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LOXL4 is a selectively expressed candidate diagnostic antigen in head and neck cancer

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ABSTRACT

Selective up-regulation of the mRNA of LOXL4, a member of the LOX matrix amine oxidase family, significantly correlated with lymph node metastases and higher tumour stages in head and neck squamous cell carcinomas (HNSCC). To evaluate the diagnostic and prognostic value of the protein we produced an antibody specific for LOXL4 and assessed the expression in 317 human HNSCC specimens. The LOXL4 protein was detected in 92.7% of primary tumours, in 97.8% of lymph node metastases and in affected oral mucosa with high-grade dysplasia, but was absent in various non-neoplastic tissues of the head and neck. TNM categories and overall survival did not link to grades of immunoreactivity. Studies in cultured primary hypopharyngeal HTB-43 carcinoma cells detected perinuclear and cell surface expression of LOXL4, but no nuclear localisation. Therefore, its interactive SRCR-domains and catalytic activity combined with tumour cell specific expression and cell surface associated location indicate multiple functions in tumour cell adhesion and interactions with the extracellular matrix. Our data suggest that LOXL4 is useful both as tumour marker and target in the treatment of HNSCC.

1. Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide. Unfortunately, local or regional disease recurs in one third of patients with advanced tumour stage, and distant metastases appear in 25% with a 5-year survival rate of around 40% despite aggressive bi-or trimodality standard treatments.[1] The availability of prognostic parameters or tumour antigens would allow the selection of patients for adjuvant treatment regimes as target therapies. Yet over the past decades, only limited success has been achieved in identifying unique targets or prognostic markers for TNM stage and behaviour of tumour growth such as invasion depth and lymphangiosis carcinomatosa along with patients' characteristics (e.g. age, co-morbidity) in HNSCC.

Lysyl oxidase (LOX) and the four lysyl oxidase-like proteins (LOXL, 2, 3 and 4) are a family of copper and lysyl-tyrosine

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quinone cofactors containing amine oxidases active in the assembly and homeostasis of the extracellular matrix (ECM). Elevated levels of LOX were reported to promote tumour cell invasion[2] and expression of the active LOX enzyme correlated with metastatic disease in head and neck cancer (HNC) and breast cancer[3] suggesting its suitability as a therapeutic target for the prevention and treatment of metastases. In differential display experiments we have noted overexpression of the latest member, LOXL4, in HNSCC cell lines.[4] Furthermore, we demonstrated significant correlation between increased LOXL4 mRNA levels and lymph node metastases and higher tumour stages in HNSCC. Our results also revealed that chromosome band 10q24.2, which contains the LOXL4 gene, was present in HNSCC cells in supernumerary copies of isochromosomes that may have contributed to LOXL4 overexpression in these cells.[5]

The significant role of ECM during tumour progression is increasingly recognised, yet only a few studies have addressed the function of ECM components in HNSCC. The matrix protein SPARC/osteonectin was identified as an independent prognostic marker for short disease-free interval in HNC.[6] Matrix metalloproteinases including MMP-2, 9, 13, and 7 have been linked to digestion of the basement membranes and infiltration of tumour cells into the surrounding tissue in HNSCC.[7] A role has been also proposed for invadopodia in HNSCC, branched actin-rich structures associated with the ECM, and the protein contactin within the invadopodia was found to be important in the regulation of MMP secretion and coupling of the dynamic actin assembly and the secretory machinery to enhance ECM degradation and invasiveness.[8] While the MMP dependent ECM degrading processes have been recognised for their roles in dissolving the matrix and promoting free cell movement, migration and invasion, results from our and other laboratories demonstrated that the LOX amine oxidases active in the assembly and maintenance of the ECM also play important roles in tumour cell invasion.

We have demonstrated that increased LOX expression and activity promoted migratory and invasive potential of breast tumour cells. Furthermore, this LOX induced mechanism, via the activation of FAK/Scr/paxillin signalling, was effective not only in invasive breast tumour cells, but in other tumour cell types including uveal melanoma[2] and invasive astrocytes.[9] In spite of these results, there is very little and contradictory information concerning the expression and functional significance of LOX family members in the upper aerodigestive tract. LOX up-regulation has been reported previously in oral submucosal fibrosis and SCC.[10] However, reduced expression of LOX– and LOXL2–mRNA expression in HNSCC was also noted and proposed as playing a role in tumour suppressive processes.[11]

In this study, we have examined the reactivity of our new anti-LOXL4 antibody[5] in subcellular compartments of cultured HNSCC cells and used this antibody to evaluate LOXL4 protein expression in a series of primary and metastatic tumour specimens, with different histopathological features and clinical parameters, to determine its prognostic value in HNSCC, and attempted to correlate the degree of LOXL4 immunoreactivity and various tumour parameters in these HNSCC samples.

2. Patients and methods

2.1. Cell lines

HTB-43 SCC cells, originally derived from the pharynx, were maintained in RPMI 1640 medium supplemented with 1% nonessential amino acids, 10% foetal calf serum (Invitrogen Corp, Grand Island, NY, USA), and 1% streptomycin-penicillin. Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2.

2.2. Head and neck tumours

A specimen collection of 233 primary tumours and 45 nodal metastases of a total of 257 different patients with invasive head and neck carcinoma obtained during primary treatment were investigated. In 30 of these patients both primary tumour and metastasis were comparatively analysed. Furthermore, nine biopsies of oral mucosa and tongue with different degrees of dysplasia (three of each) were assessed for LOXL4 expression. Histopathological features and clinical parameters were obtained from pathology reports, clinical databases and patient's files. Nearly all patients exhibited alcohol drinking (94%) and smoking (97%) behaviour. Tumour samples were collected between 1988 and 2005. In addition, normal oral mucosa samples of 30 healthy patients were also collected to serve as controls. Control samples were taken from the tonsils and palate during regular surgical treatment of tonsillar hyperplasia or snoring without additional excisions. Other non-neoplastic tissue samples including normal keratinising and non-keratinising squamous epithelium,

Table 1 – Patient data with TNM categories and histological grading of primary tumours and regional lymphnode metastases

	Primary tumour	Nodal metastasis
Age years (mean ± stdv)	59.6 (±10.7)	63.3 (±8.9)
Sex male/female (%)	195/38 (83.3% / 16.7%)	32/13 (71.1% / 28.9%)
T stage		
Tx (%)	–	6 (13.3%)
T1 (%)	41 (17.6%)	12 (26.7%)
T2 (%)	61 (26.2%)	10 (22.2%)
T3 (%)	63 (27.0%)	11 (24.4%)
T4 (%)	68 (29.2%)	6 (13.3%)
N stage		
N0 (%)	76 (32.6%)	–
N1 (%)	50 (21.5%)	13 (28.9%)
N2 (%)	100 (42.9%)	27 (60.0%)
N3 (%)	7 (3.0%)	5 (11.1%)
M stage		
M0 (%)	220 (94.4%)	41 (91.1%)
M1 (%)	13 (5.6%)	4 (8.9%)
Grading		
G1 (%)	2 (0.9%)	–
G2 (%)	151 (64.8%)	29 (72.5%)
G3 (%)	80 (34.3%)	11 (27.5%)
n.a.	–	5