are 3 known types of EMT that mediate development, wound healing, and carcinogenesis. This review summarizes studies of known EMT biomarkers in the context of HNSCC progression. The biomarkers discussed come from a wide range of proteins, including cell-surface proteins (E-cadherin, N-cadherin, and Integrins), cytoskeletal proteins (α-Smooth Muscle Actin, Vimentin, and β-catenin), extracellular matrix proteins (Collagens, Fibronectin, and Laminin), and transcription factors (SNAIL1, SNAIL2, TWIST, and LEF-1). Overall, the findings of these studies suggest that EMT mediates HNSCC progression. The mechanistic role of the EMT markers that have been associated with HNSCC should be more clearly defined if new anti-HNSCC therapies to block EMT progression are to be developed.

KEY WORDS: disease progression, extracellular matrix, oral pathology, neoplasm invasiveness, biological markers, head and neck neoplasms.

DOI: 10.1177/0022034512467352

Received August 7, 2012; Last revision October 16, 2012; Accepted October 17, 2012

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer in the world (Leemans *et al.*, 2011). In the United States, HNSCC accounts for more deaths each year than cervical cancer, melanoma, or Hodgkin's lymphoma and costs more than 2 billion dollars to treat (Lee *et al.*, 2004; Menzin *et al.*, 2007). Since patients with HNSCC often present with late-stage tumors, the five-year survival rate is only 50%, which is poorer than that for breast cancer or melanoma (Siegel *et al.*, 2011). Poor survival can be attributed to the high frequency of local recurrence, second primary tumors, and distant metastases. An understanding of the process by which tumor cells destroy the basement membrane, invade, and metastasize is essential for the advancement of HNSCC treatment strategies and improving survival.

Destruction of the basement membrane and invasion of genetically and phenotypically altered cells into the underlying stroma are required for progression of epithelial dysplasia (pre-cancer) to HNSCC (Fig. 1). The basement membrane is the first and most robust structural barrier to invasion (Rowe and Weiss, 2008). In normal and pre-cancerous mucosa, the basement membrane separates surface-stratified squamous epithelium from the underlying connective tissue. Epithelial dysplasia exhibits less organization than normal oral epithelium (pre-cancer), due to accumulation of genetically altered cells above the basement membrane. After destruction of the basement membrane, tumor cells invade locally or metastasize to distant sites. Tumor spread contributes to the lethality of the disease.

Epithelial-to-mesenchymal transition (EMT) facilitates invasion. EMT describes the development of motile cells from non-motile parent epithelial cells (Fig. 2). EMT, which occurs in embryonic development, wound healing, and cancer (Fig. 3), is classified into 3 subtypes (Zeisberg and Neilson, 2009). Type 1 occurs in gastrulation and in migration of neural crest cells; some of the migrated cells undergo mesenchymal-to-epithelial transition (MET) to become epithelial cells in organs produced by the mesoderm and endoderm. This embryological EMT occurs in the orofacial region during palatogenesis. Type 2 occurs in wound healing and can result in fibrosis when there is persistent inflammation. Cytokines generated by tissue injury induce the fibroblast phenotype from epithelial or endothelial cells. Type 3 occurs in subsets of invasive cancer cells by using some of the Type 2 EMT program for migration and aggregation of epithelial cells in wound healing. After invading, tumor cells can transition back to the epithelial morphology (MET) to proliferate and generate tumors at distant sites.

The purpose of this review is to present the growing evidence that EMT plays a significant role in the invasion and metastasis of HNSCC. Many protein types, including cell-surface proteins, cytoskeletal proteins, extracellular matrix (ECM) components, and transcription factors, contribute to EMT (Fig. 4, Table).

