

Signal Transduction Pathways Involved in Epithelial-Mesenchymal Transition in Oral Cancer Compared with Other Cancers

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Key Words

Epithelial-mesenchymal transition · Oral cancer · Signaling pathways

Abstract

Epithelial-mesenchymal transition (EMT) is a central mechanism governing destined cell movement in embryonic development. Emerging evidence reveals that EMT characterizing the progression of many carcinomas is linked to the acquisition of an invasive and metastatic phenotype. While it is established that EMT is controlled by well-conserved mechanisms, additional research is required for various tissue- or tumor-specific transitions. We review the literature related to the major components of EMT including adhesion molecules, cytoskeleton reorganization and signaling pathways in oral cancer.

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Introduction

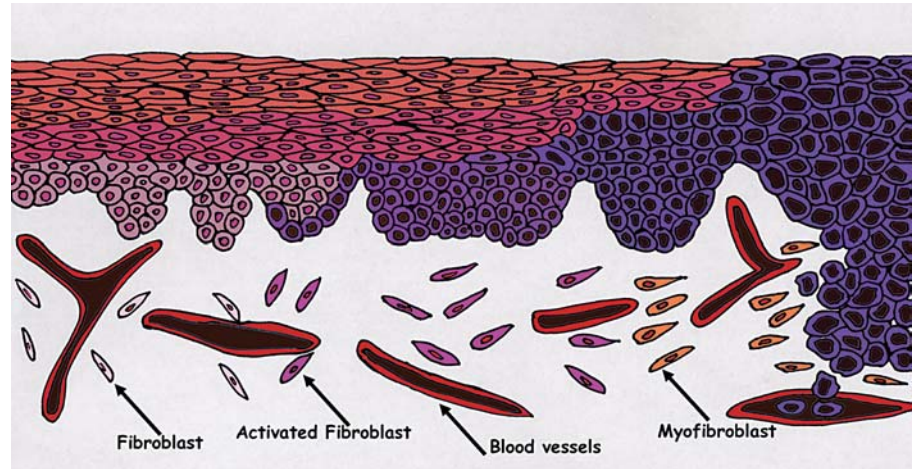
Over 90% of head and neck cancers are squamous cell carcinomas (SCC) which are the most common malignant neoplasm of the oral cavity. Oral cancer consistently ranks as one of the top ten cancers worldwide, with broad differences in geographic distribution. Worldwide, the annual incidence of new cases exceeds 300,000. In

spite of improved therapeutic procedures, SCCs generally exhibit a poor prognosis. In addition, treatment results in significant functional and cosmetic defects. Several factors contribute to a poor prognosis for upper aerodigestive tract SCC patients including the delayed detection of cancerous lesions and the tendency to de-

Abbreviations used in this paper

| | |
|--------|--|
| EGF | epidermal growth factor |
| EGFR | epidermal growth factor receptor |
| EMT | epithelial-mesenchymal transition |
| E-pal | a palindromic sequence between -75 and -86 that potentiates the activity of the proximal E-cadherin promoter |
| HGF | hepatocyte growth factor |
| HMGA | high mobility group A |
| JNK | c-Jun NH2-terminal kinase |
| LMO | LIM-only protein |
| MAPK | mitogen-activated protein kinase |
| MMP | matrix metalloproteinase |
| MT-MMP | membrane-type matrix metalloproteinase |
| NF-κB | nuclear factor κB |
| RTK | receptor tyrosine kinase |
| SCC | squamous cell carcinoma |
| TGF-β | transforming growth factor-β |
| TIMP | tissue inhibitor of metalloproteinase |
| TNF-α | tumor necrosis factor-α |
| Wnt | wingless type |

Fig. 1. Oral carcinogenesis from normal epithelium to invasive carcinoma: (1) normal epithelium with inactive, silent fibroblasts; (2) somatic mutation has occurred in the basal cells (purple) and the epithelium becomes a precursor lesion; (3) more genetic alterations are accumulated through time and the epithelium becomes dysplastic and fibroblasts become active (pink); (4) the epithelium has the following characteristics: dissociation of adhesion molecules, basement membrane breakdown, acquisition of motile features, and cancer cells invade and metastasize. Active fibroblasts can transform to myofibroblasts (orange).



velop multifocal malignancy and/or premalignant lesions, as a consequence of 'field cancerization'. It has been generally accepted that prompt detection of early oral cancer and/or epithelial dysplasia is required to improve prognosis. Therefore, it is important to identify markers for carcinoma progression (fig. 1).

