

Review

Molecules of cell adhesion and extracellular matrix proteolysis in oral squamous cell carcinoma

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Summary. Oral squamous cell carcinoma (OSCC) is the most common malignancy of the oral cavity with a poor 5-year survival rate, due in large part to the presence of metastatic disease at initial diagnosis. In recent years, a number of studies have examined the oral tumor microenvironment to assess the potential dynamic balance between extracellular matrix deposition and proteolytic degradation as well as the cellular adhesion molecules that mediate adhesion to matrix and regulate tissue cohesion. The objective of this review is to provide a brief overview of the major matrix components, adhesion molecules and proteolytic enzymes in the oral tumor microenvironment and to summarize recent findings regarding the role of these complex molecular players in oral tumor progression.

Key words: Oral cancer, Cell adhesion, Protease, Extracellular matrix

Introduction

The human oral cavity is comprised of an extensive mucosal surface histologically subdivided into masticatory mucosa on the gingiva and hard palate; a lining mucosa on lips, cheeks, alveolar mucosal surface, floor of the mouth, inferior surfaces of the tongue, and soft palate; and a specialized mucosa on the dorsal surface of the tongue (Ross and Pawlina, 2005). The lining mucosa is organized similarly to the epidermis of the skin as a stratified squamous epithelium comprised of four layers; the stratum basale, stratum spinosum, stratum granulosum, and stratum corneum. Surface keratinization varies by mucosal subsite and is absent in buccal mucosa and the floor of the mouth, is partially

present on some areas of the gingiva and parts of the palate, and full keratinization is seen in most parts of the hard palate and overlying gingival attachments to bone (Adams, 1976). Epithelial cells make contact with adjacent cells via cell surface adhesion molecules and are separated from the loose subepithelial connective tissue, or lamina propria, by a dense basement membrane. Adhesion of epithelial cells to neighboring cells and non-cellular components of the extracellular matrix (ECM) is an essential process in histogenesis, necessary to develop normal structure and function (De Arcangelis and Georges-Labouesse, 2000; Daley et al., 2008; Muller et al., 2008). Molecules governing cell adhesion and migration have profound effects on cell survival, proliferation, differentiation and motility. The dynamic cellular rearrangement that occurs in embryonic development requires disruption of tissue integrity, accompanied by ECM proteolysis and deposition. The turnover of extracellular matrix via cellular deposition and proteolysis occurs not only in developmental tissue remodeling, but during wound healing and tumor cell invasion and metastasis as well.

The majority of oral cancers originate from squamous epithelia of the oral tongue and floor of the mouth (Werning, 2007). Oral cavity cancer results in over 80,000 deaths annually and is one of the top ten most frequently diagnosed cancers worldwide (Parkin et al., 2005). Oral squamous cell carcinoma (OSCC) is the most common malignancy of the oral cavity with approximately 23,000 new cases detected yearly in the U.S. (Jemal et al., 2008). The 5-year survival rate of OSCC remains at a low 50%, predominantly due to the presence of regional and/or distant metastasis at the time of initial diagnosis. A more detailed understanding of the molecular mechanisms that coordinate OSCC progression and metastasis may result in identification of improved diagnostic and treatment strategies. Recent years have seen an intense focus on the study of molecules involved in cell adhesion, extracellular

matrix, and proteolysis in oral cancer research. The objective of this review is to provide a summary of new findings in this area to highlight the complex role(s) of these key molecular players in oral tumor progression.

Extracellular matrix components

The meshwork of proteins, proteoglycans and glycosaminoglycan surrounding cells in multicellular organisms is collectively referred as ECM, the major constituents of which include collagen, fibronectin, laminin, vitronectin and hyaluronate (Fig. 1) (De Arcangelis and Georges-Labouesse, 2000; Daley et al., 2008). These large macromolecules form a supportive framework for cells embedded therein and serve as ligands for matrix-binding integrins on the cell surface. ECM proteins also sequester growth factors within the matrix. Matrix components are produced by various cells including epithelial and stromal cells within a specific tissue type. Changes in composition and structure of the ECM, due to altered expression, secretion, or processing, often accompany oral tumor progression and have been widely investigated.

Collagen

Collagens are the dominant component of ECM and are the most abundant proteins in mammals. Assembled as hetero- and homo-trimers, there are over 20 distinct collagen types described to date, some with tissue-specific localization (Lyons and Jones, 2007). In the basement membrane of oral epithelia, collagen I and collagen IV are the most common types (Garrone et al., 1997). Collagen I and III primarily make up the dermis and collagen IV, VII and XVII are found in the dermo-epidermal interface (Garrone et al., 1997).

Collagen I and III are increased in the stroma adjacent to benign alterations in malignantly transformed keratinocytes. These collagen types are also associated with well differentiated OSCC, as the destruction of fibrillar structures is more pronounced during the transition from moderately to poorly differentiated squamous cell carcinomas (Stenback et al., 1999). Collagen IV is disrupted and discontinuous in invasive OSCC but not in carcinoma in situ (Giannelli et al., 2001). Transcriptional profiling has identified both type I and IV alpha1 and alpha2 chains as differentially expressed in OSCC relative to normal oral tissue (Chen et al., 2008; Ye et al., 2008), suggesting the potential utility of these gene products as diagnostic or prognostic indicators. Interestingly, enhanced expression of minor collagen isoforms including collagen V and IX has also been identified using microarray analysis in OSCC (Sok et al., 2003; Schmalbach et al., 2004). Downregulation of collagen XVII in basal cells in mild dysplasias and upregulation in suprabasal keratinocytes in moderate and severe dysplasias, as well as in the central cells of grade II and III squamous cell carcinomas, has been demonstrated using immunohistochemistry and *in situ*

hybridization. Furthermore, overexpression of collagen XVII is found at the tumor invasive front (Parikka et al., 2003), suggesting a correlation between expression of collagen XVII and tumor progression.

Fibronectin

Matrix fibronectin is a dimeric matrix glycoprotein with rod-like structure composed of three homologous repeats or modules, Types I, II, and III. Each module constitutes an independently folded unit and is referred to as Fn1, Fn2 and Fn3 respectively, joined by short connector sequences (Faralli et al., 2009). Fibronectin is ubiquitously expressed throughout the ECM and promotes cell-matrix adhesion through interaction with integrin $\alpha 5\beta 1$, although binding to integrins $\alpha 4\beta 1$, $\alpha 5\beta 1$, $\alpha v\beta 1$ and $\alpha v\beta 6$ has also been reported (Johansson et al., 1997).

In OSCC, fibronectin expression is prevalent both in the tumor stroma and at the invasive front, and the expression of fibronectin in primary tumors associated with metastatic cases is increased when compared with those primaries not associated with metastases (Harada et al., 1994). An isoform of fibronectin designated oncofetal fibronectin is absent in normal adult

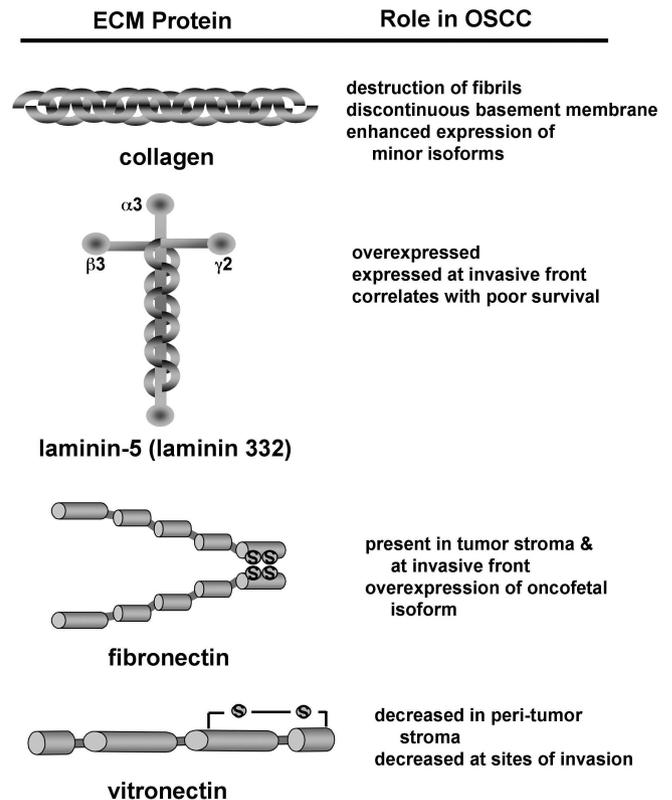


Fig. 1. Overview of extracellular matrix (ECM) proteins in OSCC. Structural features of the major ECM protein components and their role in OSCC are depicted.

epithelium but is present in cancer stroma, as detected by immunohistochemistry. Intense oncofetal fibronectin expression at regions of invasion is also observed (Bilde et al., 2009). Oncofetal fibronectin is a strong indicator for poor prognosis in OSCC (Lyons and Cui, 2000; Lyons et al., 2001).

Laminin

Laminins are high molecular weight heterotrimeric glycoproteins present in basement membrane composed of α , β and γ chains encoded by distinct genes (Ghosh and Stack, 2000). To date, 5 α , 3 β and 3 γ chains have been reported, assembling into sixteen different laminin heterotrimers (Scheele et al., 2007). Laminin isoforms are secreted by a wide variety of cells in a tissue-specific manner (Lyons and Jones, 2007) and considerable data exist regarding the involvement of laminin isoforms in oral epithelium and in OSCC. Laminin-5 (laminin-332), composed of $\alpha 3$, $\beta 3$ and $\gamma 2$ subunits, is the major laminin isoform in oral squamous epithelium. Laminin-5 is an integral constituent of hemidesmosomes, that form stable cell-matrix adhesions in normal epithelia (Goldfinger et al., 1998). The major laminin-5-binding integrins are $\alpha 3\beta 1$ and $\alpha 6\beta 4$ (Scheele et al., 2007).

As laminin-5 has been shown to be both an adhesive and a pro-migratory factor, the function of this glycoprotein remains somewhat controversial (Ziober et al., 2006). The 460 kDa precursor of laminin-5 undergoes specific proteolytic processing after secretion (Kainulainen et al., 1998) and in keratinocytes *in vivo* more than 50% of the laminin-5 is fully processed. Unprocessed laminin-5 can promote keratinocyte migration while cells in contact with processed mature laminin-5 form stable attachments to the matrix (Ziober et al., 2006). Mechanistic analysis of these apparently contradictory phenomena showed that processing of the $\alpha 3$ subunit secondary to plasminogen activation and plasmin formation resulted in dramatic changes in cellular behavior (Goldfinger et al., 1998, 2000).

In OSCC, overexpression of laminin-5 has been identified in three distinct transcriptional profiling studies, suggesting the potential utility of laminin-5 analysis as a diagnostic tool or to predict local recurrence at surgical margins (Chen et al., 2008; Ye et al., 2008; Mendez et al., 2009). Laminin-5 gene expression is also strongly correlated with poor survival and thus may find use as a prognostic indicator as well (Mendez et al., 2009). This is supported by immunohistological and cell biological studies showing higher expression of laminin-5 at regions of infiltration and invasion (Katoh et al., 2002; Kuratomi et al., 2006; Lindberg et al., 2006; Gasparoni et al., 2007; Bilde et al., 2009; Kulasekara et al., 2009). In addition, OSCC cells that are highly invasive *in vitro* show an increased motility on laminin-5 when compared with less invasive cell lines (DiPersio et al., 1997). Together these data suggest that laminin-5 may play a key role in progression of OSCC.

Vitronectin

Vitronectin is a glycoprotein mainly synthesized by the liver but widely found in loose connective tissue, blood vessel walls, and in the stroma of lymphatic tissue. It is a monomer in circulation, but converts into multimeric form when deposited in ECM (Seiffert, 1997). Vitronectin binds to plasminogen activator inhibitor-1 (PAI-1) via its somatomedin B domain, thus counter-regulating proteolysis initiated by plasminogen activation (Xu et al., 2001). Vitronectin contains an RGD sequence, through which it binds to membrane bound integrins, mainly αv integrins, anchoring cells to the extracellular matrix. The somatomedin B domain is also the site of interaction between vitronectin and urinary-type plasminogen receptor (uPAR), and this interaction has been implicated in cell migration and signal transduction (Wei et al., 1994; Zhou et al., 2003).