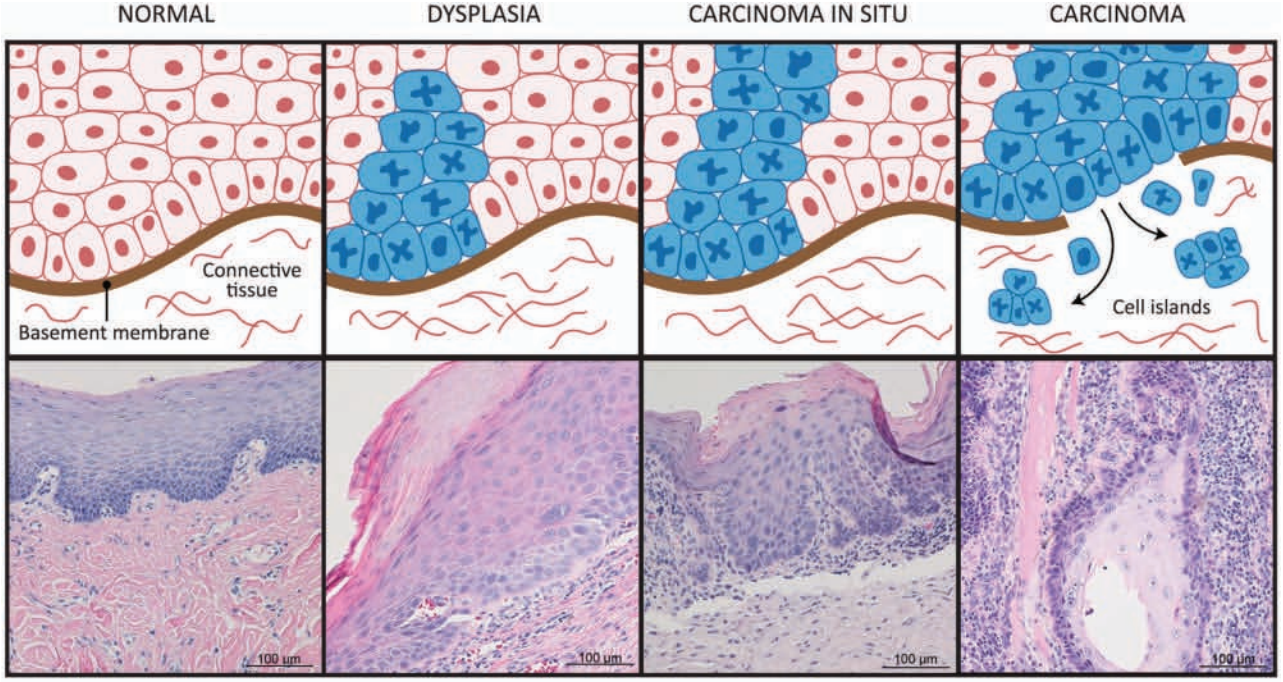

Figure 1. Head and neck squamous cell carcinoma (HNSCC) progression. Normal epithelium consists of cells with low mitotic activity and an intact basement membrane. In dysplasia, abnormal cells appear near the basement membrane, and in carcinoma *in situ* (full-thickness dysplasia), the abnormal cells are present throughout the full thickness of the epithelium. In carcinoma, the basement membrane is disrupted, and tumor cells invade the connective tissue.

CELL-SURFACE PROTEINS

Cell-surface proteins contributing to EMT in HNSCC include cadherins and integrins.

Cadherins

E-cadherin is the main protein of adherens junctions that anchor oral epithelial cells to each other. It is a calcium-dependent cell-surface protein that facilitates adhesion between and among epithelial cells. E-cadherin is characterized by long cytoplasmic and extracellular domains, which create homophilic interactions between adjacent cells to facilitate adhesion. The expression of E-cadherin is decreased during embryonic development, tumor fibrosis, and cancer progression (Zeisberg and Neilson, 2009). In oral epithelial cells, from which HNSCC develops, surface E-cadherin anchors cells to each other and links to the cytoskeleton *via* β-catenin. Loss or sequestration of E-cadherin in the nucleus impairs cell-cell adhesion and releases β-catenin, which translocates to the nucleus to induce transcription of EMT genes, such as *TWIST*.

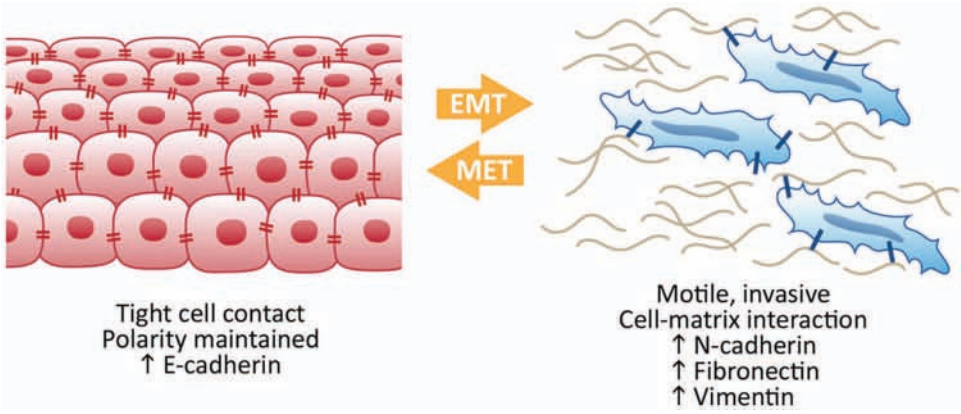

Figure 2. Epithelial-to-mesenchymal transition (EMT) to mesenchymal-to-epithelial transition (MET). Epithelial-like cells display tight cell-cell contacts and maintain polarity, whereas mesenchymal-like cells are more motile and display more contact with the extracellular matrix. Proteins associated with the epithelial-like or the mesenchymal-like states are referred to as biomarkers. As cells progress through EMT and MET, the levels of proteins associated with each state are altered, reflecting the phenotypic switch between the 2 states.

