

Epithelial-mesenchymal transition and progression of oral carcinomas

Review Article

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Summary

Oral carcinomas are devastating diseases with poor patient survival. This unfortunate outcome could partly result from that carcinoma cells frequently loose epithelial cell characteristics and gain mesenchymal cell-type features, as referred to epithelial-mesenchymal transition (EMT). Induction of EMTs makes carcinoma cells invasive and metastatic. Unveiling the mechanism for EMTs would be a challenge for development of a novel strategy for patients suffered from the disease. In this review, we will focus on transcriptional regulation of carcinoma cell-EMTs, in addition to the WNT signaling pathway, based on our recent findings.

I. Introduction

Squamous cell carcinomas are the most common malignant neoplasm of the oral cavity. Worldwide, annual incidence of new cases exceeds 300,000. However, surgery, radiotherapy and chemotherapy have not improved the five-year survival rate of this devastating disease in more than two decade (Lippman and Hong, 2001). Because of their location, treatment leads to long-term survival functional significant and cosmetic defects in survivors, which can have a significant impact on the quality of life. The high mortality rate may be due to the fact that oral carcinoma cells easily invade into territorial tissues and metastasize to the cervical lymph nodes. Treatment failures can be attributed to multiple factors but remain difficult to predict, because no reliable molecular marker is currently available in early detection or as indicators of prognosis. The tailoring of individual treatment strategies to aggressively treat those carcinomas at greatest risk of patient death would likely improve long-term survival. There is an urgent need to identify characteristics of the primary tumor that might predict aggressive tumors. Thus, it is an important issue to uncover molecular pathways of carcinoma progression to be a metastatic disease.

Metastasis of carcinoma cells requires several steps, including detachment from the primary site,

dedifferentiation, invasion of the surrounding stroma and vessel walls, embolism, and stromal invasion and proliferation in distant organs. With few exceptions, carcinomas are derived from single somatic cells and their progeny. Carcinoma cells in the emerging neoplastic clone accumulate within them a series of genetic and/or epigenetic changes that lead to changes in gene activity and hence to altered phenotype that are subjected to selection for tumor progression (Ponder, 2001). Loss of epithelial morphology and acquisition of mesenchymal characteristics, often referred to as the epithelial-mesenchymal transition (EMT), are typical for carcinoma cells and predispose tumors to a more advanced state of progression (Hay, 1995; Birchmeier et al, 1996; Thiery, 2002). The genetic instability may trigger alterations in regulatory sequences of correct gene expression and may accumulate EMTs in carcinomas from a standpoint of tumor progression. Induction of EMTs in squamous carcinoma cells drives tumor progression through enhancement of invasive and metastatic features (Oft et al, 2002; Grille et al, 2003). Identifying the mechanism(s) that is involved in EMTs provides insights into understanding the pathway of tumor progression and development of a novel strategy predicting tumor malignancy and may contribute to long-term survival of patients (Thiery, 2002).

II. Activation of WNT signaling pathway

Torrential flooding of intracellular signaling establishes the biological status of carcinoma cells, and their interaction makes difficult to understand the primary pathway for tumor progression. Among numbers of the pathway, β -catenin (CTNNB1)-mediated WNT signaling is stimulated in varieties of tumors. WNT was identified as an oncogenic gene activated by chromosomal integration of Mouse Mammary Tumor Virus, and constitutes a large gene family (19 members in human). Ligation of secreted WNT molecules to cell surface receptors (frizzled and LDL receptor-related protein) sparks signaling pathway (Seidensticker and Behrens, 2000). In this pathway, WNT-frizzled binding abrogates kinase activity of glycogen synthase kinase 3 β , liberating CTNNB1 from degradation and increasing the cytoplasmic-free CTNNB1 pool. An excess amount of CTNNB1 translocates into the nucleus and transcribes target genes. In the absence of WNT, glycogen synthase kinase-3 forces CTNNB1 to degrade, resulting in a decrease of the free CTNNB1 pool (Seidensticker and Behrens, 2000). WNT pathway directly activates expression of genes involved in proliferation, invasion and EMTs of carcinoma cells (www.stanford.edu/~rnusse/wntwindow.html). Therefore, it seems reasonable to suppose that the WNT pathway promotes progression of tumors. In oral carcinomas, carcinoma cells express keratinocyte-type WNTs (WNT 6 and 7A), but also miss-express fibroblast-type (WNT3, 11 and 16) or other cell-type (WNT3A, 4, 7B and 14). Carcinoma cells express WNT3 and activate the WNT pathway at the invasive front (**Figure 1**) (Uraguchi et al,