Analysis of vitronectin expression in primary OSCC relative to metastatic cervical lymph nodes shows a significant decrease in vitronectin levels in peri-tumor stroma at sites of invasion (Harada et al., 1994). Conversely, fibronectin deposition is enhanced at the tumor invasive front, suggesting deposition of a provisional matrix (Harada et al., 1994). Loss of matrix-associated vitronectin is supported by a proteomic analysis of serum biomarkers for OSCC (Bijian et al., 2009). In this study, sera from control mice were compared to those bearing tongue tumors using a variety of analyses. Results show enhanced levels of host-derived (i.e. murine) vitronectin in serum of tumor bearing mice (Bijian et al., 2009).

Cell surface adhesion molecules

Cell surface adhesion molecules function as receptors for components of the ECM or membrane-associated counterparts from adjacent cells, enabling cell-cell and cell-ECM communication. The most commonly expressed adhesion molecules are integrins and cadherins, that function as membrane biosensors to detect changes in the extracellular environment and transmit these signals intracellularly. Additional proteins including CD44 and urinary type plasminogen activator receptor (uPAR) also play a role in cell adhesion.

Integrins

Integrins are a family of heterodimeric transmembrane cell adhesion proteins comprised of α and β subunits that are responsible for binding of cells to ECM components. Integrins are widely distributed and have been found on virtually every cell and tissue (Virtanen et al., 1990). To date, 16 α subunits and 8 β subunits have been identified, resulting in 24 integrin heterodimers (Fig. 2) (Lyons and Jones, 2007). Many integrins can bind to several different matrix ligands, and generally, one ligand is often recognized by several integrins (De Arcangelis and Georges-Labouesse, 2000).

Through cytoplasmic tail interactions with adaptor proteins and signaling components, integrin-mediated adhesion to ECM proteins provides a functional link between the cytoskeleton and the extracellular environment. In addition to adhesive and migratory functions, integrins can also affect cell growth, differentiation, gene expression, angiogenesis, protease production, and apoptosis (De Arcangelis and Georges-Labouesse, 2000; Daley et al., 2008; Muller et al., 2008).

In the normal oral mucosa, expression of $\alpha 2\beta 1$, $\alpha 3\beta 1$, $\alpha 6\beta 4$ and $\alpha 9\beta 1$ integrins is prevalent, with staining found in suprabasal as well as basal distributions (Thomas and Speight, 2001). Although altered integrin expression profiles have been reported in many cancers, no one specific integrin has been implicated in transformation to the malignant phenotype. Indeed, changes in integrin expression vary both between and within different tumor types (Thorup et al., 1997). In tumor tissues, altered integrin profiles as well as altered integrin distribution within specific tissues have been observed; however integrin expression profiles in OSCC have not been extensively evaluated. Focal loss of staining for $\alpha 2\beta 1$, $\alpha 3\beta 1$, $\alpha v\beta 5$ and $\alpha 6\beta 4$ has been reported, but does not correlate with prognosis. In a recent study, $\alpha 3$ and $\beta 5$ integrin expression was associated with metastasis to cervical lymph nodes while $\beta 5$ expression correlated with disease-specific death (Kurokawa et al., 2008). In addition, variable loss or reduced expression of $\beta 1$ integrins and integrin $\alpha 6\beta 4$ is found in poorly differentiated tumors (Lyons and Jones, 2007). In contrast, an additional study showed that high levels of $\alpha 3$, $\alpha 6$ and $\beta 1$ integrin are associated with improved prognosis (Ohara et al., 2009). Furthermore, acquisition of $\alpha v\beta 6$, a fibronectin-, vitronectin-, and tenascin-binding integrin associated with wound healing, has been reported in OSCC (Breuss et al., 1995; Jones et al., 1997; Thomas et al., 2006). This is supported by *in vitro* data showing that forced expression of this integrin increases OSCC cell motility and promotes invasive activity (Ramos et al., 2002). These results highlight the complex roles of integrins in oral tumor progression.

Cadherins

Cadherins are transmembrane proteins that function in cell-cell adhesion via formation of adherens junctional complexes with cadherins on adjacent cells. The cytoplasmic domains of cadherins connect via catenins to the actin cytoskeleton, linking cell-cell contact to cytoskeletal function (Wheelock et al., 2008) (Fig. 3). Cadherins are essential for cell sorting during development and differentiation. The cadherin superfamily contains over 30 members (Takeichi, 1993; Charalabopoulos et al., 2004), however the most commonly expressed cadherin family members are E-cadherin found in epithelial tissue, N-cadherin in cells of neural origin and P-cadherin found predominantly in placenta.

In stratified squamous epithelia, E-cadherin is

prevalent, desmosomal cadherins are also abundant, and P-cadherin expression has been reported (Thomas and Speight, 2001). Cadherin function is essential for control of growth in gingival epithelium in culture (Kandikonda et al., 1996) and loss of E-cadherin function induces abnormal stratification of cells (Lewis et al., 1994). In many carcinomas, loss of E-cadherin expression is associated with tumor progression, such that well-differentiated tumors retain E-cadherin while poorly differentiated lesions show loss of expression (Takeichi, 1993; Mahomed et al., 2007). Loss of E-cadherin has been observed in mild and moderate dysplasia in addition to *in situ* and microinvasive carcinomas (Santos-Garcia et al., 2006), suggesting that loss of expression may be an early event in tumor progression. Reduced E-cadherin staining is associated with infiltrative growth, a more invasive histological staining, and metastasis to cervical lymph nodes (Schipper et al., 1991; Schipper et al., 1994; Diniz-Freitas et al., 2006; Hung et al., 2006; Foschini et al., 2008). In this regard, it is interesting to note that downregulation of E-cadherin function results in increased proteinase expression in oral epithelial cells (Munshi et al., 2002). Indeed, weak or absent E-cadherin expression correlates with both a shorter disease-free interval and shorter overall survival time (Mattijssen et al., 1993; Bosch et al., 2005; Diniz-Freitas et al., 2006; Wang et al., 2009).

Acquisition of expression of N-cadherin accompanies progression of many carcinomas and may be associated with loss of E-cadherin, often resulting in a more invasive mesenchymal phenotype (Wheelock et al., 2008). Although N-cadherin has not been extensively evaluated in OSCC, a recent immunohistochemical

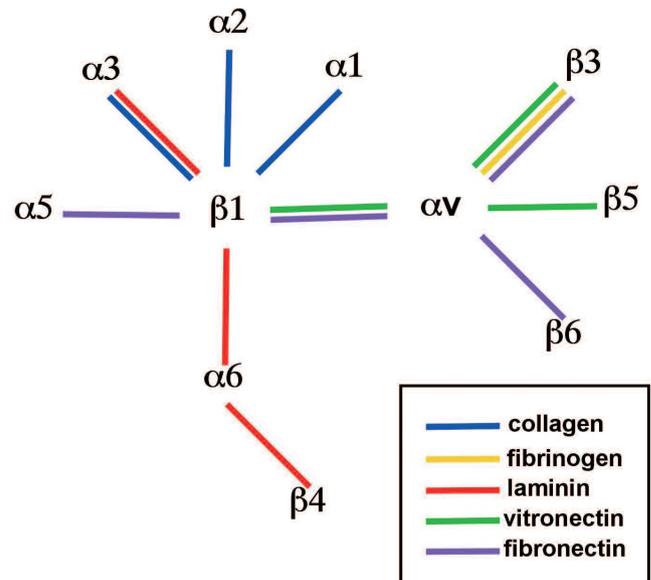


Fig. 2. Integrins expressed in OSCC. The diagram depicts integrin subunits identified in OSCC, their binding partners, and matrix ligands.

analysis of E- and N-cadherin expression in OSCC showed reduced E-cadherin immunoreactivity in primary tumors and lymph node metastases (Pyo et al., 2007). While N-cadherin staining was negative in normal oral epithelium, 37% of primary tumors and 36% of lymph node metastases exhibited positive N-cadherin staining. While not correlated with tumor grade or stage, an association was observed between positive N-cadherin staining and loss of E-cadherin expression, providing preliminary evidence for cadherin switching in OSCC. This is supported by a recent study showing that N-cadherin staining is correlated with lymph node metastasis in SCC of the tongue (Li et al., 2009). It has been reported that methylation of the 5' CpG island in the E-cadherin gene promoter coordinates reciprocal expression of E- and N-cadherins in OSCC, providing an interesting area for future investigation (Chen et al., 2004).

CD44

CD44 and its variants are a family of transmembrane glycoproteins that bind hyaluronan, ECM proteins and growth factors (Thomas and Speight, 2001; Ponta et al., 2003; Lyons and Jones, 2007). Alternative splicing of a single CD44 gene generates at least 20 isoforms of CD44, with additional variability added by post-translational glycosylation (Gunthert, 1993; Sreaton et al., 1993). CD44 isoforms share common N- and C-termini with the standard version of CD44, generated by the splicing combination of exons 1-5 and exons 16-19. The large number of variant forms arise via extensive alternative splicing insertion of exons 6-15 between the N- and C-terminal common domains (Georgolios et al., 2006). The N-terminal domain of CD44 encoded by exon 1-5 harbors the docking sites for matrix ligands

(Underhill, 1992).

Multiple CD44 isoforms are found in the normal oral epithelium (Oliveira et al., 1998; Mack and Gires, 2008), generally organized in a differentiation-related expression pattern with higher expression in the basal and suprabasal layers (Mackay et al., 1994; Hudson et al., 1996; Oliveira and Odell, 1997; Georgolios et al., 2006). A number of studies have evaluated OSCC tumors; however consistent results have not been achieved. Decreased expression of CD44 variants has been reported comparing carcinoma of the tongue to adjacent non-cancerous epithelia (Califano et al., 2000; Carinci et al., 2002) and this is supported by a study of lower lip and tongue carcinomas, that exhibit a decrease in CD44 positivity with increasing histologic grade (Cruz et al., 2009). Similarly, CD44v6 is down-regulated in the transition from normal epithelium, benign epithelial dysplasia, and carcinoma *in situ* to invasive carcinoma (Sneath and Mangham, 1998). However, in a more recent report analysis of CD44s and CD44v6 is unable to distinguish between normal, benign and malignant epithelium of the head and neck (Mack and Gires, 2008). Interestingly, it has been speculated that low expression of CD44 may be related to enhanced tumor cell invasiveness; an observation supported by data showing that low CD44s levels in tongue tumors are related to poor survival (Gonzalez-Moles et al., 2007). Low levels of tumor-associated CD44 may be related to shedding of the receptor, as a recent study demonstrated elevated levels of soluble CD44 in oral rinses of oral cancer patients relative to those with benign diseases of the upper aerodigestive tract (Franzmann et al., 2007). The metalloproteinase ADAM-17 may participate in CD44 ectodomain shedding, as indicated by a study combining immunohistochemistry and western blotting, that

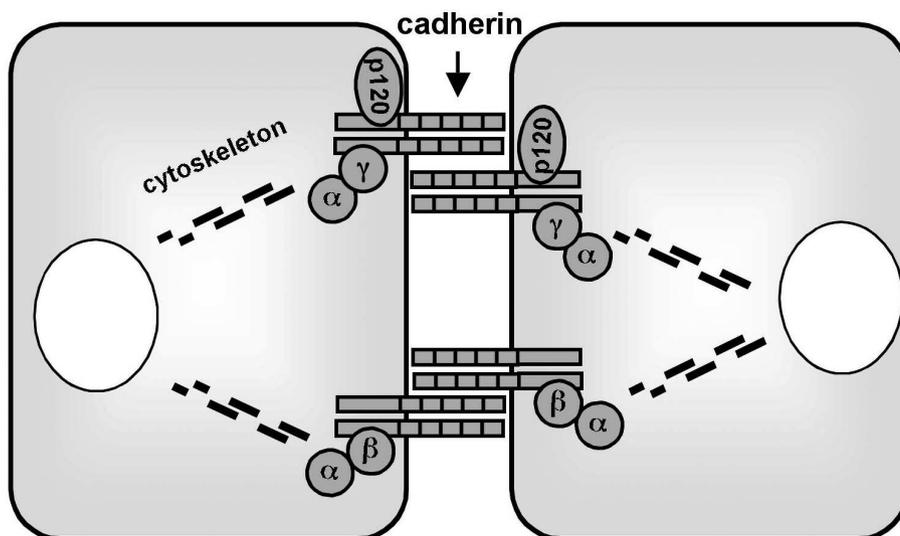


Fig. 3. Cadherin-mediated cell-cell adhesion. The diagram depicts cell-cell adhesion mediated by cadherin dimers on adjacent cells. Also shown are cytoplasmic components of the adhesive complex α , β , γ and p120 catenin.

showed a correlation between high levels of ADAM-17 and CD44 cleavage (Takamune et al., 2007).

uPAR

The urinary-type plasminogen activator receptor (uPAR, CD87) was originally identified as a surface-anchored binding protein for urinary type plasminogen activator (uPA), a serine proteinase involved in activation of circulating plasminogen to the broad spectrum proteinase plasmin (Blasi and Carmeliet, 2002; Shi and Stack, 2007; D'Alessio and Blasi, 2009). The presence of uPAR at the cell membrane enables generation of efficient and focused pericellular proteolytic activity. As such, this system has been widely implicated in many physiologic and pathologic processes including fibrinolysis, matrix remodeling, cell proliferation, invasion and migration as discussed further below (Dass et al., 2008).