As with other cancers, most of the oral cancer deaths result from local invasion and distant metastasis. The landmark of carcinoma progression during the invasive and metastatic phases is epithelial cell plasticity and dedifferentiation, which is similar to epithelial-mesenchymal transition (EMT) that occurs during embryonic development. EMT is the process that cells undergo to switch from a polarized, epithelial phenotype to a motile mesenchymal phenotype (fig. 2). This process can occur during embryonic development, wound healing, fibrosis, and cancer progression and has been extensively reviewed over the last 10 years [Hay, 1995; Kalluri and Neilson, 2003; Thiery, 2003; Schiller et al., 2004; Thiery and Sleeman, 2006]. Loss of epithelial cell polarity and acquisition of motility results from the disappearance of cell junction adherence molecules, reorganization of cytoskeleton and redistribution of organelles [Thiery, 2002; Thiery and Sleeman, 2006] (fig. 2). Uncovering the mechanism for EMTs would be one strategy to predict tumor progression and possibly develop therapeutic intervention. However, this is complicated by the diversity of molecular mechanisms contributing to the plasticity of epithelial cells in different tissues [Gotzmann et al., 2004]. In this review, we will focus on the literature related to the major components of EMT including adhesion molecules, cytoskeleton reorganization and known signaling pathways in oral cancer.

Cell Adhesion Molecules and Oral Cancer

Many characteristics of tumor cells can be attributed to the aberrant expression or function of cell adhesion molecules [Thomas and Speight, 2001; Peinado et al., 2004]. In addition, loss of cell-cell adhesion is one of the most important hallmarks of EMT [Gotzmann et al., 2004]. EMT is associated with the functional loss of E-cadherin, which plays a major role in the establishment and maintenance of intercellular adhesion, cell polarity, and tissue architecture [Takeichi, 1993; Gumbiner, 1996]. In cancer, downregulation of E-cadherin is a key step towards an invasive phenotype of carcinoma. Reduced expression of E-cadherin is frequently associated with dedifferentiation, invasion, and lymph nodes or distant metastasis, and ultimately poor prognosis in various human malignancies [Jiang, 1996; Jiao et al., 2001; Mukai et al., 2001; Tanaka et al., 2002]. Several reports have demonstrated that in cases of head and neck SCC, downregulation of E-cadherin expression is significantly correlated with histological grade, invasiveness and lymph node metastasis [Mattijssen et al., 1993; Bagutti et al., 1998; Shinohara et al., 1998; Thomas and Speight, 2001; Kudo et al., 2004]. Our preliminary data showed aberrant E-cadherin in the invasive front but not the differentiated tumor (fig. 3). E-cadherin mRNA levels may not change, so further studies which focus on the invasive front using laser capture microdissection are needed to clarify the mRNA status.

During tumor progression, E-cadherin can be functionally inactivated by different mechanisms, including somatic mutation and downregulation of gene expression through promoter methylation and/or transcriptional re-

