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Correspondence

LMO4 expression in squamous cell carcinoma of the anterior tongue

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Sir: Ninety per cent of head and neck cancers are squamous cell carcinomas (SCC), the majority representing SCC of the anterior tongue. Understanding the biology of these SCC may assist in the clinical management of the disease and reveal novel potential targets for therapy.

LMO4 is a member of the LIM-only (LMO) family of transcriptional regulators, consisting of LMO1–4. These proteins act as molecular adaptors, providing a scaffold for multiprotein complexes of DNA binding factors and transcriptional regulatory proteins, which play essential roles in cell fate determination, tissue patterning and organ development.[1] Aberrant expression of LMO4 has been demonstrated in a number of cancer types, with particular attention being paid to its prognostic value and contribution towards tumorigenesis.[2,3]

Table 1. Clinicopathological parameters and molecular data, and its association with LMO4 expression, for all patients in the cohort

	Number of patients	LMO4*			*P*-value
Clinicopathological parameter	(*n* = 140) (%)	0–≤55%	55–≤70%	>70%	χ^2
Tumour stage†					
I	75 (53.6)	25	25	25	0.842
II	53 (37.9)	17	21	15	
III	10 (7.1)	4	2	4	
IV	2 (1.4)	1	0	1	
Overall stage†					
I	72 (51.4)	25	24	23	0.953
II	37 (26.4)	13	14	10	
III	16 (11.4)	4	5	7	
IV	15 (10.8)	5	5	5	
Lymph node stage†					
N_0	119 (85.0)	41	39	39	0.666
$\geq N_1$	21 (15.0)	6	9	6	
Tumour grade‡					
Well differentiated	37 (26.4)	14	12	11	0.948
Moderately differentiated	72 (51.4)	24	24	24	
Poorly differentiated	31 (22.1)	9	12	10	
Adjuvant radiotherapy					
Yes	53 (37.8)	30	31	26	0.762
No	87 (62.1)	17	17	19	
Died of disease					
Yes	33 (23.6)	9	12	12	0.669
No	107 (76.4)	38	36	33	

Table 1. (*Continued*)

Clinicopathological parameter	Number of patients (*n* = 140) (%)	LMO4* 0–≤55%	55–≤70%	>70%	*P*-value χ^2
Recurrence					
Yes	41 (29.3)	11	17	19	
No	99 (70.7)				
pRb					
<50%		33	33	19	0.0074
≥50%		13	14	25	
$p14^{ARF}$					
Positive		38	36	38	0.5154
Negative		9	12	7	
E2F-1					
>35%		8	10	14	0.2451
≤35%		38	37	30	
$p16^{INK4A}$					
≥1%		24	26	26	0.8114
0%		23	22	19	
Cyclin D1					
>10%		28	33	30	0.6188
≤10%		19	15	15	
p53					
0–≤10%		21	17	15	0.6072
>10–≤70%		12	19	16	
>70%		14	12	14	
Ki-67					
0–≤20%		22	18	15	0.1612
>20–≤60%		13	23	23	
60%		12	7	7	
$p21^{WAF1/CIP1}$					
0–≤25%		23	18	4	0.1274
>25–≤60%		14	23	8	
>60%		14	18	11	

*Scoring was based on the percentage of positively stained nuclei.
†Tumour and lymph node stage were determined by pathological analysis according to the tumour–node–metastasis (TNM) system. Complete clinicopathological and molecular marker data sets from the 140 patient cohort is presented.
‡Tumour grade was determined by pathological analysis.