2004). Analogous findings are reported in colorectal carcinomas (Kirchner and Brablets, 2000; Brabletz et al, 2001; Takahashi et al, 2002). Since the WNT pathway triggers EMTs (Eger et al, 2000), activation of WNT expression and signaling in oral carcinoma cells may stimulate EMTs and progression of tumors. However, activation of the WNT pathway also plays a pivotal role in developmental and non-tumorigenic events (Okuse et al, manuscript submitted). In these situations, the pathway will be terminated after completion of the events. Thus, if the WNT pathway takes an indispensable part in tumorigenesis and/or tumor progression, we should address a mechanism responsible for the sustained expression of WNTs in carcinoma cells.

III. Miss-expression of mesenchyme-specific transcription factors

It is widely accepted that many of molecular pathways underlying tumorigenesis represent aberrations of the normal developmental processes. In a majority of tumors, transcription factors can be re-expressed that are derivatives of embryonic cells in which the transcription factors are normally expressed during embryogenesis. However, transcription factors can be expressed in tumorigenic cells derived from those in which a particular transcription factors are not normally expressed during development (Abate-Shen, 2002). PAX5 is expressed in medulloblastoma, but not in the cerebellum from which this tumor is derived (Kozmik, 1995). Miss-expression of transcription factors, as opposed to the re-expression,

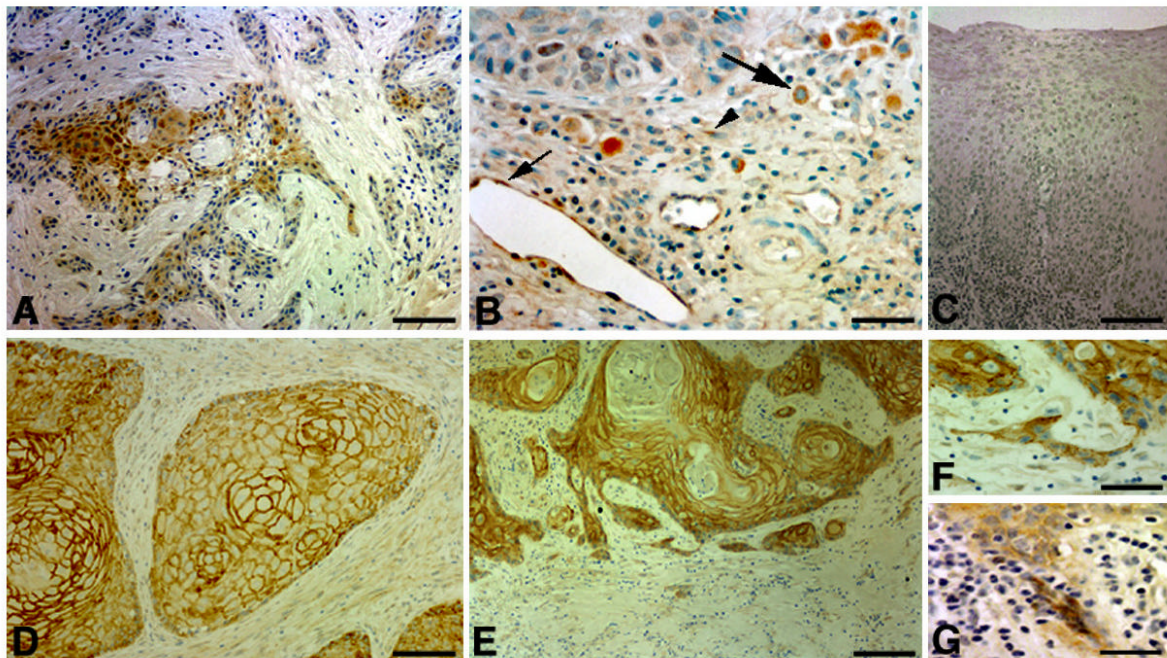


Figure 1. Immunolocalization of WNT3 and CTNNB1 in oral carcinoma tissues. (A) WNT3 was localized to carcinoma cells at the invasive front. (B) Endothelial cells (small arrow), fibroblasts (arrowhead), and macrophage-like cells (large arrow) adjacent to carcinoma cells were also positively stained. (C) Normal gingiva did not react to WNT3 antibody. (D, E) CTNNB1 stained cell-cell junction of carcinoma cells. (F, G) At the extremity of carcinoma invasion, CTNNB1 showed diffuse cytoplasmic or nuclear staining. Bar = 100 μ m (A, C-E) and 50 μ m (B, F, G). Reproduced from Uraguchi et al, 2004 with kind permission from Journal of Dental Research.

could provide phenotypic alterations and accumulate the cellular trans-differentiation, especially EMTs, in carcinomas. In this section, we provide evidence for mis-expression of mesenchyme-specific transcriptional co-factors in oral carcinoma cells and contribution for the pathology of diseases.