Several studies have shown reduced E-cadherin in HNSCC, with lowest E-cadherin levels in poorly differentiated tumors (Wu *et al.*, 2000). E-cadherin expression is similar between primary tumors and metastases, perhaps because of MET in metastatic tumors. The promoter region of the E-cadherin gene (*CDH1*) is hypermethylated in HNSCC, but hypermethylation is not correlated with advanced stage (Calmon *et al.*, 2007), mortality, or second primary tumor (Dikshit *et al.*, 2007). Meta-analysis of E-cadherin studies in HNSCC showed that abnormal E-cadherin expression is predictive of diminished disease-specific survival (Zhao *et al.*, 2012).

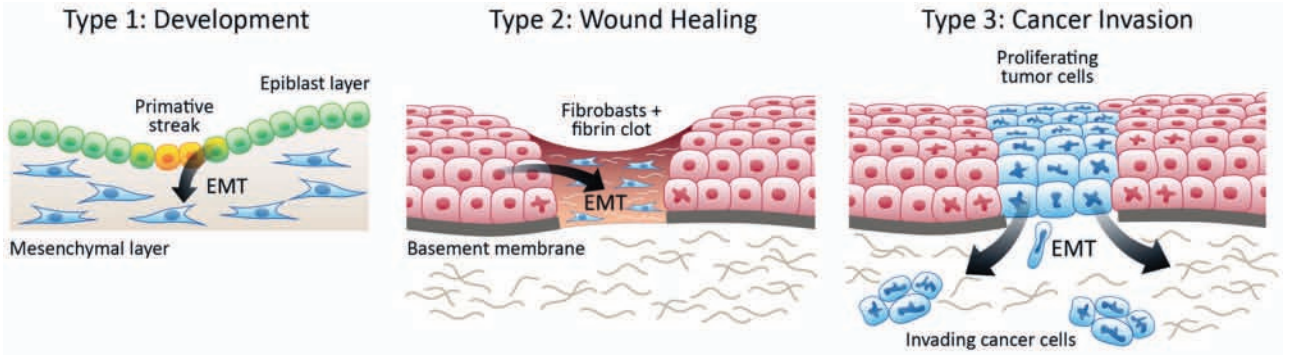

Figure 3. Three types of Epithelial-to-mesenchymal transition (EMT). Type 1 EMT occurs in development, for example, when gastrulation epithelial cells transition to motile mesenchymal cells. Type 2 EMT occurs when secondary epithelial or endothelial cells move to interstitial spaces in wound healing or chronic inflammation, resulting in fibrosis. Type 3 EMT occurs when epithelial tumor cells migrate beyond a primary tumor and metastasize.

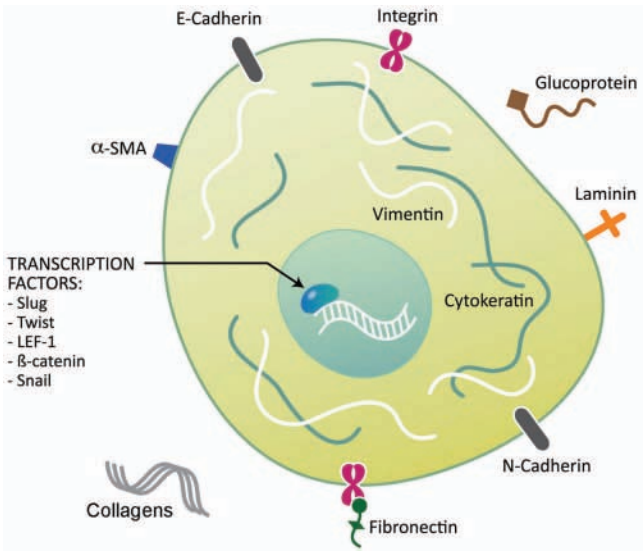

Figure 4. Proteins involved in Epithelial-to-mesenchymal transition (EMT). Several proteins have been identified as biomarkers of EMT. These proteins include cell-surface proteins, cytoskeletal proteins, extracellular matrix proteins, and transcription factors.