The uPAR is a highly glycosylated cell surface protein (apparent molecular weight 55-60KD) expressed by both epithelial and stromal cells and is anchored to the outer layer of the plasma membrane via a glycosylphosphatidylinositol linkage at the C-terminus (Blasi and Carmeliet, 2002). In addition to its role in regulation of pericellular proteolysis, uPAR has been

implicated in a number of non-proteolytic adhesive functions. Ligand- (i.e., uPA-) occupied uPAR can bind directly to the adhesive glycoprotein vitronectin (Wei et al., 1994; Waltz et al., 1997), providing a functional link between adhesive function and proteinase regulation. In addition, following the initial observation that uPAR can complex with and modify the function of cellular integrins (Wei et al., 1996), a number of studies have confirmed this observation in a variety of cell types (Chapman, 1997; Ghosh et al., 2000; Ossowski and Aguirre-Ghiso, 2000). Studies have shown that the role of uPAR as a non-traditional 'lateral' integrin ligand, binding a site distinct from the integrin-matrix contact site, may be to modulate integrin function. Indeed, uPAR/integrin association can modify integrin signaling through both focal adhesion kinase (FAK) and Src to regulate downstream signal transduction pathways (Fig. 4A) (Wei et al., 1994; Aguirre Ghiso et al., 1999; Nguyen et al., 1999, 2000). In oral keratinocytes and OSCC cells, uPAR binds to $\alpha 3\beta 1$ integrin, activating a Src/MEK/ERK signaling pathway (Ghosh et al., 2000, 2006, 2010). This leads to transcriptional activation of a number of target genes associated with tumor progression including uPA, multiple kallikrein proteinases, and laminin-5 (Ghosh et al., 2006, 2010; Pettus et al., 2009). Furthermore, uPAR interaction with

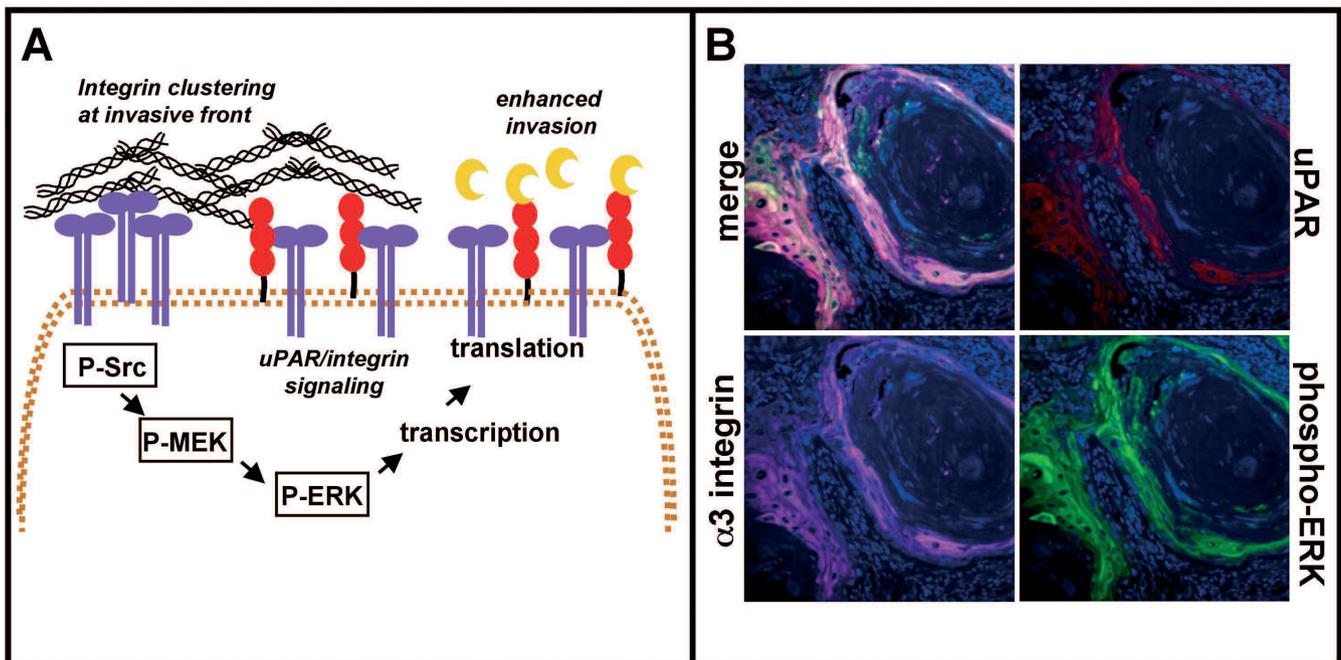


Fig. 4. uPAR/integrin association in OSCC. **A.** Model for uPAR/ $\alpha 3\beta 1$ integrin association and regulation of gene expression. Matrix induced clustering of $\alpha 3\beta 1$ integrin recruits uPAR to sites of integrin aggregation. Formation of a uPAR/ $\alpha 3\beta 1$ complex modulates $\alpha 3\beta 1$ integrin signaling, leading to alterations in transcription of genes associated with OSCC progression. $\alpha 3\beta 1$ integrin (purple); uPAR (red); uPA (yellow). **B.** Four-color immunofluorescence analysis of uPAR/ $\alpha 3\beta 1$ integrin interaction in murine orthotopic oral tumors. SCC25-uPAR cells were injected into the murine tongue, allowed to form tumors and analyzed for co-localization of uPAR (red), $\alpha 3$ integrin (purple) and activated phospho-ERK (green) (Ghosh et al., 2009). Multiple areas of colocalization are observed (merge), suggesting that uPAR/ $\alpha 3\beta 1$ integrin association observed *in vitro* can also occur *in vivo* and may modulate gene expression.

$\alpha 3\beta 1$ regulates focal adhesion dynamics and Rho family GTPase activation in oral cancer cells, providing a mechanism for uPAR-regulated alterations in motility and invasion (Shi et al., 2010). Evidence for co-localization of uPAR, $\alpha 3\beta 1$ integrin, and activated ERK *in vivo* was provided by analysis of orthotopic murine tongue tumors formed from OSCC25 cells that overexpress uPAR, using a 4-color immunofluorescence analysis. These results show multiple areas of co-localization between uPAR and $\alpha 3\beta 1$ integrin in areas also exhibiting ERK phosphorylation (Fig. 4B) (Ghosh et al., 2010). Similar results were obtained from analysis of human OSCC tumors, demonstrating a significant positive correlation between staining for uPAR, $\alpha 3$ integrin and phospho-ERK (Ghosh et al., 2010). Together these data provide *in vivo* evidence to support the hypothesis that uPAR/ $\alpha 3\beta 1$ integrin association regulates gene expression and oral tumor progression.

Proteolytic enzymes and their inhibitors

Approximately 550 proteolytic enzymes are encoded in human genome (Overall and Kleifeld, 2006). Many participate in ECM remodeling, a process controlled not only at the level of proteinase expression, but post-translationally as well. Most matrix-degrading proteinases are secreted as zymogens, requiring activation to attain catalytic competence. Furthermore, proteinase inhibitors also function to regulate matrix proteolysis. It is generally accepted that zymogen activation, proteinase activity and proteinase inhibition are well-regulated in normal tissues, such that a balance between matrix deposition and degradation is maintained. Under pathological conditions, such as occur during tumor progression from *in situ* to invasive carcinoma or to distant metastasis, uncontrolled proteinase activity may catalyze matrix degradation and thereby facilitate progression. A number of proteinases from multiple mechanistic classes have been identified in the oral tumor milieu, as well as changes in expression of proteinase inhibitors. In addition to ECM proteolysis, matrix degradation may release growth factors and matrikines embedded within the ECM to regulate tumor cell growth (Tran et al., 2005). Proteolytic processing of adhesion molecules, receptors, cytokines and chemokines may also regulate tumor progression.

Plasminogen activators (PA) and inhibitors (PAI)

The serine proteinase urinary type plasminogen activator (uPA) binds to uPAR, whereupon it efficiently catalyzes activation of circulating plasminogen to the broad-spectrum proteinase plasmin. The activity of uPA is controlled by PA inhibitor (PAI)-1 and -2, members of the serpin family of proteinase inhibitors (Binder et al., 2007; Croucher et al., 2008; Dass et al., 2008). Interaction of PAI-1 with uPAR-associated uPA precipitates internalization via the low density lipoprotein receptor-related protein-1 (LRP-1) system

(Binder et al., 2007; Lillis et al., 2008). Fibrin overlay zymography was initially used to detect uPA activity in sulcus or gingival epithelium and blood vessels on frozen OSCC sections. Further analysis with tissue explants of corresponding tumors revealed the co-expression of uPA and PAI-1 (Barlow and Southam, 1992). Recently, a novel method was developed combining laser capture microdissection with plasminogen-casein zymography to analyze uPA and uPA-PAI-1 complexes in oral cancer and adjacent non-cancerous epithelium, showing that tumor tissue uPA activity is highly increased compared with adjacent non-cancerous tissue (Curino et al., 2004). Positive expression of uPAR is found in approximately 40% of cases in a retrospective immunohistochemical study, and positive expression correlates with poor histological grade and poor prognosis (de Bock and Wang, 2004; Bacchiocchi et al., 2008). Similarly, immunohistochemical analyses correlate expression of both uPA and uPAR with invasion and metastasis to regional lymph nodes (Nozaki et al., 1998). More recently, analyses of paired tissue samples from OSCC and normal oral epithelia using enzyme-linked immunosorbent assays (ELISA) for uPA, uPAR, PAI-1, and PAI-2 show that concentrations of all four proteins are significantly higher in tumors than in normal oral tissue. Strong correlations are observed between uPA, uPAR, and PAI-1 with clinical and pathological indices of cancer aggressiveness (Baker et al., 2007). An additional ELISA study shows significant elevation of uPA in primary tumors as well as in tumor positive lymph nodes relative to normal oral mucosa, and protease levels correlate with increased likelihood of tumor relapse (Hundsdoerfer et al., 2005). Somewhat counterintuitive are studies showing that high levels of PAI-1 positively correlate with cancer invasion, angiogenesis and poor clinical outcome, while loss of PAI-1 is associated with less aggressive tumor growth (Yasuda et al., 1997; Dass et al., 2008). A PAI-1 promoter 4G/5G polymorphism may account for its high expression in cancers. A study on the 4G/5G polymorphism in the PAI-1 gene in oral cancer patients found that the mutant 4G allele is more common in early stages of oral carcinogenesis compared to healthy controls (Vairaktaris et al., 2006).

Maspin

Mammary serine proteinase inhibitor (maspin) is an additional member of the serpin superfamily first identified as a candidate tumor suppressor gene in human breast cancer (Zou et al., 1994). Maspin shares sequence homology with both inhibitory and non-inhibitory serpins, yet is more closely related to noninhibitory clade B serpins. The reactive center loop, a crucial structure for targeting proteases, is shorter than that of most inhibitory serpins, but it is responsible for promoting cell adhesion (Khalkhali-Ellis, 2006). Maspin expression is found in the cytoplasm, ECM and associated with membrane (Khalkhali-Ellis, 2006). The

involvement of maspin in tumor progression is complex and remains poorly understood. However recent data suggest that maspin may interfere with cell detachment via interaction with the uPA/uPAR system, providing a novel mechanism for regulation of focal adhesion turnover (Yin et al., 2006). Immunohistochemical analysis of maspin expression in OSCC shows that maspin is better associated with tumor differentiation grade and functions as a prognostic indicator (Iezzi et al., 2007). Maspin expression patterns are differentially localized in oral tumors, with strong staining in the middle third of the epithelium indicative of mild-to-moderate dysplasia while strong staining in the upper third correlates with carcinoma (Vered et al., 2009). Maspin expression is not correlated with recurrence in early stage tongue cancer (Cho et al., 2007). However, negative maspin immunoreactivity in carcinoma cells shows promise as a predictor of cervical lymph node metastasis (Marioni et al., 2008).