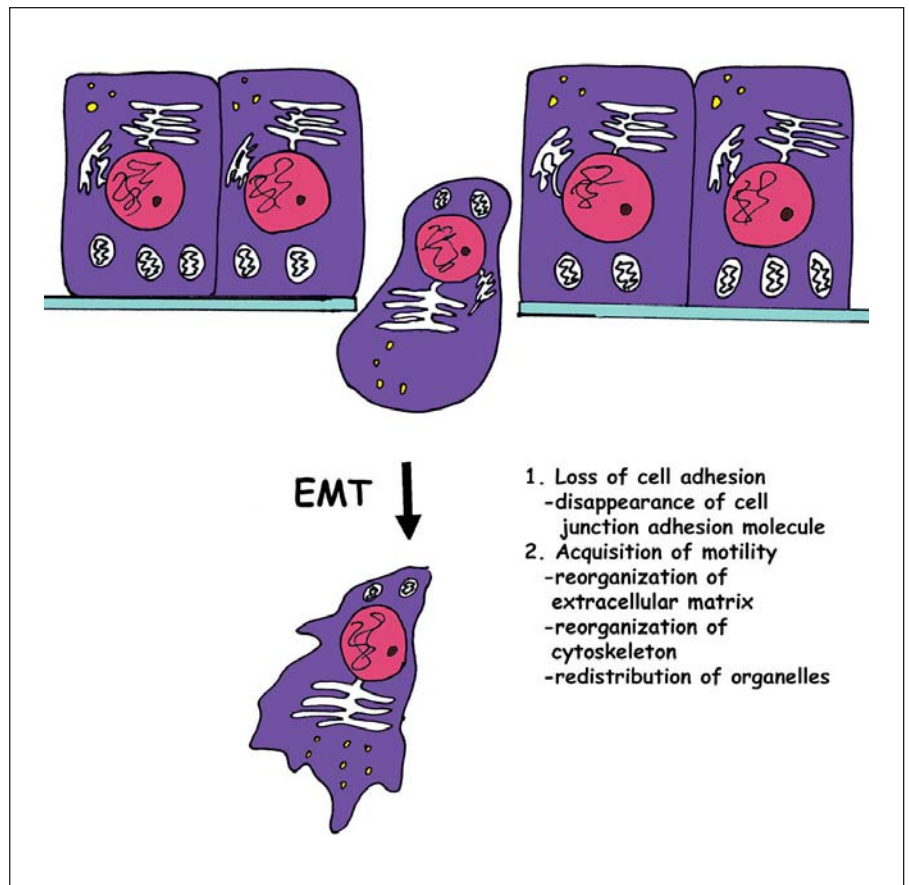
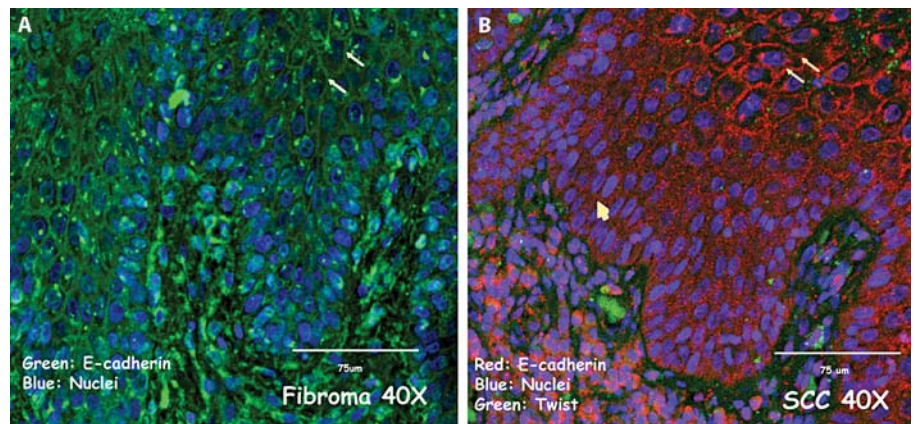


Fig. 2. EMT occurs during invasion and metastasis: the loss of cell-cell adhesion and acquisition of mobile characteristics require changes in gene expression and cell-matrix interactions.

Fig. 3. Projected confocal images of E-cadherin distribution in normal epithelial cells (**A**: green) and SCC (**B**: red). **A** E-cadherin (green) and nuclei (blue) in normal epithelial cells from a fibroma had a membranous staining pattern (arrows). **B** E-cadherin (red), Twist (green) and nuclei (blue) from an SCC specimen. Apical carcinoma cells had membranous E-cadherin staining (arrows), but the invasive front (basal cells, arrowhead) had a cytoplasmic distribution. Twist was expressed primarily in the stromal cells, but occasional epithelial cells were positive. Scale bar = 75 μ m.