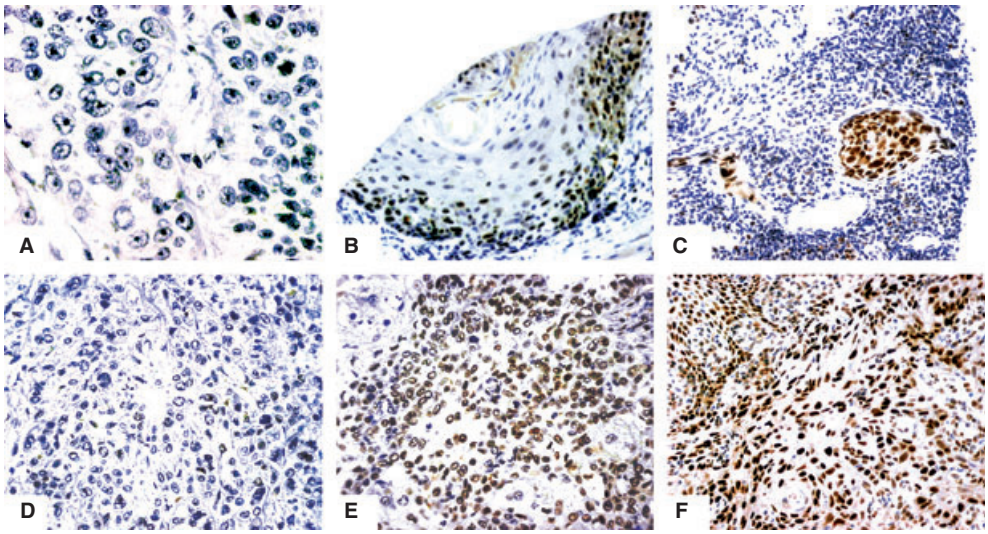

Figure 1. Squamous cell carcinoma (SCC) of the anterior tongue: poorly differentiated tumours display a nucleolar component (**A**), while strong nuclear staining of LMO4 is observed at the invasive front of the tumour cell islands (**B**). Strong nuclear staining is also evident in lymph node metastases (**C**). LMO4 nuclear expression intensity was scored 0–3, where 0 represents no staining, 1 mild nuclear staining (**D**), 2 moderate nuclear staining (**E**) and 3 strong nuclear staining (**F**).

We performed an immunohistochemical analysis of LMO4 protein expression in a large cohort of patients (n = 140; Table 1) with primary operable SCC of the anterior tongue treated with curative intent in the Department of Head and Neck Surgery at the St Vincent's Hospital and Westmead Hospital, Sydney, Australia,[4] and correlated expression with clinicopathological parameters (tumour stage, nodal stage, grade, pathological stage, treatment), patient survival and expression of previously characterized molecular markers ($p14^{ARF}$, $p16^{INK4A}$, cyclin D1, E2F-1, pRb, p53, $p21^{WAF1/CIP1}$, Ki-67).[4,5] Construction of SCC tissue microarrays, immunohistochemistry conditions and scoring (both staining intensity and percentage of cells with positive nuclei) and statistical analyses were performed as described previously.[4,5]

We demonstrated increased LMO4 expression throughout the cohort, particularly at the invasive front of the tumour cell islands and lymph node metastases when compared to the corresponding primary tumour (Figure 1). These data strongly support and extend a report by Mizunuma *et al.*,[2] who demonstrated that both LMO4 and LIM domain-binding protein 1 (LDB1) interact and were localized preferentially in the nuclei of carcinoma cells at the invasive front, with increased immunoreactivity in the lymph node metastases compared to the primary site.[2] Together, these data suggest that LMO4-LDB1 complexes may be involved in progression of the carcinoma, possibly through dedifferentiation of squamous cells of the oral cavity.