Matrix metalloproteinases

Matrix metalloproteinases (MMPs) are a family of 23 structurally related zinc dependent metallo-endopeptidases that are encoded by distinct genes. MMPs are involved in a wide variety of physiologic and pathologic processes including embryonic development, tissue remodeling, wound healing, tumor invasion and metastasis. Classification based on molecular structure and putative substrate specificity subdivides the family into collagenases (MMP-1, MMP-8, MMP-13), gelatinases (MMP-2, MMP-9), stromelysins (MMP-3, MMP-10, -11), membrane-type (MMP-14, -15, -16, -17, -24, -25) and other MMPs (Thomas et al., 1999; Sorsa et al., 2004). MMPs are secreted, by both stromal and epithelial cells, largely as inactive zymogens that require removal of an N-terminal prodomain that inhibits catalytic function (Thomas et al., 1999). Tissue inhibitor of metalloproteinase (TIMP) activity was first identified on the basis of collagenase inhibitory activity in human serum and in human fibroblast conditioned medium. To date, four TIMPs have been identified varying in molecular weight between 21-29 kDa (Sorsa et al., 2004; Verstappen and Von den Hoff, 2006). TIMPs regulate MMP activity by forming high affinity, non-covalent, stoichiometric 1:1 complexes with active MMPs. X-ray crystallographic studies have shown that TIMP-1 and TIMP-2 have a wedge shape structure that fits into the active site cleft of MMPs in a substrate-like manner (Visse and Nagase, 2003). In addition to functioning in MMP inhibition, TIMP-2 also plays a role in the activation of proMMP-2 via formation of a trimolecular proMMP-2/MT1-MMP/TIMP-2 complex. TIMPs are increasingly recognized as multifunctional proteins due to evidence showing that they are involved in stimulating cell growth, apoptosis and immunological interactions (Verstappen and Von den Hoff, 2006; Stetler-Stevenson, 2008).

MMP-1 (collagenase) mRNA expression is found in

head and neck cancer cells within tumor islands and in adjacent stromal cells while normal stromal cells have no expression (Polette et al., 1991). Expression is higher in tumor tissue relative to matched normal oral tissues (Baker et al., 2006). Analysis of mRNA levels in tumors relative to normal gingival tissue suggests that MMP-1 as well as MMP-10 have potential utility as oral cancer markers (Yen et al., 2009). This is supported by an additional molecular profiling study of tongue tumors relative to normal mucosa, indicating a high level of increase in MMP-1 expression (Estilo et al., 2009). Subtractive hybridization and microarray analyses combined with immunohistochemistry identified increased expression of MMP-1, -3, -7, and -10 as associated with reduced survival (Chiang et al., 2008). A specific polymorphism of MMP-7 (-181A/G) is associated with increased risk of early stage oral cancer (Vairaktaris et al., 2007) and protein levels are associated with survival of OSCC patients (de Vicente et al., 2007). Gelatinase levels (MMP-2 and -9) are also elevated in malignant oral tissues relative to adjacent normal tissue and evidence for zymogen activation has also been reported (Kim et al., 2006; Patel et al., 2007). Oral cancer patients with increased MMP-2 and MMP-9 activity have shorter disease-free survival than those with lower activity in a study based on carefully microdissected specimens and gelatin zymography (Yorioka et al., 2002). Furthermore, levels of MMP-2 and -9 are higher in patients with lymph node metastasis (de Vicente et al., 2005; Gao et al., 2005). Expression of both MMP-9 and TIMP-2 has predictive value for tumor metastases and poor survival, whereas TIMP-2 is the only independent predictor of poor prognosis in early-stage OSCC (Katayama et al., 2004). Overexpression of MMP-10 and MMP-11 in oral cancer is correlated with poor cancer differentiation and local invasiveness, but not to lymph node metastasis (Thomas et al., 1999). High MMP-13 expression is also associated with aggressive oral tumors and may have value as a prognostic indicator (Luukkaa et al., 2006). In addition, expression of the membrane-anchored MMP designated MT1-MMP (MMP-14) is also a predictive factor for lymph node metastasis, particularly when evaluated in conjunction with laminin-5 expression (Kawano and Yanagisawa, 2006). We have performed a preliminary analysis of the effect of MT1-MMP expression on tumor growth *in vivo* (Stack laboratory, unpublished results). In this study, SCC25 cells were generated that overexpress MT1-MMP and were compared to vector control cells (SCC25-vec) in an orthotopic murine tongue tumor model (Fig. 5). Analysis of H&E sections shows that tumors formed from SCC25-vec cells exhibit circumscribed nests of well-differentiated SCC. In contrast, tumors formed from SCC25-MT1-MMP cells were moderately differentiated SCC. SCC25-MT1-MMP tumors also exhibited sheets of tumor cells with an infiltrative pattern of growth, including tumor infiltration of underlying muscle. These data support the conclusion that MT1-MMP may play a role in OSCC

progression.

Kallikreins

Human kallikreins (KLK) are a large family of proteinases subdivided into two categories: plasma kallikreins and tissue kallikreins (Borgono et al., 2004). The former is encoded by a single gene on chromosome 4q35 and is an enzyme that releases bioactive peptide bradykinin from high molecular weight kininogen (Pampalakis and Sotiropoulou, 2007). In contrast, tissue kallikreins are encoded by the largest uninterrupted cluster of serine protease genes (15 in total) in the human genome, located on chromosome 19q13.4

(Clements et al., 2004; Pampalakis and Sotiropoulou, 2007). Tissue kallikreins possess either trypsin-like (KLK1, KLK2, KLK4, KLK5, KLK6, KLK8, KLK10-15) or chymotrypsin-like (KLK3, KLK7, KLK9) activity and are widely involved in ECM degradation (Pampalakis and Sotiropoulou, 2007). KLKs are expressed in a wide range of tissues and many tissues co-express multiple KLKs.

Tissue KLKs have been widely investigated as potential cancer biomarkers. KLK3 (also called prostate specific antigen or PSA) is a well known biomarker for prostate cancer screening, diagnosis and recurrence monitoring (Grubb and Kibel, 2007). Similarly, in early stage ovarian cancer patients, pre-surgery serum KLK6

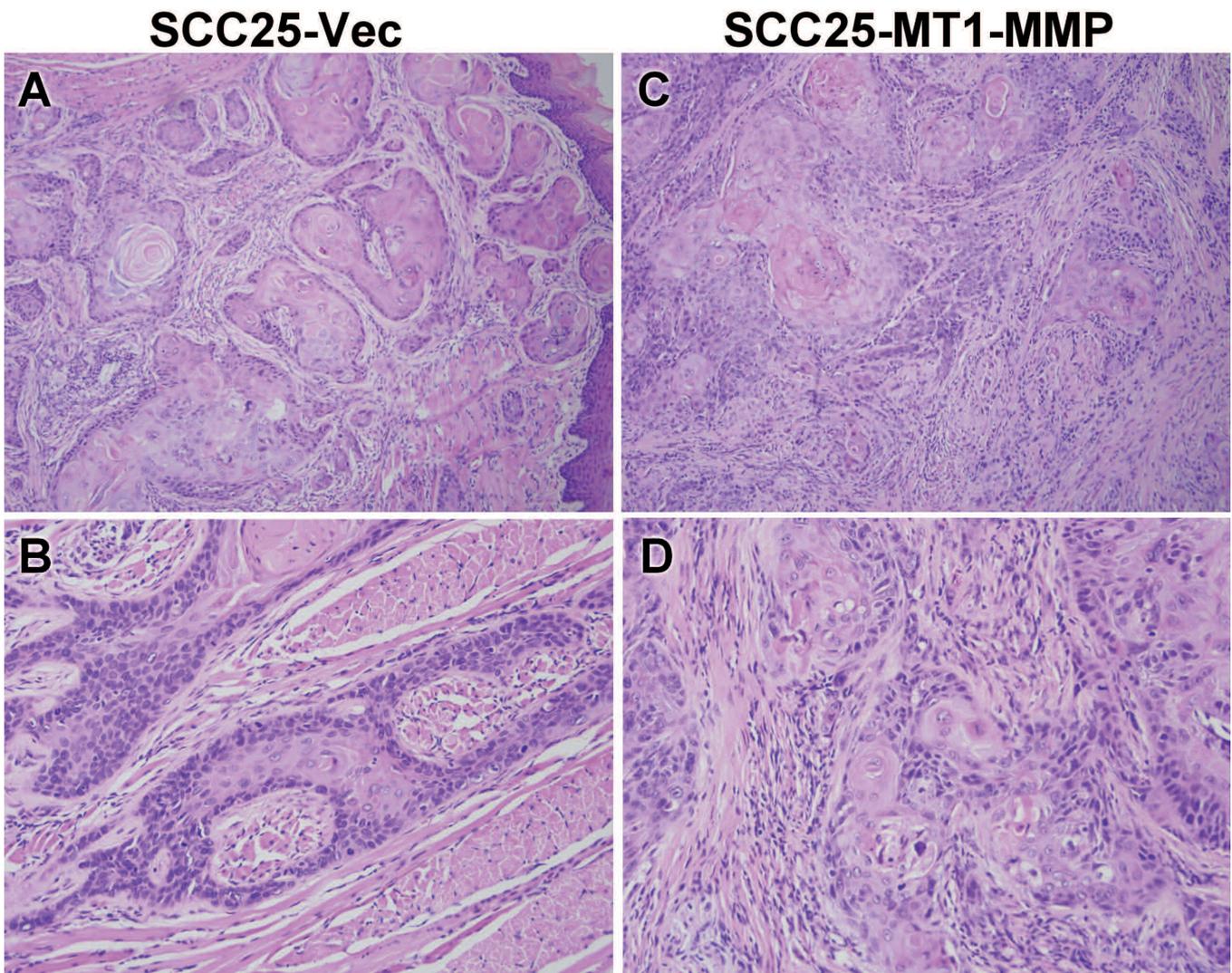


Fig. 5. Overexpression of MT1-MMP alters progression of murine OSCC. SCC25 cells expressing empty vector (**A, B** - SCC25-vec) or MT1-MMP (**C, D** - SCC25-MT1-MMP) were injected into the murine tongue and H&E sections of resulting tumors were analyzed. Tumors formed from SCC25-vec cells exhibited circumscribed nests of well-differentiated SCC. Tumors formed from SCC25-MT1-MMP were moderately differentiated SCC and cells exhibited sheets of tumor cells with an infiltrative pattern of growth. Tumor infiltration of underlying muscle and increased mitotic figures were also evident. A, C, x 200; B, D, x 400

and KLK10 detection increases the sensitivity of CA125 in diagnosis (Borgono et al., 2004). The role of KLK expression as a potential biomarker for OSCC has not been examined. Despite substantial advances in the application of KLK family members as cancer biomarkers, their specific role in cancer pathogenesis and progression remains largely unknown (Emami and Diamandis, 2008). However, many kallikreins are directly or indirectly involved in the degradation of ECM proteins, and therefore likely facilitate tumor invasion and metastasis (Borgono et al., 2004). In OSCC, expression of KLKs has not been extensively evaluated. However, a recent cDNA microarray analysis of highly aggressive OSCC cells that overexpress uPAR identified enhanced expression of four human tissue

kallikreins, KLK 5, 7, 8, and 10 (Ghosh et al., 2010; Pettus et al., 2009). This was confirmed by immunohistochemical analysis of orthotopic murine tumors as well as human OSCC tissues (Fig. 6) (Pettus et al., 2009). The functional significance of KLK expression in OSCC is currently unknown.