The use of E-cadherin expression to personalize anti-HNSCC therapy has been explored (Eriksen *et al.*, 2005; DH Huang *et al.*, 2009). Higher E-cadherin expression is correlated with better sensitivity toward the EGFR-tyrosine kinase inhibitors. The 'E-cadherin to N-cadherin' switch, which occurs during embryonic development and cancer progression, is used to monitor EMT. E-cadherin is expressed in epithelial cells, and N-cadherin is up-regulated by TWIST in type 3 EMT in gastric cancer (Z Yang *et al.*, 2007). In HNSCC, high expression of N-cadherin correlated with malignant behaviors such as a high-grade pattern of invasion and poorly differentiated cancer cells (Nguyen *et al.*, 2011). Cadherin switching (high expression of N-cadherin and low expression of E-cadherin) was observed in 30 of the 80 cases and correlated with invasion and lymph node metastasis, as well as EMT features. Thus, cadherin switching may be a critical event in the progression of HNSCC through EMT.

Integrins

EMT facilitates relocation of cells from above the basement membrane into the ECM, which involves a change in expression of integrins (Zeisberg and Neilson, 2009). Integrins are heterodimeric adhesion receptors composed of α and β subunits. There are 18 α and 8 β subunits that variously combine into 24 different integrins. Integrins bind to ligands, including collagens, laminins, and fibronectin in the ECM. Ligand-bound integrins induce several signaling cascades that control cell polarity, motility, survival, shape, proliferation, and differentiation.

Integrins mediate interactions between cells and the surrounding ECM by making transmembrane connections between the cytoskeleton and the ECM. The interaction between the α5 integrin and fibronectin is necessary for metastasis of a melanoma cell line (Qian *et al.*, 2005). Blocking the α5 subunit in HNSCC decreases adhesion to collagen IV and fibronectin (Dyce *et al.*, 2002). The α5β1 integrin has been associated with cisplatin resistance and enhanced adhesion to fibronectin, which is abolished when the integrin is blocked by a neutralizing antibody (Nakahara *et al.*, 2003). The β1 integrin increases invasion of HNSCC cells, which decreases significantly when the integrin is blocked (Koontongkaew *et al.*, 2011).

CYTOSKELETAL MARKERS

Cytoskeletal proteins that contribute to EMT in HNSCC include alpha-smooth-muscle actin (α-SMA), vimentin, and β-catenin.

α-SMA

Cells expressing α-SMA contribute to EMT in embryogenesis and wound healing in normal epithelial cells (Zeisberg and Neilson, 2009). In squamous cell carcinoma (SCC), the tumor tissue is surrounded by reactive stroma, made up mostly of cancer-associated fibroblasts (CAFs), also known as myofibroblasts because they acquire characteristics of muscle fibers, including expression of α-SMA. Expression of α-SMA is controlled by growth factors and specialized ECM proteins. α-SMA is incorporated into stress fibers of fibroblasts, thereby augmenting their contractile ability, which is critical to tissue remodeling. CAFs are known to potentiate the

Table. Known Biomarkers of EMT in HNSCC

Biomarker Category	Proteins	References
Cell-surface proteins	E-Cadherin	Biddle *et al.*, 2011
	N-Cadherin	Nguyen *et al.*, 2011
	Integrins	Dyce *et al.*, 2002
		Nakahara *et al.*, 2003
		Koontongkaew *et al.*, 2011
Cytoskeletal proteins	α-SMA	Lim *et al.*, 2011
	Vimentin	Yoon *et al.*, 2007
		Paccione *et al.*, 2008
		Chen *et al.*, 2011
	β-catenin	Chang *et al.*, 2008
		Tsai *et al.*, 2009
		Goto *et al.*, 2010
Extracellular Matrix proteins	Collagen (I)	Chen *et al.*, 2008
		Koontongkaew *et al.*, 2011
	Collagen (III)	Kauppila *et al.*, 1998
		Tapper *et al.*, 2001
	Collagen (IV)	Chen *et al.*, 2008
	Fibronectin	Dooley *et al.*, 2003
		Tijink *et al.*, 2006
		Warawdekar *et al.*, 2006
	Laminin 5	Marinkovich, 2007
		Chen *et al.*, 2008
		Mendez *et al.*, 2009
Transcription factors	SNAIL1	MH Yang *et al.*, 2007
		Lyons *et al.*, 2008
		Hayry *et al.*, 2010
		Hsu *et al.*, 2010
		Mendelsohn *et al.*, 2012
	SNAIL2	CH Huang *et al.*, 2009
	TWIST	Ou *et al.*, 2008
	LEF-1	Segrelles *et al.*, 2006

Abbreviations: EMT, epithelial-to-mesenchymal transition; HNSCC, head and neck squamous cell carcinoma.

development and progression of epithelial cancers. Fibroblasts can be distinguished based on the stage of tumor development by differences in α-SMA expression, which is expressed more highly in mature fibroblasts than newly transitioning cells. Fibroblasts from HNSCC tumors grow more slowly compared with normal fibroblasts from the oral cavity (Lim *et al.*, 2011).