A. HMGA2

The high mobility group A (HMGA) family consists of three members, HMGA2, HMGA1a and HMGA1b. A prominent feature of the HMGA family is the three DNA-binding domains, termed AT-hooks, that bind to AT-rich DNA in the minor groove. They have no transcriptional activity per se, but through binding with other transcription factors, they organize the framework of the nucleoprotein-DNA transcriptional complex and enhance the transcription of several genes, which are specifically expressed in mesenchymal cells (Thanos 2002, Carey et al, 1988). Because HMGA2 is predominantly expressed in undifferentiated mesenchymal cells during development (Zhou et al, 1996), it has been hypothesized that inappropriate activation of the HMGA2 gene in terminally differentiated mesenchymal cells that initiate the tumorigenic pathway and leads to a mesenchymal tumor (Ashar et al, 1995; Schoenmakers et al, 1995). However, very little is known about a role of HMGA2 expression in carcinomas of epithelial origin. Quantitative analysis of HMGA2 gene expression demonstrated that oral carcinomas ectopically express the gene at levels 163.4 ± 90.4 (mean \pm 1 S.D.)-fold greater than that of normal counterparts. HMGA2 protein expression is identified in 73.8% of carcinomas and predominantly seen in most carcinoma cells at the invasive front, where carcinoma

cells gain the characteristics of EMTs and facilitate tumor invasion (**Figure 2**). In addition, HMGA2 protein expression is closely associated with tumor recurrence and patient survival. This is highlighted by the fact that 100% of patients who died of tumor recurrence express HMGA2 protein, and every HMGA2-expressing patient without lymph node metastasis died of tumor recurrence. Furthermore, protein expression is closely associated with the long-term patient survival rate independent of other risk factors (**Figure 3**). Although survival of clinically metastasis-negative patients free from disease recurrence is limited to 51.7%, 100% of HMGA2-negative patients survive without tumor recurrence (Miyazawa et al, 2004). Treatment of clinically metastasis-negative patients with chemotherapy or radiotherapy with neck dissection is a controversial issue (Lippman and Hong, 2001). HMGA2 may be a novel superior marker for tumor recurrence and examination of HMGA2 protein expression by immunostaining on incisional biopsy specimens would predict tumor aggressiveness and stratify patients into risk group.

B. LMO4 and LDB1

The LIM-only protein (LMO) carries two tandemly repeat LIM zinc-binding domain, which acts as an adaptor for transcription factors facilitating assembly of large transcriptional complexes (Breen et al, 1998; Sugihara et al, 1998). LMO4 gene is widely distributed in embryonic tissues (Kenny et al, 1998; Sugihara et al, 1998), and involved in negative regulation of breast carcinoma cell differentiation (Visvader et al, 2001). LMO4 binds with a high affinity to the LIM domain-binding protein 1 (LDB1), which binds to transcription factors and bridge a