ADAMs

Metalloproteinases in the a disintegrin and metalloproteinase (ADAM) and structurally related ADAM-with thrombospondin motifs (ADAMTS) family are membrane associated proteins consisting of a prodomain, a metalloproteinase domain, a disintegrin domain, a cysteine-rich domain, an EGF-like domain, a

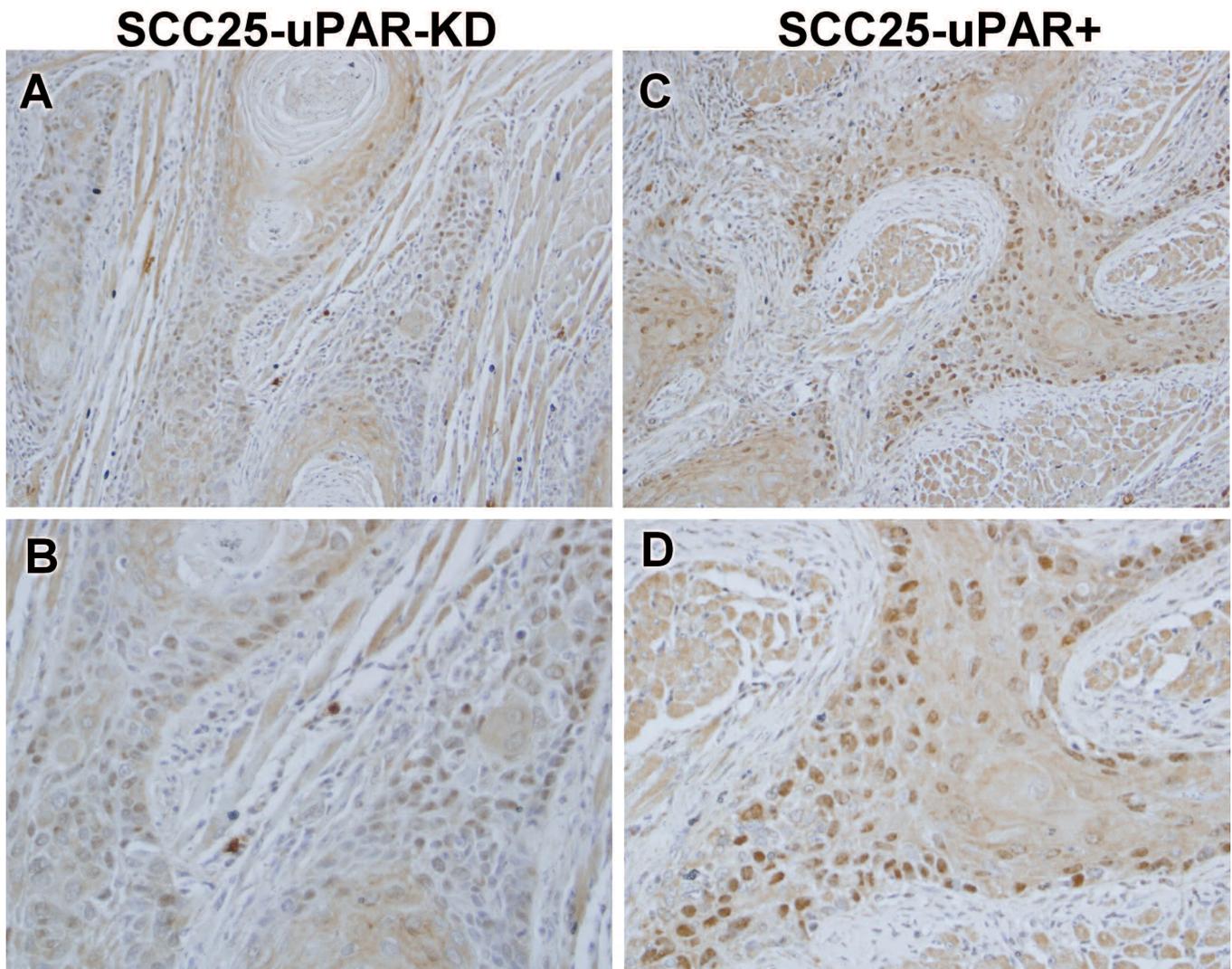


Fig. 6. Example of enhanced KLK staining in orthotopic murine oral tongue tumors. Tumors generated from SCC25 cells in which expression of uPAR was down-regulated with siRNA (A, B – SCC25-uPAR-KD) or was upregulated (C, D – SCC25-uPAR+) were immunostained with antibodies against KLK5 as described in (Pettus et al., 2009). Enhanced expression of KLK5 is evident in tumors formed from SCC25-uPAR+ cells (brown stain). A, C, x 200; B, D, x 400

transmembrane domain, and a cytoplasmic tail (Seals and Courtneidge, 2003; Rocks, Paulissen et al., 2008). ADAMTSs are largely secreted and have a characteristic thrombospondin type I motifs, but no EGF-like, transmembrane, and cytoplasmic domains (Rocks et al., 2008). There are 13 ADAMs and 19 ADAMTSs that have been identified in human (Seals and Courtneidge, 2003). ADAMs often function as sheddases, catalyzing ectodomain cleavage of a wide variety of adhesion molecules, growth factors and cytokines. For example, processing of cell surface growth factors such as TGF- α and heparin-binding (HB)-EGF can lead to enhanced cell proliferation (Rocks, Estrella et al., 2008; Van Schaeybroeck et al., 2008). These proteases also control cell adhesion, migration, and proliferation by acting on various membrane-associated and extracellular substrates (Turner et al., 2009). Some ADAMTSs process proteoglycans in the ECM, thereby modulating the tumor microenvironment (Turner et al., 2009).

Aberrant regulation of ADAMs and ADAMTSs has been reported in many cancer types, and both positive and negative regulation of progression have been described (Turner et al., 2009). Expression in OSCC has not been extensively evaluated. ADAM10 expression is found elevated in OSCC relative to matched non-malignant tissue (Ko et al., 2007). An antisense oligonucleotide against ADAM10 reduced growth in an OSCC cell line, suggesting that ADAM10 expression may regulate tumor cell proliferation (Ko et al., 2007). A cDNA microarray study found ADAM-12 upregulation in OSCC and it was further confirmed by real-time polymerase chain reaction and immunohistochemistry on fresh-frozen tissues (Kornberg et al., 2005). ADAM-17 expression correlates significantly with lymph node metastasis and recurrence in OSCC. In addition, paired immunohistochemical and western blot analyses provide evidence that ADAM17 may participate in CD44 ectodomain shedding in OSCC (Takamune et al., 2007, 2008). Furthermore, ADAM-17 siRNA strongly inhibited TNF- α induced OSCC invasion (Takamune et al., 2008).

Cathepsins

Cathepsins are largely intracellular lysosomal proteases, but several cathepsins have extracellular proteolytic activities. Most cathepsins are cysteine proteinases, however several members of other mechanistic classes have also been identified including serine and aspartic proteinases (Kuester et al., 2008). The activity of cathepsins is regulated by proteinaceous inhibitors designated cystatins, as well as some serpins. Both intracellular and extracellular substrates have been identified.

Immunohistochemical analysis of oral cancer specimens shows a strong causal relationship between the expression levels of cathepsin B and D and tumor invasiveness and metastasis (Vigneswaran et al., 2000; Kawasaki et al., 2002). In a follow-up study, the

potential contribution of cathepsin B to cellular invasiveness was evaluated in OSCC cells *in vitro* (Wickramasinghe et al., 2005). Downregulation of cathepsin B diminishes the invasiveness and motility of this cell line, suggesting cathepsin B as a potential therapeutic target (Wickramasinghe et al., 2005). Cathepsin L expression is found in dysplastic lesions as well as in OSCC and may be used to identify those dysplasias that may progress (Macabeo-Ong et al., 2003). An additional study has identified cathepsin O as a potential marker of OSCC metastasis using cDNA microarray analysis of tongue tumors and normal tongue tissues (Carinci et al., 2005). Several studies have linked cathepsins with apoptosis in OSCC. By blocking the activity of cathepsin B, cystatin M was shown to rescue tumor cells from TNF-induced apoptosis (Vigneswaran et al., 2003). Cathepsin inhibition also blocks shedding of the Fas death receptor (Johansson et al., 2006). Subsequent studies showed that TRAIL-induced cell death is mediated through cathepsin B, suggesting a complex role of cathepsins in tumor progression vs tumor suppression (Nagaraj et al., 2006).

Conclusion

In recent years, increasing attention has been paid to the role of the tumor microenvironment as an active component in regulation of tumor progression and metastasis. Deposition and degradation of the ECM modulate the mechanical properties of a tumor and the surrounding stroma and thereby control gene expression. Proteolysis can also release matrix-bound growth factors and alter the function of adhesion molecules, thereby contributing to changes in tissue cohesion. Further detailed clinical and mechanistic analyses of the precise functional interplay between matrix macromolecules, adhesive proteins, proteolytic enzymes and their inhibitors in oral tumor progression has the potential to uncover new diagnostic and/or prognostic indicators as well as promising new molecular targets for novel patient-directed therapeutics.

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References

- Adams D. (1976). Keratinization of the oral epithelium. *Ann. R. Coll. Surg. Engl.* 58, 351-358.
- Aguirre Ghiso J.A., Kovalski K. and Ossowski L. (1999). Tumor dormancy induced by downregulation of urokinase receptor in human carcinoma involves integrin and mapk signaling. *J. Cell Biol.* 147, 89-104.
- Bacchiocchi R., Rubini C., Pierpaoli E., Borghetti G., Procacci P., Nocini P.F., Santarelli A., Rocchetti R., Ciavarella D., Lo Muzio L. and Fazioli F. (2008). Prognostic value analysis of urokinase-type plasminogen activator receptor in oral squamous cell carcinoma: An immunohistochemical study. *BMC Cancer* 8, 220.