HNSCC characterized by extensive genetic copy number alterations, loss of heterozygosity, and inactivation of p53 and p16INK4A had higher α-SMA expression, which correlated with poor prognosis in an independent dataset of HNSCC samples when compared with tumors with less genetic instability (Lim *et al.*, 2011). However, the mechanistic link between α-SMA expression and EMT and HNSCC is unknown.

Vimentin

Vimentin is an intermediate filament that is used as a marker of mesenchymal cells to distinguish them from epithelial cells (Zeisberg and Neilson, 2009). Vimentin is expressed at sites of cellular elongation and is associated with a migratory phenotype. Increased vimentin expression is frequently used as an EMT marker in cancer.

In HNSCC cell lines, Chen *et al*. isolated an ALDH1-rich subpopulation of cells and characterized their invasive potential and EMT phenotype (Chen *et al.*, 2011). Spheroid-derived cells had increased vimentin when compared with monolayer-derived cells from different cell lines. Furthermore, vimentin expression decreased when cells were grown as a monolayer. Vimentin expression is higher in nodal metastatic cells than in the primary HNSCC tumors, and is enhanced by epidermal growth factor and TGF-β (Paccione *et al.*, 2008). Reducing vimentin levels by RNA interference decreased the proliferation, migration, and invasion of metastatic cells compared with control cells (Paccione *et al.*, 2008). Yoon *et al*. developed an orthotopic model of HNSCC metastasis and selected HNSCC cells through 4 rounds of serial metastasis to obtain a highly metastatic subpopulation (Yoon *et al.*, 2007). The metastatic population acquired mesenchymal features, including increased vimentin and integrin β1, and reduced epithelial expression, including reduced E-cadherin and involucrin. In contrast, non-metastatic parental cells had low vimentin expression.

β-Catenin

The Wnt/β-catenin pathway has a critical role in invasion in HNSCC (Goto *et al.*, 2010). E-cadherin is anchored to the cytoskeleton *via* β-catenin, a cytoplasmic plaque protein (Wheelock and Johnson, 2003). In loss of cell adhesion, as occurs in invasion, E-cadherin is endocytosed and β-catenin is released. In normal and non-invasive cells, β-catenin is usually localized to cell membranes. In cells undergoing EMT, β-catenin is located in the cytoplasm (reflective of its dissociation from E-cadherin). This cytosolic (free) β-catenin translocates to the nucleus to promote transcription of genes that induce EMT. Nuclear β-catenin is a transcriptional co-activator with T-cell factor (TCF)/Lymphoid enhancer-binding factor (LEF), which controls transcription of SNAIL1 (Zeisberg and Neilson, 2009).

Nuclear β-catenin is correlated with a poor prognosis in patients with metastasic HNSCC (Tsai *et al.*, 2009). Rap1, a ras-like protein, stabilized β-catenin and increased its nuclear localization; more advanced N-stage lesions were associated with high free β-catenin and high active Rap1 (Goto *et al.*, 2010).

ECM PROTEINS

The ECM proteins that promote EMT in HNSCC include collagen, fibronectin, and laminin.

Collagens

While migration of normal cells is strictly controlled by limited proteolysis of the ECM, in cancer, proteolytic remodeling of the ECM facilitates invasion (Egeblad *et al.*, 2010). Collagens are the major structural components of the ECM. There are 28 types of collagen that have a triple-helical structure. Collagen I and II are fibrillary collagens, while collagen IV constitutes a sheet-like structure that is the major component of basement membranes. There is increased expression of collagen I (α1) and collagen III (α1) in type 1 and 3 EMT, while collagen IV (α1) is down-regulated in all 3 types of EMT (Zeisberg and Neilson, 2009).