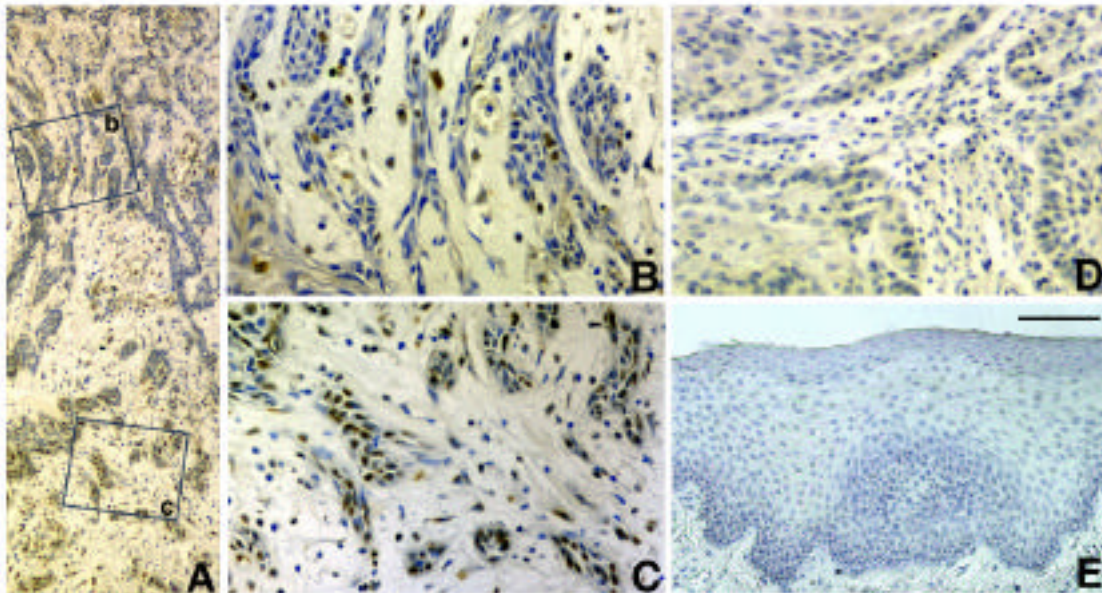


Figure 2 Immunolocalization of HMGA2 in squamous cell carcinomas. A shows a low-power view of HMGA2 staining. A high-power view of the staining at the center and invasive front is shown in **B** (depicted in inset b in **A**) and **C** (depicted in inset c in **A**), respectively. **D**, a carcinoma tissue section was reacted with nonimmune IgG instead of anti-HMGA2 antibody as a negative control reaction. **E**, normal epithelial cell of the gingiva was negatively stained. Bar, 250 (**A**), 50 (**C and D**), and 150 μ m (**E**). Reproduced from Miyazawa et al, 2004 with kind permission from Cancer Research.

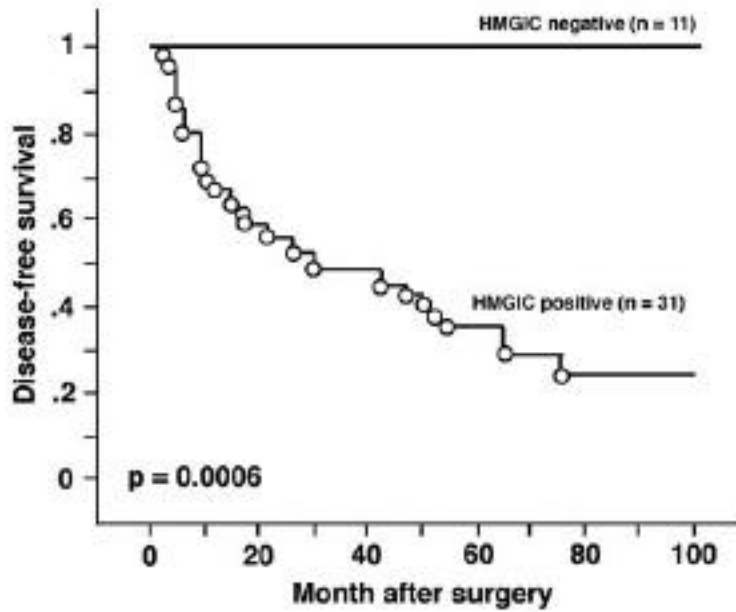


Figure 3 Disease-specific survival in oral carcinoma patients based on the expression of HMGA2. The graph summarizes Kaplan-Meier survival analysis for patients with positive or negative HMGA2 staining. Statistically significant differences were examined between negative and positive HMGA2 staining ($P = 0.0006$). Reproduced from Miyazawa et al, 2004 with kind permission from Cancer Research.