- Baker E.A., Leaper D.J., Hayter J.P. and Dickenson A.J. (2006). The matrix metalloproteinase system in oral squamous cell carcinoma. *Br. J. Oral Maxillofac. Surg.* 44, 482-486.
- Baker E.A., Leaper D.J., Hayter J.P. and Dickenson A.J. (2007). Plasminogen activator system in oral squamous cell carcinoma. *Br. J. Oral Maxillofac. Surg.* 45, 623-627.
- Barlow Y. and Southam J.C. (1992). Plasminogen activators in normal and malignant oral epithelium *in vivo* and *in vitro*. *Arch. Oral Biol.* 37, 749-756.
- Bijian K., Mlynarek A.M., Bally R.L., Jie S., Xu Y., Hier M.P., Black M.J., Di Falco M.R., LaBoissiere S. and Alaoui-Jamali M.A. (2009). Serum proteomic approach for the identification of serum biomarkers contributed by oral squamous cell carcinoma and host tissue microenvironment. *J. Proteome Res.* 8, 2173-2185.
- Bilde A., von Buchwald C., Dabelsteen E., Therkildsen M.H. and Dabelsteen S. (2009). Molecular markers in the surgical margin of oral carcinomas. *J. Oral Pathol. Med.* 38, 72-78.
- Binder B.R., Mihaly J. and Prager G.W. (2007). Upar-upa-pai-1 interactions and signaling: A vascular biologist's view. *Thromb. Haemost.* 97, 336-342.
- Blasi F. and Carmeliet P. (2002). uPAR: A versatile signalling orchestrator. *Nat. Rev. Mol. Cell Biol.* 3, 932-943.
- Borgono C.A., Michael I.P. and Diamandis E.P. (2004). Human tissue kallikreins: Physiologic roles and applications in cancer. *Mol. Cancer Res.* 2, 257-280.
- Bosch F.X., Andl C., Abel U. and Kartenbeck J. (2005). E-cadherin is a selective and strongly dominant prognostic factor in squamous cell carcinoma: A comparison of e-cadherin with desmosomal components. *Int. J. Cancer* 114, 779-790.
- Breuss J.M., Gallo J., DeLisser H.M., Klimanskaya I.V., Folkesson H.G., Pittet J.F., Nishimura S.L., Aldape K., Landers D.V., Carpenter W., Gillet N., Sheppard D., Matthy M.A., Albelda S.M., Kramer R. H. and Pytela R. (1995). Expression of the beta 6 integrin subunit in development, neoplasia and tissue repair suggests a role in epithelial remodeling. *J. Cell Sci.* 108, 2241-2251.
- Califano J., Westra W.H., Meininger G., Corio R., Koch W.M. and Sidransky D. (2000). Genetic progression and clonal relationship of recurrent premalignant head and neck lesions. *Clin. Cancer Res.* 6, 347-352.
- Carinci F., Stabellini G., Calvitti M., Pelucchi S., Targa L., Farina A., Pezzetti F. and Pastore A. (2002). Cd44 as prognostic factor in oral and oropharyngeal squamous cell carcinoma. *J. Craniofac. Surg.* 13, 85-89.
- Carinci F., Lo Muzio L., Piattelli A., Rubini C., Chiesa F., Ionna F., Palmieri A., Maiorano E., Pastore A., Laino G., Dolci M. and Pezzetti F. (2005). Potential markers of tongue tumor progression selected by cDNA microarray. *Int. J. Immunopathol. Pharmacol.* 18, 513-524.
- Chapman H.A. (1997). Plasminogen activators, integrins, and the coordinated regulation of cell adhesion and migration. *Curr. Opin. Cell Biol.* 9, 714-724.
- Charalabopoulos K., Gogali A., Kostoula O.K. and Constantopoulos S.H. (2004). Cadherin superfamily of adhesion molecules in primary lung cancer. *Exp. Oncol.* 26, 256-260.
- Chen C., Mendez E., Houck J., Fan W., Lohavanichbutr P., Doody D., Yueh B., Futran N.D., Upton M., Farwell D.G., Schwartz S.M. and Zhao L.P. (2008). Gene expression profiling identifies genes predictive of oral squamous cell carcinoma. *Cancer Epidemiol. Biomarkers Prev.* 17, 2152-2162.
- Chen Q., Lipkina G., Song Q. and Kramer R.H. (2004). Promoter methylation regulates cadherin switching in squamous cell carcinoma. *Biochem. Biophys. Res. Commun.* 315, 850-856.
- Chiang Y.Y., Tsai M.H., Lin T.Y. and Chiang I.P. (2008). Expression profile of metastasis-related genes in invasive oral cancers. *Histol. Histopathol.* 23, 1213-1222.
- Cho J.H., Kim H.S., Park C.S., Kim J.K., Jung K.Y., Shin B.K. and Kim H.K. (2007). Maspin expression in early oral tongue cancer and its relation to expression of mutant-type p53 and vascular endothelial growth factor (vegf). *Oral Oncol.* 43, 272-277.
- Clements J.A., Willemsen N.M., Myers S.A. and Dong Y. (2004). The tissue kallikrein family of serine proteases: Functional roles in human disease and potential as clinical biomarkers. *Crit. Rev. Clin. Lab. Sci.* 41, 265-312.
- Croucher D.R., Saunders D.N., Lobov S. and Ranson M. (2008). Revisiting the biological roles of pai2 (serpinb2) in cancer. *Nat. Rev. Cancer* 8, 535-545.
- Cruz M.C., Pereira A.L., Lopes F.F., Nonaka C.F., Silva R.R., Freitas Rde A., Souza L.B. and Pinto L.P. (2009). Immunohistochemical expression of e-cadherin and cd44v6 in squamous cell carcinomas of the lower lip and tongue. *Braz. Dent. J.* 20, 64-69.
- Curino A., Patel V., Nielsen B.S., Iskander A.J., Ensley J.F., Yoo G.H., Holsinger F.C., Myers J.N., El-Nagaar A., Kellman R.M., Shillitoe E.J., Molinolo A.A., Gutkind J.S. and Bugge T.H. (2004). Detection of plasminogen activators in oral cancer by laser capture microdissection combined with zymography. *Oral Oncol.* 40, 1026-1032.
- D'Alessio S. and Blasi F. (2009). The urokinase receptor as an entertainer of signal transduction. *Front. Biosci.* 14, 4575-4587.
- Daley W.P., Peters S.B. and Larsen M. (2008). Extracellular matrix dynamics in development and regenerative medicine. *J. Cell Sci.* 121, 255-264.
- Dass K., Ahmad A., Azmi A.S., Sarkar S.H. and Sarkar F.H. (2008). Evolving role of upa/upar system in human cancers. *Cancer Treat. Rev.* 34, 122-136.
- De Arcangelis A. and Georges-Labouesse E. (2000). Integrin and ecm functions: Roles in vertebrate development. *Trends Genet.* 16, 389-395.
- de Bock C.E. and Wang Y. (2004). Clinical significance of urokinase-type plasminogen activator receptor (upar) expression in cancer. *Med. Res. Rev.* 24, 13-39.
- de Vicente J.C., Fresno M.F., Villalain L., Vega J.A. and Hernandez Vallejo G. (2005). Expression and clinical significance of matrix metalloproteinase-2 and matrix metalloproteinase-9 in oral squamous cell carcinoma. *Oral Oncol.* 41, 283-293.
- de Vicente J.C., Lequerica-Fernandez P., Santamaria J. and Fresno M.F. (2007). Expression of mmp-7 and mt1-mmp in oral squamous cell carcinoma as predictive indicator for tumor invasion and prognosis. *J. Oral Pathol. Med.* 36, 415-424.
- Diniz-Freitas M., Garcia-Caballero T., Antunez-Lopez J., Gandara-Rey J.M. and Garcia-Garcia A. (2006). Reduced e-cadherin expression is an indicator of unfavourable prognosis in oral squamous cell carcinoma. *Oral Oncol.* 42, 190-200.
- DiPersio C.M., Hodivala-Dilke K.M., Jaenisch R., Kreidberg J.A. and Hynes R.O. (1997). Alpha3beta1 integrin is required for normal development of the epidermal basement membrane. *J. Cell Biol.* 137, 729-742.
- Emami N. and Diamandis E.P. (2008). Human kallikrein-related peptidase 14 (klk14) is a new activator component of the klk proteolytic cascade. Possible function in seminal plasma and skin. *J.*

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- Biol. Chem. 283, 3031-3041.
- Estilo C.L., O-charoenrat P., Talbot S., Socci N.D., Carlson D.L., Ghossein R., Williams T., Yonekawa Y., Ramanathan Y., Boyle J.O., Kraus D.H., Patel S., Shaha A.R., Wong R.J., Hurn J.M., Shah J.P. and Singh B. (2009). Oral tongue cancer gene expression profiling: Identification of novel potential prognosticators by oligonucleotide microarray analysis. *BMC Cancer* 9, 11.
- Faralli J.A., Schwinn M.K., Gonzalez J.M. Jr, Filla M.S. and Peters D.M. (2009). Functional properties of fibronectin in the trabecular meshwork. *Exp. Eye Res.* 88, 689-693.
- Foschini M.P., Cocchi R., Morandi L., Marucci G., Pennesi M.G., Righi A., Tosi A.L., de Biase D., Pession A. and Montebugnoli L. (2008). E-cadherin loss and delta n731 expression in oral squamous cell carcinomas showing aggressive behavior. *Head Neck* 30, 1475-1482.
- Franzmann E.J., Reategui E.P., Pedrosa F., Pernas F.G., Karakullucu B.M., Carraway K.L., Hamilton K., Singal R. and Goodwin W.J. (2007). Soluble cd44 is a potential marker for the early detection of head and neck cancer. *Cancer Epidemiol. Biomarkers Prev.* 16, 1348-1355.
- Gao Z.B., Duan Y.Q., Zhang L., Chen D.W. and Ding P.T. (2005). Expression of matrix metalloproteinase 2 and its tissue inhibitor in oral squamous cell carcinoma. *Int. J. Mol. Med.* 16, 599-603.
- Garrone R., Lethias C. and Le Guellec D. (1997). Distribution of minor collagens during skin development. *Microsc. Res. Tech.* 38, 407-412.
- Gasparoni A., Della Casa M., Milillo L., Lorenzini G., Rubini C., Urso R. and Lo Muzio L. (2007). Prognostic value of differential expression of laminin-5 gamma2 in oral squamous cell carcinomas: Correlation with survival. *Oncol. Rep.* 18, 793-800.
- Georgiolas A., Batistatou A., Charalabopoulos A., Manolopoulos L. and Charalabopoulos K. (2006). The role of cd44 adhesion molecule in oral cavity cancer. *Exp. Oncol.* 28, 94-98.
- Ghosh S. and Stack M.S. (2000). Proteolytic modification of laminins: Functional consequences. *Microsc. Res. Tech.* 51, 238-246.
- Ghosh S., Brown R., Jones J.C., Ellerbroek S.M. and Stack M.S. (2000). Urinary-type plasminogen activator (upa) expression and upa receptor localization are regulated by alpha 3beta 1 integrin in oral keratinocytes. *J. Biol. Chem.* 275, 23869-23876.
- Ghosh S., Johnson J.J., Sen R., Mukhopadhyay S., Liu Y., Zhang F., Wei Y., Chapman H.A. and Stack M.S. (2006). Functional relevance of urinary-type plasminogen activator receptor-alpha3beta1 integrin association in proteinase regulatory pathways. *J. Biol. Chem.* 281, 13021-13029.
- Ghosh S., Koblinski J., Johnson J.J., Liu Y., Ericsson A., Davis J.W., Shi Z., Ravosa M., Crawford S., Frazier S. and Stack M.S. (2010). Urinary type plasminogen activator receptor alpha3 beta1 integrin signaling altered gene expression and oral tumor progression. *Mol. Cancer Res.* 8, 145-158.
- Giannelli G., Milillo L., Marinosci F., Lo Muzio L., Serpico R. and Antonaci S. (2001). Altered expression of integrins and basement membrane proteins in malignant and pre-malignant lesions of oral mucosa. *J. Biol. Regul. Homeost. Agents* 15, 375-380.
- Goldfinger L.E., Stack M.S. and Jones J.C. (1998). Processing of laminin-5 and its functional consequences: Role of plasmin and tissue-type plasminogen activator. *J. Cell Biol.* 141, 255-265.
- Goldfinger L.E., Jiang L., Hopkinson S.B., Stack M.S. and Jones J.C. (2000). Spatial regulation and activity modulation of plasmin by high affinity binding to the g domain of the alpha 3 subunit of laminin-5. *J. Biol. Chem.* 275, 34887-34893.
- Gonzalez-Moles M.A., Gil-Montoya J.A., Ruiz-Avila I., Esteban F., Delgado-Rodriguez M. and Bascones-Martinez A. (2007). Prognostic significance of p21waf1/cip1, p16ink4a and cd44s in tongue cancer. *Oncol. Rep.* 18, 389-396.
- Grubb R.L. 3rd and Kibel A.S. (2007). Prostate cancer: Screening, diagnosis and management in 2007. *Mo. Med.* 104, 408-413; quiz 413-404.
- Gunthert U. (1993). Cd44: A multitude of isoforms with diverse functions. *Curr. Top. Microbiol. Immunol.* 184, 47-63.
- Harada T., Shinohara M., Nakamura S. and Oka M. (1994). An immunohistochemical study of the extracellular matrix in oral squamous cell carcinoma and its association with invasive and metastatic potential. *Virchows Arch.* 424, 257-266.
- Hudson D.L., Speight P.M. and Watt F.M. (1996). Altered expression of cd44 isoforms in squamous-cell carcinomas and cell lines derived from them. *Int. J. Cancer* 66, 457-463.
- Hundsdoerfer B., Zeilhofer H.F., Bock K.P., Dettmar P., Schmitt M., Kolk A., Pautke C. and Horch H.H. (2005). Tumour-associated urokinase-type plasminogen activator (upa) and its inhibitor pai-1 in normal and neoplastic tissues of patients with squamous cell cancer of the oral cavity - clinical relevance and prognostic value. *J. Craniomaxillofac. Surg.* 33, 191-196.
- Hung K.F., Chang C.S., Liu C.J., Lui M.T., Cheng C.Y. and Kao S.Y. (2006). Differential expression of e-cadherin in metastatic lesions comparing to primary oral squamous cell carcinoma. *J. Oral Pathol. Med.* 35, 589-594.
- Iezzi G., Piattelli A., Rubini C., Goteri G., Artese L., Perrotti V. and Carinci F. (2007). Masp expression in oral squamous cell carcinoma. *J. Craniofac. Surg.* 18, 1039-1043.
- Jemal A., Siegel R., Ward E., Hao Y., Xu J., Murray T. and Thun M.J. (2008). Cancer statistics, 2008. *CA Cancer J. Clin.* 58, 71-96.
- Johansson A.C., Norberg-Spaak L. and Roberg K. (2006). Role of lysosomal cathepsins in naphthazarin- and fas-induced apoptosis in oral squamous cell carcinoma cells. *Acta. Otolaryngol.* 126, 70-81.
- Johansson S., Svineng G., Wennerberg K., Armulik A. and Lohikangas L. (1997). Fibronectin-integrin interactions. *Front. Biosci.* 2, d126-146.
- Jones J., Watt F.M. and Speight P.M. (1997). Changes in the expression of alpha v integrins in oral squamous cell carcinomas. *J. Oral Pathol. Med.* 26, 63-68.
- Kainulainen T., Hakkinen L., Hamidi S., Larjava K., Kallioinen M., Peltonen J., Salo T., Larjava H. and Oikarinen A. (1998). Laminin-5 expression is independent of the injury and the microenvironment during reepithelialization of wounds. *J. Histochem. Cytochem.* 46, 353-360.
- Kandikonda S., Oda D., Niederman R. and Sorkin B.C. (1996). Cadherin-mediated adhesion is required for normal growth regulation of human gingival epithelial cells. *Cell Adhes. Commun.* 4, 13-24.
- Katayama A., Bandoh N., Kishibe K., Takahara M., Ogino T., Nonaka S. and Harabuchi Y. (2004). Expressions of matrix metalloproteinases in early-stage oral squamous cell carcinoma as predictive indicators for tumor metastases and prognosis. *Clin. Cancer Res.* 10, 634-640.
- Katoh K., Nakanishi Y., Akimoto S., Yoshimura K., Takagi M., Sakamoto M. and Hirohashi S. (2002). Correlation between laminin-5 gamma2 chain expression and epidermal growth factor receptor expression and its clinicopathological significance in squamous cell carcinoma of the tongue. *Oncology* 62, 318-326.