Collagen type I is the most prevalent form in the interstitial matrix. In HNSCC, collagen type I RNA transcripts are more widely expressed in HNSCC than in pre-cancerous or normal tissue. Collagen I stimulated cytokine secretion in genetically matched primary and metastatic HNSCC cell lines, but cytokine secretion was significantly up-regulated in metastatic cells when seated on collagen I (α1) gel (Koontongkaew *et al.*, 2011). The cytokines released (IL-1α, IL-1β, IL-6, TNF-α, and TNF-β) stimulated MMP activity and invasion of the HNSCC cells. IL-6 is overexpressed in HNSCC and is a biomarker of poor disease-specific survival (Van Tubergen *et al.*, 2011). Moreover, collagen I enhanced MMP-2 and MMP-9 secretion in both primary and metastatic cell lines (Koontongkaew *et al.*, 2011). MMP-9 is a biomarker that contributes to invasion of HNSCC and is correlated with poor disease-specific survival (Mitra *et al.*, 2008).

Collagen III (α1) is an ECM component that promotes cancer progression in ovarian (Tapper *et al.*, 2001) and breast cancers (Kauppila *et al.*, 1998). In HNSCC, collagen III (α1) cDNA was found to be highly expressed in a Paclitaxel-resistant cell line (Schmidt *et al.*, 2006). Altered collagen IV (α1) was linked to invasion and motility of cancer cells. Laminin in tumor cells binds collagen IV (α1) and then secretes gelatinases that break down collagen IV (α1) to facilitate migration of tumor cells (Zeisberg and Neilson, 2009). Surprisingly, Chen *et al.* found that collagen IV RNA is increased in HNSCC surgical specimens compared with dysplastic and normal tissues (Chen *et al.*, 2008). Further investigation is necessary to determine the role of collagen IV in invasion of HNSCC.

Fibronectin

Fibronectin is a glycoprotein scaffold for fibrillar ECM (Zeisberg and Neilson, 2009). It is composed of a dimer of similar subunits of repetitive sequences covalently linked by 2 disulfide bonds at their C-termini. In normal cells, fibronectin mediates cellular interactions with the ECM and is important in migration, differentiation, growth, and adhesion of cells. Although fibronectin is up-regulated in all 3 types of EMT, its use as an EMT biomarker is limited because it is produced by many cell types, such as epithelial cells, fibroblasts, and mononuclear cells (Z Yang *et al.*, 2007). Fibronectin can be up-regulated by SNAIL and TWIST in type 3 EMT.

In HNSCC, there are contradictory reports with respect to expression of fibronectin. Dooley *et al.* showed that fibronectin and its receptor are strongly up-regulated with α5β6 integrin in SCC cell lines and tissues (Dooley *et al.*, 2003). Three fibronectin isoforms (extra domain A, extra domain B, and IIICS) are generated *via* alternative splicing, depending on cytokine and pH conditions. Fibronectin fragment ED-B is not expressed in normal tissues (except those undergoing wound healing), but expression is correlated with HNSCC and tumor cell aggressiveness (Tijink *et al.*, 2006). The ED-A and IIICS isoforms are expressed in blood plasma of HNSCC patients, suggesting that hydrolytic enzyme-aided invasion leads to degradation of EMC components (Warawdekar *et al.*, 2006).

Laminin

Similar to collagen, laminin is a major component of the basement membrane. Laminins are glycoproteins made up of one α chain, one β chain, and one γ chain. There are 15 known heterotrimers of laminin. Laminin 1 (α1β1γ1) is the laminin of greatest interest in EMT types I and II, where it is down-regulated or disrupted. Laminin 5 (α3β3γ2) has been linked to EMT in metaplastic carcinoma of the breast and hepatocarcinoma (Zeisberg and Neilson, 2009).

The role of laminin 5 in HNSCC is clearly significant. Laminin 5 has been shown to be a major component of the ECM, and laminin 5 expression has been correlated with invasion and patient prognosis. Interestingly, the laminin 332 G4 domain, a proteolytic product of laminin 5, promotes laminin 5 deposition, and may have a role in wound healing and SCC formation (Marinkovich, 2007). In a study comparing HNSCC, pre-cancer, and normal tissues, overexpression of transcripts of LAMC2 encoding laminin-γ2 chain and COL4A1 collagen type IV α1 distinguished HNSCC from pre-cancerous and normal tissues that had lower LAMC2 (Chen *et al.*, 2008). In another study, high LAMC2 predicted poor HNSCC-specific survival (Mendez *et al.*, 2009).

TRANSCRIPTION FACTORS

The transcription factors that promote EMT in HNSCC include SNAIL, TWIST, and LEF-1.