pression [Peinado et al., 2004]. Downregulation of E-cadherin expression is observed in many carcinomas, while inactivating mutations, frequent in diffuse gastric carcinomas and in lobular breast carcinomas, are rarely observed in other types of tumors [Berx et al., 1998a, b; Peinado et al., 2004]. Besides regulation of E-cadherin by

promoter hypermethylation and/or genetic alterations, direct transcriptional control of E-cadherin has emerged in the last years as an important regulatory mechanism [Peinado et al., 2004]. Four transcriptional repressors have been identified: Snail, Slug, SIP1 and Twist (fig. 4). It remains unresolved if cooperation between repressors

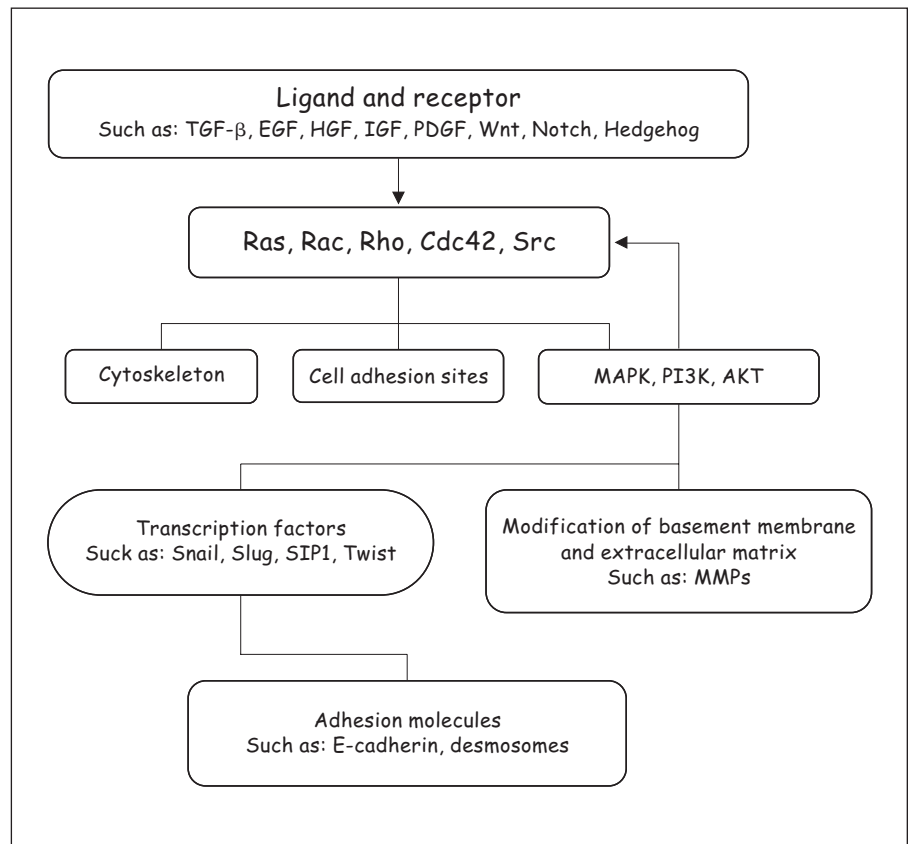


Fig. 4. Pathways and factors involved in EMT. See text for abbreviations.

occurs or if there is a functional hierarchy in E-cadherin downregulation during tumor progression. Analyzing the expression of different repressors in several carcinoma cell lines has rendered some apparently contradictory results. Expression of different repressors has been found in the tumors arising from different organs or even in the same tissue. A comparative analysis of the binding affinities for the E-pal element of three E-cadherin repressors, Snail, Slug and E47, revealed that Snail binds with a higher affinity than the other two repressors. From this study, a hierarchy might exist in terms of the participation of the repressors when present in the same biological context [Peinado et al., 2004]. A recent study in gastric carcinomas has shown an association between E-cadherin repression and Snail expression in diffuse gastric carcinomas while SIP1 expression was linked to E-cadherin repression in intestinal-type gastric carcinomas [Rosivatz et al., 2002]. These studies indicate that different repressors may participate in silencing E-cadherin in different types of tumors or, perhaps, at defined stages of tumor progression [Peinado et al., 2004]. The distinct expression patterns observed between different E-cadherin