- Kawano K. and Yanagisawa S. (2006). Predictive value of laminin-5 and membrane type 1-matrix metalloproteinase expression for cervical lymph node metastasis in t1 and t2 squamous cell carcinomas of the tongue and floor of the mouth. *Head Neck* 28, 525-533.
- Kawasaki G., Kato Y. and Mizuno A. (2002). Cathepsin expression in oral squamous cell carcinoma: Relationship with clinicopathologic factors. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 93, 446-454.
- Khalkhali-Ellis Z. (2006). Maspin: The new frontier. *Clin Cancer Res* 12, 7279-7283.
- Kim S.H., Cho N.H., Kim K., Lee J.S., Koo B.S., Kim J.H., Chang J.H. and Choi E.C. (2006). Correlations of oral tongue cancer invasion with matrix metalloproteinases (mmps) and vascular endothelial growth factor (vegf) expression. *J. Surg. Oncol.* 93, 330-337.
- Ko S.Y., Lin S.C., Wong Y.K., Liu C.J., Chang K.W. and Liu T.Y. (2007). Increase of disintegrin metalloprotease 10 (adam10) expression in oral squamous cell carcinoma. *Cancer Lett.* 245, 33-43.
- Kornberg L.J., Villaret D., Popp M., Lui L., McLaren R., Brown H., Cohen D., Yun J. and McFadden M. (2005). Gene expression profiling in squamous cell carcinoma of the oral cavity shows abnormalities in several signaling pathways. *Laryngoscope* 115, 690-698.
- Kuester D., Lippert H., Roessner A. and Krueger S. (2008). The cathepsin family and their role in colorectal cancer. *Pathol. Res. Pract.* 204, 491-500.
- Kulasekara K.K., Lukandu O.M., Neppelberg E., Vintermyr O.K., Johannessen A.C. and Costea D.E. (2009). Cancer progression is associated with increased expression of basement membrane proteins in three-dimensional in vitro models of human oral cancer. *Arch. Oral Biol.* 54, 924-931.
- Kuratomi Y., Kumamoto M., Kidera K., Toh S., Masuda M., Nakashima T. and Inokuchi A. (2006). Diffuse expression of laminin gamma2 chain in disseminating and infiltrating cancer cells indicates a highly malignant state in advanced tongue cancer. *Oral Oncol.* 42, 73-76.
- Kurokawa A., Nagata M., Kitamura N., Noman A.A., Ohnishi M., Ohyama T., Kobayashi T., Shingaki S. and Takagi R. (2008). Diagnostic value of integrin alpha3, beta4, and beta5 gene expression levels for the clinical outcome of tongue squamous cell carcinoma. *Cancer* 112, 1272-1281.
- Lewis J.E., Jensen P.J. and Wheelock M.J. (1994). Cadherin function is required for human keratinocytes to assemble desmosomes and stratify in response to calcium. *J. Invest. Dermatol.* 102, 870-877.
- Li S., Jiao J., Lu Z. and Zhang M. (2009). An essential role for n-cadherin and beta-catenin for progression in tongue squamous cell carcinoma and their effect on invasion and metastasis of tca8113 tongue cancer cells. *Oncol. Rep.* 21, 1223-1233.
- Lillis A.P., Van Duyn L.B., Murphy-Ullrich J.E. and Strickland D.K. (2008). LDL receptor-related protein 1: Unique tissue-specific functions revealed by selective gene knockout studies. *Physiol. Rev.* 88, 887-918.
- Lindberg P., Larsson A. and Nielsen B.S. (2006). Expression of plasminogen activator inhibitor-1, urokinase receptor and laminin gamma-2 chain is an early coordinated event in incipient oral squamous cell carcinoma. *Int. J. Cancer* 118, 2948-2956.
- Luukka M., Vihinen P., Kronqvist P., Vahlberg T., Pyrhonen S., Kahari V.M. and Grenman R. (2006). Association between high collagenase-3 expression levels and poor prognosis in patients with head and neck cancer. *Head Neck* 28, 225-234.
- Lyons A.J. and Cui N. (2000). Salivary oncofetal fibronectin and oral squamous cell carcinoma. *J. Oral Pathol. Med.* 29, 267-270.
- Lyons A.J. and Jones J. (2007). Cell adhesion molecules, the extracellular matrix and oral squamous carcinoma. *Int. J. Oral Maxillofac. Surg.* 36, 671-679.
- Lyons A.J., Bateman A.C., Spedding A., Primrose J.N. and Mandel U. (2001). Oncofetal fibronectin and oral squamous cell carcinoma. *Br. J. Oral Maxillofac. Surg.* 39, 471-477.
- Macabeo-Ong M., Shiboski C.H., Silverman S., Ginzinger D.G., Dekker N., Wong D.T. and Jordan R.C. (2003). Quantitative analysis of cathepsin I mrna and protein expression during oral cancer progression. *Oral Oncol.* 39, 638-647.
- Mack B. and Gires O. (2008). Cd44s and cd44v6 expression in head and neck epithelia. *PLoS ONE* 3, e3360.
- Mackay C.R., Terpe H.J., Stauder R., Marston W.L., Stark H. and Gunthert U. (1994). Expression and modulation of cd44 variant isoforms in humans. *J. Cell Biol.* 124, 71-82.
- Mahomed F., Altini M. and Meer S. (2007). Altered e-cadherin/beta-catenin expression in oral squamous carcinoma with and without nodal metastasis. *Oral Dis.* 13, 386-392.
- Marioni G., Gaio E., Giacomelli L., Bertolin A., D'Alessandro E., Stramare R., Facco E., Staffieri A. and Blandamura S. (2008). Maspin subcellular localization and expression in oral cavity squamous cell carcinoma. *Eur. Arch. Otorhinolaryngol.* 265 (Suppl 1), S97-104.
- Mattijssen V., Peters H.M., Schalkwijk L., Manni J.J., van 't Hof-Grootenboer B., de Mulder P.H. and Ruiter D.J. (1993). E-cadherin expression in head and neck squamous-cell carcinoma is associated with clinical outcome. *Int. J. Cancer* 55, 580-585.
- Mendez E., Houck J.R., Doody D.R., Fan W., Lohavanichbutr P., Rue T.C., Yueh B., Futran N.D., Upton M.P., Farwell D.G., Heagerty P.J., Zhao L.P., Schwartz S.M. and Chen C. (2009). A genetic expression profile associated with oral cancer identifies a group of patients at high risk of poor survival. *Clin. Cancer Res.* 15, 1353-1361.
- Muller E.J., Williamson L., Kolly C. and Suter M.M. (2008). Outside-in signaling through integrins and cadherins: A central mechanism to control epidermal growth and differentiation? *J. Invest. Dermatol.* 128, 501-516.
- Munshi H.G., Ghosh S., Mukhopadhyay S., Wu Y.I., Sen R., Green K.J. and Stack M.S. (2002). Proteinase suppression by e-cadherin-mediated cell-cell attachment in premalignant oral keratinocytes. *J. Biol. Chem.* 277, 38159-38167.
- Nagaraj N.S., Vigneswaran N. and Zacharias W. (2006). Cathepsin b mediates trail-induced apoptosis in oral cancer cells. *J. Cancer Res. Clin. Oncol.* 132, 171-183.
- Nguyen D.H., Catling A.D., Webb D.J., Sankovic M., Walker L.A., Somlyo A.V., Weber M.J. and Gonias S.L. (1999). Myosin light chain kinase functions downstream of ras/erk to promote migration of urokinase-type plasminogen activator-stimulated cells in an integrin-selective manner. *J. Cell Biol.* 146, 149-164.
- Nguyen D.H., Webb D.J., Catling A.D., Song Q., Dhakephalkar A., Weber M.J., Ravichandran K.S. and Gonias S.L. (2000). Urokinase-type plasminogen activator stimulates the ras/extracellular signal-regulated kinase (erk) signaling pathway and mcf-7 cell migration by a mechanism that requires focal adhesion kinase, src, and shc. Rapid dissociation of grb2/sps-shc complex is associated with the transient phosphorylation of erk in urokinase-treated cells. *J. Biol. Chem.* 275, 19382-19388.
- Nozaki S., Endo Y., Kawashiri S., Nakagawa K., Yamamoto E., Yonemura Y. and Sasaki T. (1998). Immunohistochemical