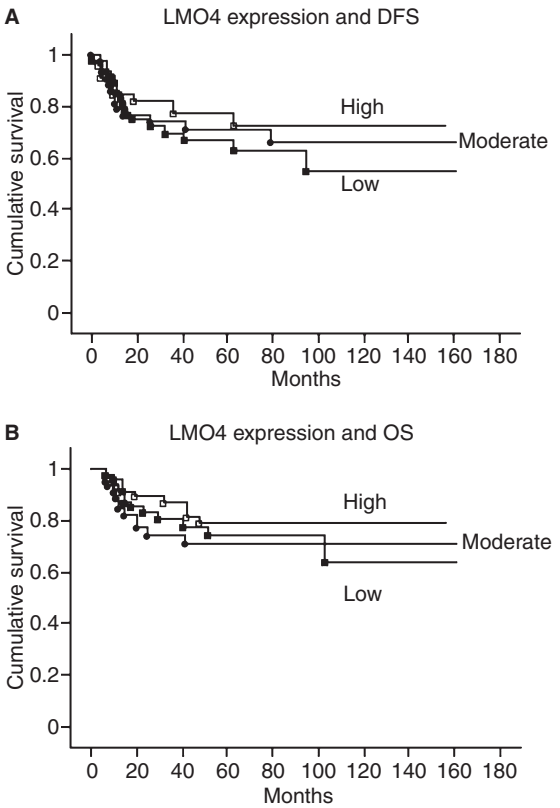

Figure 2. Kaplan–Meier survival curves for low versus moderate versus high LMO4 nuclear expression and (**A**) disease-free survival and (**B**) overall survival. Scores are given as a percentage of cells with positive nuclear immunoreactivity within the representative area of the tissue microarray core, as outlined in Table 1.

Deregulation of LMO4 has been described previously in several tumour types; in particular, high LMO4 nuclear expression has been reported as an independent predictor of death from breast cancer.[3] The precise role played by LMO4 in breast oncogenesis remains to be elucidated. One mechanism by which LMO4 may contribute to tumorigenesis is via repression of the BRCA1 tumour suppressor gene.[6] LMO4 interacts physically with the CtBP-interacting protein (CtIP), as well as BRCA1 to repress BRCA1 mediated transcriptional activity.[6] Thus, negative regulation of BRCA1 expression by LMO4 has been suggested as an additional mechanism by which BRCA1 activity is downregulated and contributes to the pathogenesis of breast cancer. Indeed, oncogenesis of SCC of the anterior tongue may undergo a similar mechanism, as our data demonstrated the trend that reduced LMO4 expression was associated with improved outcome; however, this failed to achieve statistical significance (Figure 2).

In our cohort, there were no significant associations between LMO4 intensity and clinicopathological parameters or with molecular markers; however, low expression of LMO4 correlated significantly with low pRb expression ($P = 0.0074$; chi-square). Low pRb has been associated with improved disease-free survival in patients with SCC of the anterior tongue.[4] In head and neck squamous cell cancer (HNSCC) the association between pRb and disease outcome is contentious, with Pavelic *et al.*[7] demonstrating a correlation between reduced expression of pRb and a more aggressive biological phenotype with reduced survival in SCC of the oral cavity, whereas Pande *et al.*[8] have demonstrated an adverse outcome in patients with overexpression of pRb in oral cancers. In our study, low expression of LMO4 was observed in approximately 34% of SCC of the anterior tongue; however, this was not associated significantly with disease-free or overall survival ($P = 0.5351$ and $P = 0.5747$, respectively; log-rank; Figure 2) despite the significant cohort size and the site-specific lesion. Clearly, further studies to elucidate the pathways regulated by LMO4 and mechanisms by which LMO4 contributes to the development and invasive progression of SCC of the anterior tongue will be an essential step towards assessing the clinical management of the disease and may reveal novel potential targets for therapy.

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Rhonda A Kwong[1]*
Christopher J Scarlett[1]*
Larry H Kalish[1]
Ian E Cole[2]
James G Kench[1,3]
Eleanor YM Sum[1]
Elizabeth A Musgrove[1]
Susan M Henshall[1]
Geoffrey J Lindeman[4]
Andrew V Biankin[1,5]
Jane E Visvader[4]
Robert L Sutherland[1]

[1]Cancer Research Program, Garvan Institute of Medical Research, Darlinghurst, [2]Department of ENT Surgery, St Vincent's Hospital, Darlinghurst, Sydney, [3]Department of Anatomical Pathology, Royal Prince Alfred Hospital, University of Sydney, Camperdown, NSW, [4]The Walter and Eliza Hall Institute of Medical Research, Parkville, Vic. and [5]Division of Surgery, Bankstown Hospital, Bankstown, Sydney, NSW, Australia

**These authors contributed equally to this work.*

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