repressors during development suggest a hierarchy. Snail plays a prominent role in inducing EMT, SIP1 displays a more restricted role in migration, and Slug has a maintenance role. Since regulation of E-cadherin expression is similar during normal development and tumor progression [Cano et al., 2000], these developmental analyses strongly support the idea that specific and hierarchical participation of different transcription factors may exist in the repression of E-cadherin during invasion. In oral cancer cell lines or SCC patients, transcriptional repressors, Snail and SIP1, and promoter hypermethylation have been implicated in E-cadherin downregulation [Yokoyama et al., 2001; Kudo et al., 2004; Maeda et al., 2005]. However, our preliminary data showed Snail and Slug were related to slightly increased or unchanged E-cadherin mRNA levels; instead, Twist and SIP1 were related to decreased E-cadherin mRNA levels. However, there were limited case numbers, so further investigation is needed to confirm the results.

Aberrant expression or function of other cell adhesion molecules in oral cancer progression, such as integrin and CD44, has also been reported [Thomas and Speight,

2001]. In Bates' colon carcinoma model of EMT, it was discovered that expression of the integrin $\alpha\beta6$ was increased after EMT. This integrin enhances the tumorigenic properties of colon carcinoma, including activation of autocrine transforming growth factor- β (TGF- β) and migration on interstitial fibronectin [Bates, 2005; Bates et al., 2005; Bates and Mercurio, 2005]. Loss of E-cadherin function was a primal event for EMT. Although $\alpha\beta6$ integrin expression was not a primal event in EMT, it enabled invasive cells to interact with interstitial matrices and to sustain activation of TGF- β . $\alpha\beta6$ expression in tumors was prognostic for tumors that would progress more rapidly to terminal disease [Bates and Mercurio, 2005].

Interestingly, $\alpha\beta6$ is not expressed constitutively in healthy oral epithelia, but is upregulated during tissue remodeling, including wound healing and carcinogenesis [Thomas et al., 2006]. $\alpha\beta6$ is upregulated in transformed lesions, particularly at the invasive front, which is consistent with the proposal that $\alpha\beta6$ promotes invasion of oral carcinoma [Thomas et al., 2006]. $\alpha\beta6$ promotes adhesion and migration on several different extracellular matrix ligands, promotes increased matrix metalloproteinase (MMP) secretion, activates TGF- $\beta1$ and TGF- $\beta3$ and promotes the survival of oral SCC cells [Thomas et al., 2006]. A high level of $\alpha\beta6$ expression was described most consistently in oral SCC, and often its expression was concentrated at the invading edge of tumor cells [Thomas et al., 2006]. $\alpha\beta6$ was also found in some leukoplakia specimens where expression correlated with progression to malignant disease [Hamidi et al., 2000]. It has been suggested that $\alpha\beta6$ expression may be useful in predicting malignant transformation and may play an active role in this process [Thomas et al., 2006]. However, expression of $\alpha\beta6$ per se was insufficient to drive malignant progression since epithelium in lichen planus also showed increased $\alpha\beta6$ expression [Hamidi et al., 2000]. Ramos et al. [2002] demonstrated that $\alpha\beta6$ was also expressed in dysplastic epithelium prior to invasive change and concluded that transgression of the basement membrane required additional molecular changes. The mechanism for the increased invasion was, in part, through $\alpha\beta6$ -dependent upregulation of the type IV collagenase MMP-9 [Thomas et al., 2006].

Another cell surface molecule, CD44, has also been identified in cancer metastasis. But a consistent pattern of CD44v expression has not been demonstrated in head and neck SCC [Thomas and Speight, 2001].