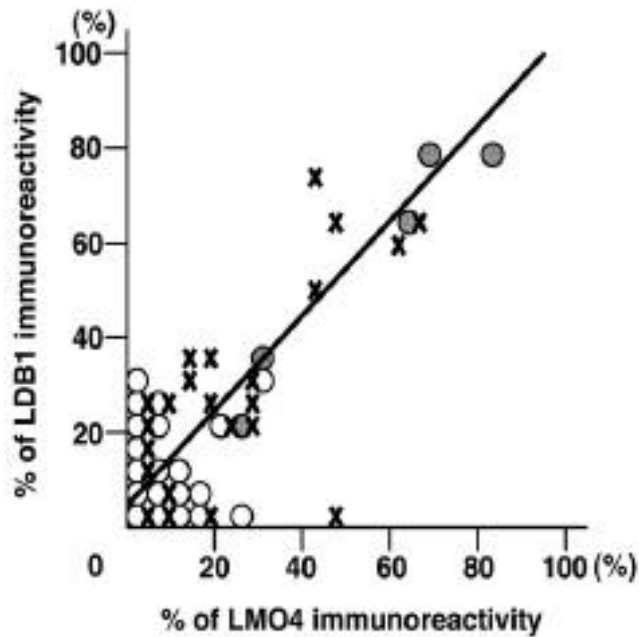


Figure 4 Immunoreactivity of LMO4 and LDB1 at the primary site of oral carcinomas. A positive direct correlation between LMO4 (horizontal line) and LDB1 (vertical line) immunoreactivity was found by simple linear regression ($r^2 = 0.669$, $P < 0.01$). Open circles, crossed and shaded circles indicated well, moderately, and poorly differentiated carcinomas, respectively. Reproduced from Mizunuma et al, 2003 with kind permission from British Journal of Cancer.

unique bipartite DNA sequence separated by about one helix turn from each other (Jurata et al, 1996; Wadman et al, 1997). Both LMO and LDB proteins appear to have essential functions in cell proliferation and lineage determination, and oncogenesis (Jurata et al, 1998; Thaler et al, 2002). The LIM domain of LDB1 contributes to the

binding of transcription factors, including LIM-homeodomain, zinc-finger and basic helix-loop-helix proteins (Jurata et al, 1996; Morcillo et al, 1997). Formation of protein complexes synergistically activates the expression of target genes (Jurata et al, 1998). However, in the presence of LMO protein, it competes

direct binding between LDB and the transcription factor (Rabbitts, 1998; Thaler et al, 2002). Miss-expression of LMOs by the chromosomal translocation is observed in T-cell leukemia and inhibits differentiation of neuronal cells (Thaler et al, 2002). We observed that normal keratinocyte and oral carcinoma cells express LDB1, but LMO4 expression is only detected in carcinoma cells. These proteins are predominantly expressed in carcinoma cells at the invasive front, and upregulated in parallel with tumor dedifferentiation (**Figure 4**) (Mizunuma et al, 2003). Although biological consequences of LMO4 expression in oral carcinoma cells is not certain, we are currently investigating the LMO4-binding transcription factor and target genes.

IV. Downregulation of E-cadherin expression

First step of carcinoma progression is dissociation from cell-cell adhesion, which is mediated by E-cadherin (CDH1). An animal model of pancreatic carcinoma demonstrated that a direct role of CDH1 in adenoma-to-carcinoma conversion (Perl et al, 1998), indicating CDH1 as a tumor suppressor gene. Disruption of CDH1-mediated cell-cell adhesion by anti-CDH1 antibodies induces EMTs of carcinoma cells (Imhof et al, 1983). Although loss of tumor suppressor gene expression has been believed to result from the classical Knudson's two-hit hypothesis, emerging evidence indicate that somatic mutation with loss of heterozygosity is extremely rare in sporadic cancer and that epigenetic pathways are responsible for the lack of CDH1 in the majority of sporadic carcinomas (Cheng et al, 2001; Graff et al, 2000). Recently, transcriptional repressor, SNAIL, and promoter hypermethylation are considered to be primary cause of CDH1 downregulation (Cano et al, 2000; Batlle et al, 2000; Graff et al, 2000). However, other CDH1 repressors, including SLUG, SIP1 and E12/47, are also known. We considered what epigenetic aberrations could repress CDH1 in oral carcinoma cells. Unexpectedly, SNAIL expression was not related to the CDH1 expression status. However, SIP1 expressing cells negligibly expressed CDH1. Promoter hypermethylation was also predominantly observed in CDH1-negative cells (Maeda et al, manuscript submitted). Synergistic action and balance between SIP1 expression and promoter hypermethylation may be critical determinant for the epigenetic loss of CDH1 during oral carcinoma progression and plays a role in an induction of EMTs.

V. Conclusion

In this review, we focused on molecular pathway for an induction of EMTs in oral carcinoma cells. Transient alterations in cell proliferation, differentiation, and migration activities initiated through changes of microenvironments are observed in pathological and physiological conditions. However, in the case of malignant tumors, it is well within the realm of possibility that tumor cells have been destined for undergoing to EMTs by more upstream critical aberrations. Aberrant

expression of transcription factors or activation of oncogenic signaling would be a candidate. Understanding the molecular mechanism(s) for EMTs is not only an interesting issue cell biologically but also may provide a novel strategy for cancer therapy.

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