SNAIL Family

SNAIL proteins regulate various aspects of the EMT phenotype, including overexpression of mesenchymal markers fibronectin and vitronectin, and suppression of epithelial markers, including E-cadherin (Zeisberg and Neilson, 2009). In addition, SNAIL blocks cell-cycle progression and contributes to cell movement and survival. Other targets of SNAIL proteins include genes regulating cell polarity and apoptosis. SNAIL family proteins are evolutionarily conserved in vertebrates, where they have a conserved role in embryonic mesoderm formation.

There are 3 transcription factors in the SNAIL family, of which SNAIL1 and SNAIL2 are functionally equivalent (Zeisberg and Neilson, 2009). SNAIL1 is important in mediating invasion and inflammation through transcriptional regulation of cytokines, and also in preventing terminal differentiation of HNSCC cells (Lyons *et al.*, 2008). EMT phenotypes and invasion in HNSCC are reduced with siRNA-mediated knockdown of SNAIL1 (MH Yang *et al.*, 2007). SNAIL1 expression in primary tumors of HNSCC patients is correlated with metastasis and poor prognosis. SNAIL1 expression correlates with histopathologic grade and depth of invasion in HNSCC (Hayry *et al.*, 2010) and is correlated with poor differentiation of the tumors, lymphovascular invasion, and regional metastasis (Mendelsohn *et al.*, 2012). Knockdown of SNAIL1 in HNSCC cell lines attenuated cisplatin resistance by facilitating DNA excision repair by stabilizing ERCC1, which is necessary for

nucleotide excision repair (Hsu *et al.*, 2010). Lysyl Oxidase–Like 2, a marker of poor prognosis in SCC, regulates EMT in part by stabilizing SNAIL1 to facilitate tumor progression (Peinado *et al.*, 2008).

Hypoxia contributes to tumor metastasis by initiating EMT through activation of SNAIL2 in HNSCC. SNAIL2 is critical for the induction of MT4-MMP in a hypoxic environment (CH Huang *et al.*, 2009). Overall, there is strong evidence to suggest that SNAIL proteins play a role in EMT in HNSCC.

TWIST

TWIST is a basic helix-loop-helix protein that modulates many target genes through E-box-responsive elements. There are 2 TWIST genes (*TWIST1* and *TWIST 2*) which are well-conserved in vertebrates. TWIST is activated in all 3 types of EMT and is up-regulated in cancer metastases (Zeisberg and Neilson, 2009). While mesoderm formation is controlled by SNAIL family proteins, TWIST1 is important in mesoderm differentiation. TWIST proteins can act as either transcriptional repressors (*e.g.*, E-cadherin) or activators (*e.g.*, N-cadherin and Fibronectin).

In HNSCC, TWIST expression is positively correlated with lymph node metastasis and clinical stage (Ou *et al.*, 2008). Hypoxia-inducible factor-1α (HIF-1α) promotes EMT and metastatic phenotypes in HNSCC *via* up-regulation of TWIST expression. Repression of TWIST reverses the EMT and metastatic phenotypes. BMI1, a polycomb-group protein frequently overexpressed in cancers, is regulated by TWIST in HNSCC (Yang *et al.*, 2010). BMI1 and TWIST expression lead to down-regulation of E-cadherin, which is associated with poor prognosis (Yang *et al.*, 2010). In a study of 109 HNSCC patients, negative SNAIL1 and TWIST immunostaining was significantly correlated with improved five-year disease-specific survival (Jouppila-Matto *et al.*, 2011).

LEF-1

LEF-1, a co-transcriptional activator with TCF, mediates WNT signaling. Through this regulation, LEF-1 plays a role in deciding the fate of cells in normal embryonic development (Zeisberg and Neilson, 2009). In EMT, the β-catenin/LEF-1 complex is localized to the nucleus, where it controls SNAIL gene expression, along with other markers associated with EMT. LEF-1 functions with β-catenin to promote cell survival and proliferation during mammary gland development and in breast cancer (Hatsell *et al.*, 2003). LEF-1 and β-catenin are up-regulated and translocate to the nucleus in Akt-transformed keratinocytes (Segrelles *et al.*, 2006).

FUTURE DIRECTIONS

In addition to proteins that have a role in HNSCC progression or a correlation with HNSCC, there are several potential biomarkers that should be explored in HNSCC. These proteins have been shown to have a role in EMT in other cancers, but to our knowledge have not been investigated in HNSCC.