Cytoskeletal Rearrangement and Oral Cancer

Cell migration is a critical step in tumor invasion and metastasis. Reorganization of the actin cytoskeleton is the primary mechanism of cell motility and is essential for most types of cell migration. The Rho GTPases, including Rac, Cdc42, and Rho, have been implicated in the establishment of cell-cell contacts and of cell-matrix interactions crucial to attaining a fully polarized epithelial state, and they are known for their regulation of the actin cytoskeleton and transcriptional activation [Schmitz et al., 2000; Yamazaki et al., 2005]. Under aberrant conditions, however, they have been implicated in motility, invasion, and some aspects of metastasis. Rho GTPases are activated by different classes of transmembrane receptors and they transmit signals to their effector proteins (fig. 4). These downstream targets include not only adaptor proteins and kinases that affect the actin cytoskeleton, but also transcription factors leading to expression of genes necessary for the drastic morphological changes that accompany these processes [Schmitz et al., 2000]. The study of Yang et al. [2003] showed activation of Rac and Rho GTPases is related to the 'fibroblastoid' oral cancer cells in vitro. In another study, increased Rac-1 immunoreactivity was reported in oral SCC compared with noncancerous matched tissue [Liu et al., 2004]. In addition, Rho regulated the hepatocyte growth factor (HGF)/scatter factor-stimulated cell motility of human oral SCC cells in vitro [Kitajo et al., 2003]. Therefore these studies demonstrated that Rho and Rac were related to the morphologic changes and increase of motility in oral cancer cases. Cdc42 mRNA expression has not been reported in oral cancer. However, the study of Baba et al. [2000] showed inostamycin reduced epidermal growth factor (EGF)-induced in vitro invasion of a tongue carcinoma cell line and inhibited EGF-induced formation of actin polymerization and filopodia through suppressing the level of EGF-induced cdc42 expression using Western blot analysis.

Other Signaling Pathways

The synergism between activation of Ras and TGF- β signaling plays a pivotal role in inducing EMT of various epithelial types [Janda et al., 2002; Gotzmann et al., 2004; Huber et al., 2005; Thiery and Sleeman, 2006]. Effectors upstream or downstream of Ras could substitute for activated receptor tyrosine kinases (RTKs) or Ras in working with TGF- β signaling to cause EMT. Several addi-

tional signal transduction pathways emerged as important for EMT, such as autocrine factors: EGF, HGF, insulin-like growth factor, platelet-derived growth factor, Wnt signaling, Notch signaling, and Hedgehog signaling (fig. 4) [Kang and Svoboda, 2005; Spears and Svoboda, 2005]. These pathways are also controlled by crosstalk between each other and with RTK/Ras and TGF- β /bone morphogenic protein signaling. EMT requires continuous TGF- β receptor and oncogenic Ras signaling and is stabilized by autocrine TGF- β production. In contrast, fibroblast growth factor, HGF/scatter factor, or TGF- β alone induce scattering, a spindle-like cell phenotype fully reversible after factor withdrawal, which does not involve sustained marker changes [Janda et al., 2002]. Similar signaling pathways have been reported related to morphologic changes in head and neck squamous carcinoma cells. For example, EGFR inhibition reversed the fibroblastic phenotype to the epithelial phenotype [Lorch et al., 2004], laminin-5 inhibition led to increased motility and morphologic change [Yuen et al., 2005], and HGF/c-Met overexpression disrupted E-cadherin junctions, showing phenotype modulation and enhanced invasion and metastasis [Murai et al., 2004].

Invasion requires the proteolytic digestion of basement membranes [Yu and Stamenkovic, 1999, 2000; Morris-Wiman et al., 2000; Brown et al., 2002; Duong and Erickson, 2004]. MMPs or membrane assembly inhibitors initiate the process by dismantling the local basement membrane. It appears unlikely that a single specific MMP has a leading role in creating a malignant phenotype since MMP expression varies between different types of tumors and between tumors of the same type. In addition to the role of modulating epithelial cell plasticity, TGF- β also participates in the regulation of extracellular matrix degradation by modulating the production of tissue inhibitor of metalloproteinase-2 (TIMP-2), metalloproteinases 2 and 13 (MMP-2, and MMP-13) [Blavier et al., 2001].