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- localization of a urokinase-type plasminogen activator system in squamous cell carcinoma of the oral cavity: Association with mode of invasion and lymph node metastasis. *Oral Oncol.* 34, 58-62.
- Ohara T., Kawashiri S., Tanaka A., Noguchi N., Kitahara H., Okamune A., Kato K., Hase T., Nakaya H. and Yoshizawa K. (2009). Integrin expression levels correlate with invasion, metastasis and prognosis of oral squamous cell carcinoma. *Pathol. Oncol. Res.* 15, 429-436.
- Oliveira D.T. and Odell E.W. (1997). Expression of cd44 variant exons by normal oral epithelia. *Oral Oncol.* 33, 260-262.
- Oliveira D.T., Sherriff M. and Odell E.W. (1998). Expression of cd44 variant exons by primary and metastatic oral squamous carcinomas. *J. Oral. Pathol. Med.* 27, 303-307.
- Ossowski L. and Aguirre-Ghiso J.A. (2000). Urokinase receptor and integrin partnership: Coordination of signaling for cell adhesion, migration and growth. *Curr. Opin. Cell Biol.* 12, 613-620.
- Overall C.M. and Kleinfeld O. (2006). Tumour microenvironment - opinion: Validating matrix metalloproteinases as drug targets and anti-targets for cancer therapy. *Nat. Rev. Cancer* 6, 227-239.
- Pampalakis G. and Sotiropoulos G. (2007). Tissue kallikrein proteolytic cascade pathways in normal physiology and cancer. *Biochim. Biophys. Acta* 1776, 22-31.
- Parikka M., Kainulainen T., Tasanen K., Vaananen A., Bruckner-Tuderman L. and Salo T. (2003). Alterations of collagen xvii expression during transformation of oral epithelium to dysplasia and carcinoma. *J. Histochem. Cytochem.* 51, 921-929.
- Parkin D.M., Bray F., Ferlay J. and Pisani P. (2005). Global cancer statistics, 2002. *CA Cancer J. Clin.* 55, 74-108.
- Patel B.P., Shah S.V., Shukla S.N., Shah P.M. and Patel P.S. (2007). Clinical significance of mmp-2 and mmp-9 in patients with oral cancer. *Head Neck* 29, 564-572.
- Pettus J.R., Johnson J.J., Shi Z., Davis J.W. and Stack M.S. (2009). Multiple kallikrein (klk5, 7, 8 and 10) expression in squamous cell carcinoma of the oral cavity. *Histol. Histopathol.* 24, 197-207.
- Polette M., Clavel C., Muller D., Abecassis J., Binnering I. and Birembaut P. (1991). Detection of mrnas encoding collagenase i and stromelysin 2 in carcinomas of the head and neck by *in situ* hybridization. *Invasion Metastasis* 11, 76-83.
- Ponta H., Sherman L. and Herrlich P.A. (2003). Cd44: From adhesion molecules to signalling regulators. *Nat. Rev. Mol. Cell Biol.* 4, 33-45.
- Pyo S.W., Hashimoto M., Kim Y.S., Kim C.H., Lee S.H., Johnson K.R., Wheelock M.J. and Park J.U. (2007). Expression of e-cadherin, p-cadherin and n-cadherin in oral squamous cell carcinoma: Correlation with the clinicopathologic features and patient outcome. *J. Craniomaxillofac. Surg.* 35, 1-9.
- Ramos D.M., But M., Regezi J., Schmidt B.L., Atakilil A., Dang D., Ellis D., Jordan R. and Li X. (2002). Expression of integrin beta 6 enhances invasive behavior in oral squamous cell carcinoma. *Matrix Biol.* 21, 297-307.
- Rocks N., Paulissen G., El Hour M., Quesada F., Crahay C., Gueders M., Foidart J.M., Noel A. and Cataldo D. (2008). Emerging roles of adam and adamts metalloproteinases in cancer. *Biochimie* 90, 369-379.
- Rocks N., Estrella C., Paulissen G., Quesada-Calvo F., Gilles C., Gueders M.M., Crahay C., Foidart J.M., Gosset P., Noel A. and Cataldo D.D. (2008). The metalloproteinase adam-12 regulates bronchial epithelial cell proliferation and apoptosis. *Cell Prolif.* 41, 988-1001.
- Ross M.H. and Pawlina W. (2005). *Histology: A text and atlas.* 5th ed. Philadelphia. Lippincott Williams & Wilkins.
- Santos-Garcia A., Abad-Hernandez M.M., Fonseca-Sanchez E., Julian-Gonzalez R., Galindo-Villardón P., Cruz-Hernandez J.J. and Bullón-Sopelana A. (2006). E-cadherin, laminin and collagen iv expression in the evolution from dysplasia to oral squamous cell carcinoma. *Med. Oral Patol. Oral Cir. Bucal.* 11, E100-105.
- Scheele S., Nystrom A., Durbeek J., Talts J.F., Ekblom M. and Ekblom P. (2007). Laminin isoforms in development and disease. *J. Mol. Med.* 85, 825-836.
- Schipper J.H., Unger A. and Jahnke K. (1994). E-cadherin as a functional marker of the differentiation and invasiveness of squamous cell carcinoma of the head and neck. *Clin. Otolaryngol. Allied Sci.* 19, 381-384.
- Schipper J.H., Frixen U.H., Behrens J., Unger A., Jahnke K. and Birchmeier W. (1991). E-cadherin expression in squamous cell carcinomas of head and neck: Inverse correlation with tumor dedifferentiation and lymph node metastasis. *Cancer Res.* 51, 6328-6337.
- Schmalbach C.E., Chepeha D.B., Giordano T.J., Rubin M.A., Teknos T.N., Bradford C.R., Wolf G.T., Kuick R., Mizek D.E., Trask D.K. and Hanash S. (2004). Molecular profiling and the identification of genes associated with metastatic oral cavity/pharynx squamous cell carcinoma. *Arch Otolaryngol. Head Neck Surg.* 130, 295-302.
- Screaton G.R., Bell M.V., Bell J.I. and Jackson D.G. (1993). The identification of a new alternative exon with highly restricted tissue expression in transcripts encoding the mouse pgp-1 (cd44) homing receptor. Comparison of all 10 variable exons between mouse, human, and rat. *J. Biol. Chem.* 268, 12235-12238.
- Seals D.F. and Courtneidge S.A. (2003). The adams family of metalloproteases: Multidomain proteins with multiple functions. *Genes Dev.* 17, 7-30.
- Seiffert D. (1997). Constitutive and regulated expression of vitronectin. *Histol. Histopathol.* 12, 787-797.
- Shi Z. and Stack M.S. (2007). Urinary-type plasminogen activator (upa) and its receptor (upar) in squamous cell carcinoma of the oral cavity. *Biochem. J.* 407, 153-159.
- Shi Z., Liu Y., Johnson J.J. and Stack M.S. (2010). Urinary type plasminogen activator receptor modulates cellular invasive behavior and integrin function in oral cancer cells via alteration in focal adhesion proteins. (in press).
- Sneath R.J. and Mangham D.C. (1998). The normal structure and function of cd44 and its role in neoplasia. *Mol. Pathol.* 51, 191-200.
- Sok J.C., Kuriakose M.A., Mahajan V.B., Pearlman A.N., DeLacure M.D. and Chen F.A. (2003). Tissue-specific gene expression of head and neck squamous cell carcinoma *in vivo* by complementary DNA microarray analysis. *Arch. Otolaryngol. Head Neck Surg.* 129, 760-770.
- Sorsa T., Tjaderhane L. and Salo T. (2004). Matrix metalloproteinases (mmps) in oral diseases. *Oral Dis.* 10, 311-318.
- Stenback F., Makinen M.J., Jussila T., Kauppila S., Risteli J., Talve L. and Risteli L. (1999). The extracellular matrix in skin tumor development-a morphological study. *J. Cutan. Pathol.* 26, 327-338.
- Stetler-Stevenson W.G. (2008). Tissue inhibitors of metalloproteinases in cell signaling: Metalloproteinase-independent biological activities. *Sci. Signal.* 1, re6.
- Takamune Y., Ikebe T., Nagano O. and Shinohara M. (2008). Involvement of nf-kappab-mediated maturation of adam-17 in the invasion of oral squamous cell carcinoma. *Biochem. Biophys. Res. Commun.* 365, 393-398.
- Takamune Y., Ikebe T., Nagano O., Nakayama H., Ota K., Obayashi T.,

- Saya H. and Shinohara M. (2007). Adam-17 associated with cd44 cleavage and metastasis in oral squamous cell carcinoma. *Virchows Arch.* 450, 169-177.
- Takeichi M. (1993). Cadherins in cancer: Implications for invasion and metastasis. *Curr. Opin. Cell Biol.* 5, 806-811.
- Thomas G.J. and Speight P.M. (2001). Cell adhesion molecules and oral cancer. *Crit. Rev. Oral Biol. Med.* 12, 479-498.
- Thomas G.J., Nystrom M.L. and Marshall J.F. (2006). Alphavbeta6 integrin in wound healing and cancer of the oral cavity. *J. Oral Pathol. Med.* 35, 1-10.
- Thomas G.T., Lewis M.P. and Speight P.M. (1999). Matrix metalloproteinases and oral cancer. *Oral Oncol.* 35, 227-233.
- Thorup A.K., Dabelsteen E., Schou S., Gil S.G., Carter W.G. and Reibel J. (1997). Differential expression of integrins and laminin-5 in normal oral epithelia. *APMIS* 105, 519-530.
- Tran K.T., Lamb P. and Deng J.S. (2005). Matrikines and matricryptins: Implications for cutaneous cancers and skin repair. *J. Dermatol. Sci.* 40, 11-20.
- Turner S.L., Blair-Zajdel M.E. and Bunning R.A. (2009). Adams and adamss in cancer. *Br. J. Biomed. Sci.* 66, 117-128.
- Underhill C. (1992). Cd44: The hyaluronan receptor. *J. Cell Sci.* 103 (Pt 2), 293-298.
- Vairaktaris E., Yapijakis C., Serefoglou Z., Vylliotis A., Ries J., Nkenke E., Wiltfang J., Derka S., Vassiliou S., Springer I., Kessler P. and Neukam F.W. (2006). Plasminogen activator inhibitor-1 polymorphism is associated with increased risk for oral cancer. *Oral Oncol.* 42, 888-892.
- Vairaktaris E., Serefoglou Z., Yapijakis C., Vylliotis A., Nkenke E., Derka S., Vassiliou S., Avgoustidis D., Neukam F.W. and Patsouris E. (2007). High gene expression of matrix metalloproteinase-7 is associated with early stages of oral cancer. *Anticancer Res.* 27, 2493-2498.
- Van Schaeybroeck S., Kelly D.M., Kyula J., Stokesberry S., Fennell D.A., Johnston P.G. and Longley D.B. (2008). Src and adam-17-mediated shedding of transforming growth factor-alpha is a mechanism of acute resistance to trail. *Cancer Res.* 68, 8312-8321.
- Vered M., Allon I. and Dayan D. (2009). Maspin, p53, p63, and ki-67 in epithelial lesions of the tongue: From hyperplasia through dysplasia to carcinoma. *J. Oral Pathol. Med.* 38, 314-320.
- Verstappen J. and Von den Hoff J.W. (2006). Tissue inhibitors of metalloproteinases (timp)s: Their biological functions and involvement in oral disease. *J. Dent. Res.* 85, 1074-1084.
- Vigneswaran N., Zhao W., Dassanayake A., Muller S., Miller D.M. and Zacharias W. (2000). Variable expression of cathepsin b and d correlates with highly invasive and metastatic phenotype of oral cancer. *Hum. Pathol.* 31, 931-937.
- Vigneswaran N., Wu J. and Zacharias W. (2003). Upregulation of cystatin m during the progression of oropharyngeal squamous cell carcinoma from primary tumor to metastasis. *Oral Oncol.* 39, 559-568.
- Virtanen I., Korhonen M., Kariniemi A.L., Gould V.E., Laitinen L. and Ylanne J. (1990). Integrins in human cells and tumors. *Cell Differ. Dev.* 32, 215-227.
- Visse R. and Nagase H. (2003). Matrix metalloproteinases and tissue inhibitors of metalloproteinases: Structure, function, and biochemistry. *Circ. Res.* 92, 827-839.
- Waltz D.A., Natkin L.R., Fujita R.M., Wei Y. and Chapman H.A. (1997). Plasmin and plasminogen activator inhibitor type 1 promote cellular motility by regulating the interaction between the urokinase receptor and vitronectin. *J. Clin. Invest.* 100, 58-67.
- Wang X., Zhang J., Fan M., Zhou Q., Deng H., Aisharif M.J. and Chen X. (2009). The expression of e-cadherin at the invasive tumor front of oral squamous cell carcinoma: Immunohistochemical and rt-pcr analysis with clinicopathological correlation. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 107, 547-554.
- Wei Y., Waltz D.A., Rao N., Drummond R.J., Rosenberg S. and Chapman H.A. (1994). Identification of the urokinase receptor as an adhesion receptor for vitronectin. *J. Biol. Chem.* 269, 32380-32388.
- Wei Y., Lukashev M., Simon D.I., Bodary S.C., Rosenberg S., Doyle M.V. and Chapman H.A. (1996). Regulation of integrin function by the urokinase receptor. *Science* 273, 1551-1555.
- Werning J.W. (2007). *Oral cancer: Diagnosis, management, and rehabilitation*, 1st ed. New York. Thieme New York.
- Wheelock M.J., Shintani Y., Maeda M., Fukumoto Y. and Johnson K.R. (2008). Cadherin switching. *J. Cell Sci.* 121, 727-735.
- Wickramasinghe N.S., Nagaraj N.S., Vigneswaran N. and Zacharias W. (2005). Cathepsin b promotes both motility and invasiveness of oral carcinoma cells. *Arch. Biochem. Biophys.* 436, 187-195.
- Xu D., Baburaj K., Peterson C.B. and Xu Y. (2001). Model for the three-dimensional structure of vitronectin: Predictions for the multi-domain protein from threading and docking. *Proteins* 44, 312-320.
- Yasuda T., Sakata Y., Kitamura K., Morita M. and Ishida T. (1997). Localization of plasminogen activators and their inhibitor in squamous cell carcinomas of the head and neck. *Head Neck* 19, 611-616.
- Ye H., Yu T., Temam S., Ziober B.L., Wang J., Schwartz J.L., Mao L., Wong D.T. and Zhou X. (2008). Transcriptomic dissection of tongue squamous cell carcinoma. *BMC Genomics* 9, 69.
- Yen C.Y., Chen C.H., Chang C.H., Tseng H.F., Liu S.Y., Chuang L.Y., Wen C.H. and Chang H.W. (2009). Matrix metalloproteinases (mmp) 1 and mmp10 but not mmp12 are potential oral cancer markers. *Biomarkers* 14, 244-249.
- Yin S., Lockett J., Meng Y., Biliran H., Jr., Blouse G.E., Li X., Reddy N., Zhao Z., Lin X., Anagli J., Cher M.L. and Sheng S. (2006). Maspin retards cell detachment via a novel interaction with the urokinase-type plasminogen activator/urokinase-type plasminogen activator receptor system. *Cancer Res.* 66, 4173-4181.
- Yorioka C.W., Coletta R.D., Alves F., Nishimoto I.N., Kowalski L.P. and Graner E. (2002). Matrix metalloproteinase-2 and -9 activities correlate with the disease-free survival of oral squamous cell carcinoma patients. *Int. J. Oncol.* 20, 189-194.
- Zhou A., Huntington J.A., Pannu N.S., Carrell R.W. and Read R.J. (2003). How vitronectin binds pai-1 to modulate fibrinolysis and cell migration. *Nat. Struct. Biol.* 10, 541-544.
- Ziober A.F., Falls E.M. and Ziober B.L. (2006). The extracellular matrix in oral squamous cell carcinoma: Friend or foe? *Head Neck* 28, 740-749.
- Zou Z., Anisowicz A., Hendrix M.J., Thor A., Neveu M., Sheng S., Rafidi K., Seftor E. and Sager R. (1994). Maspin, a serpin with tumor-suppressing activity in human mammary epithelial cells. *Science* 263, 526-529.