Forkhead box protein c2 (FOXC2) is a transcription factor that is expressed in type I EMT and is important in angiogenesis, musculogenesis, and the development of the heart, kidney, and urinary tract. FOXC2 is expressed in ductal breast cancers and metastatic breast cancer (Zeisberg and Neilson, 2009). Overexpression of EMT transcription factors such as SNAIL and TWIST increase FOXC2 expression. Furthermore, the overexpression of FOXC2 can induce EMT, which suggests that FOXC2 may play a role in type 3 EMT.

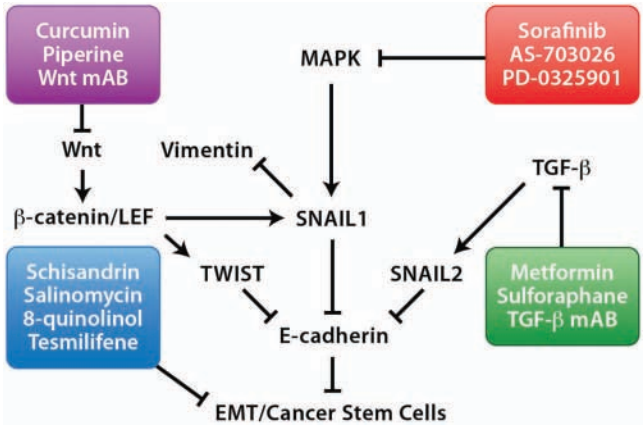

Figure 5. Targeted therapies against epithelial-to-mesenchymal transition (EMT) pathways. EMT progression involves many signaling pathways that may be targeted in the clinical setting, which include monoclonal antibodies (mAB) and small molecule inhibitors (boxed).

Expression of zona-occludens 1 (ZO-1) occurs in all 3 types of EMT (Zeisberg and Neilson, 2009). ZO-1 is a tight junction protein that is usually located at cell-cell adhesion membrane complexes in normal epithelial cells. During EMT, ZO-1 relocates from the adhesion membrane complexes to the cytoplasm and then to the nucleus, depending on the degree of differentiation and migration of the cell (Polette *et al.*, 2007). ZO-1 is involved in the EMT process in colorectal and bile duct cancers, but has not yet been linked to EMT in HNSCC (Hirakawa *et al.*, 2009; Nemeth *et al.*, 2009).

Zinc finger E-box binding homeobox 1 (ZEB1) is an E-cadherin transcriptional repressor that is down-regulated by miR-200 microRNAs in cancer cells that display an EMT phenotype (Zeisberg and Neilson, 2009). ZEB1 has been investigated in prostate cancer, non-small-cell lung carcinoma, and invasive ductal breast cancer, but has not been investigated in HNSCC (Drake *et al.*, 2010; Gemmill *et al.*, 2011; Montserrat *et al.*, 2011).

Osteoblast cadherin (OB-cadherin) is a definitive marker for activated fibroblasts (Zeisberg and Neilson, 2009). While in cancer and embryonic development, an 'E-cadherin to N-cadherin' switch is used to monitor EMT progression, an 'E-cadherin to OB-cadherin' switch may indicate EMT progression in type II EMT specifically. In cancer, OB-cadherin has an association with prostate and breast cancer, where it is hypothesized to be involved in metastasis; its expression in HNSCC is unknown (Farina *et al.*, 2009).

Cancer stem cells (CSC) are a small population of tumor cells that can both initiate a tumor and repopulate a tumor following treatment, contributing to treatment resistance. In HNSCC, a subpopulation of CD44+ cancer stem cells displays a phenotypic switch to become either proliferative or migratory (Biddle *et al.*, 2011). The migratory population, designated

$CD44^{high}ESA^{low}$, displays reduced E-cadherin and increased Vimentin, TWIST, SNAIL1, and SNAIL2, features of EMT cells. Given the striking similarities between EMT and this subpopulation of CSCs, it is likely that future research will elucidate the role of EMT in maintaining the CSC population.

EMT progression involves many signaling pathways that may be targeted in the clinical setting (Foroni *et al.*, 2012; Prud'homme, 2012). Fig. 5 summarizes some of the pathways and anti-EMT therapies. The mechanistic role of the EMT markers associated with HNSCC should be clearly defined in the development of new anti-HNSCC therapies to block HNSCC progression. An understanding of the role of EMT in HNSCC will allow clinicians to personalize treatment for patients with particularly aggressive tumors and improve treatment outcomes.

ACKNOWLEDGMENTS

The authors thank Chris Jung and Kenneth Rieger for their artistic assistance with illustrations. This work was supported by National Institute of Dental and Craniofacial Research grants DE018512, DE019513, and DE017977 (NJD), F30 DEO21293 (CSS), and F32 DE213052 (EVT). The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

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