In oral cancer, gelatinases (MMP-2, MMP-9), stromelysins (MMP-3, MMP-10, MMP-11), collagenases (MMP-1, MMP-13) and membrane type 1 matrix metalloproteinase (MT1-MMP) were expressed either in tumor cells or stromal cells [Johansson et al., 1997]. Johansson et al. [1997] showed MMP-13 was expressed by tumor cells at the invading front and in a subset of SCCs. MMP-13 mRNA was also expressed by stromal fibroblasts. MMP-13 mRNA levels in SCC cells were enhanced by TGF- β , tumor necrosis factor- α (TNF- α) and keratinocyte growth factor. In the study of Ikebe et al. [2004], MMP-9 was secreted by tumor cells, and was correlated with the number of cocultured fibroblasts. The cocultured fibroblasts en-

hanced the induction of the active MMP-9, cell motility and activation of NF- κ B in tumor cells by presence of TNF- α . This interaction was not specific to the peritumor fibroblasts, because a murine stromal cell line and noninflammatory human gingival fibroblasts also had the same effect on tumor cells. The molecule signaling from fibroblasts to tumor cells remains to be identified. Coculture of tumor epithelial cells with fibroblasts or treatment of fibroblasts with conditioned media from tumor cells can activate the MMP expression at transcriptional level. In the study of Westermarck et al. [2000], fibroblasts treated with conditioned media from tumor cells activated fibroblast MMP-1 expression. The induction of MMP-1 expression correlated with activation of JNK and p38 MAPK. In an older review it was noted that all tumors that expressed TIMP-1 mRNA had well-differentiated invasive cancer cell clusters that had a basement membrane [Thomas et al., 1999]. TIMP-1 mRNAs were also located in endothelial cells near tumor islands and low expression in stromal cells was observed. TIMP-2 mRNA was expressed in the majority of tumors with expression predominantly limited to endothelial cells and occasional stromal cells near tumor islands [Thomas et al., 1999]. De Vicente et al. [2005] showed TIMP-1 protein by immunohistochemistry was found in 66% of oral SCC cases. TIMP-1 was expressed in tumor tissue, and 19% of the samples were also positive in surrounding stroma. TIMP-2 was detected in 56% of oral SCC cases and most were also in tumor tissue, only 13% were detected in stroma. Both TIMP-1 and TIMP-2 correlated with cyclin D1 and p53 expression patterns, but only TIMP-2 was correlated to poor survival and local recurrence.

Another signaling pathway that has been identified in EMT is the Wnt signaling pathway. Wnt signaling initiates proliferation, dedifferentiation, and EMT in various types of carcinoma cells [Eger et al., 2000; Lo Muzio, 2001; Kim et al., 2002; Taki et al., 2003], including oral carcinoma cells [Uraguchi et al., 2004]. In the study of Uraguchi et al. [2004], it was demonstrated that Wnt-expressing carcinoma cells exhibited increased β -catenin levels in the cytoplasmic pool and translocation to the nucleus. The activation state of signaling correlated with the expression of MT1-MMP. Wnt 3 expression and nuclear localization of β -catenin were prominent in carcinoma cells at the invasive front, where EMT occurs. Expression of some mesenchymal specific transcription factors, such as HMGA2 [Miyazawa et al., 2004] and LMO4 [Mizunuma et al., 2003], has been correlated with poor differentiation, invasion and metastasis of oral cancer [Imai et al., 2004].

Conclusion

In this review, we focused on molecular pathways related to the major events of EMT in oral cancer, such as cell-cell dissociation, cytoskeleton rearrangement, and basement membrane degradation. Because there are only a few studies, small sample sizes in each study and heterogeneity of cancer types, EMT mechanisms in oral cancer and/or precancerous lesions have not been clearly established. However, these few investigations have uncovered evidence that E-cadherin may be downregulated via Snail, SIP1 and hypermethylation. Rho and Rac appear to have a role in tumor cell mobility and many MMPs are upregulated at the leading edge. Future investigations should concentrate on analyzing E-cadherin transcrip-

tion repression, cytoskeleton changes, and mesenchymal transcriptional factor expression to understand EMT mechanisms, which may provide a strategy for treatment by targeting these signal transduction pathways.

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