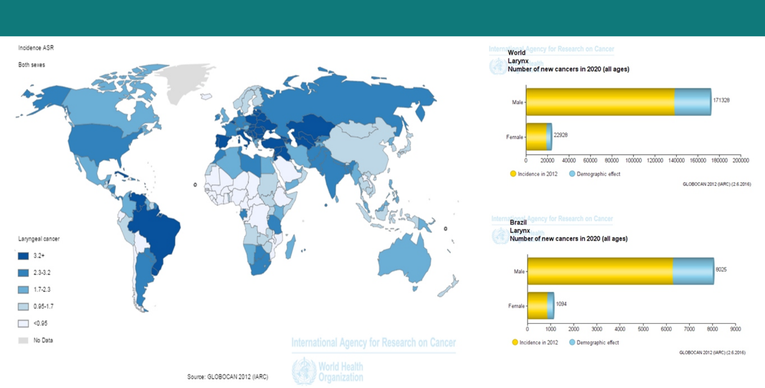
Transcriptome analysis identified *ALCAM* expression as potential biomarker to LSCC patient's outcome

Pedro Nicolau Neto[a], **Paulo Thiago de Souza Santos**[a], **Flavia Nascimento de Carvalho**[a], **Fernando Dias**[b], **Izabela Costa**[b], **Sheila Coelho Soares Lima**[a], **Tatiana de Almeida Simão**[c], **Luis Felipe Ribeiro Pintoa**[c]

Authors affiliation: a – Molecular Carcinogenesis Program – National Cancer Institute, Rio de Janeiro, Brazil; b – Head and Neck Surgery Section – National Cancer Institute, Rio de Janeiro, Brazil ; c – Biochemistry Department – Rio de Janeito State University, Rio de Janeiro Brazil

INTRODUCTION

- Laryngeal squamous cell carcinoma (LSCC) is one of the most incidence tumors in the world, especially in developing countries, such as Brazil;
- The main risk factors for LSCC are tobacco and alcohol consumption and it usually occurs in patients older than 60 years;
- Similarly to other head and neck tumors, LSCC is a major health problem because of poor prognosis and slight improvement in the five-year survival during the past four decades;
- It is necessary to increase knowledge about LSCC molecular alterations aiming to the identification of targets for new therapeutic approaches



OBJECTIVE

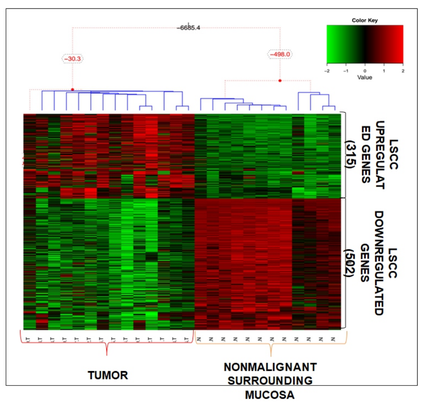
To develop biomarkers for LSCC prognosis

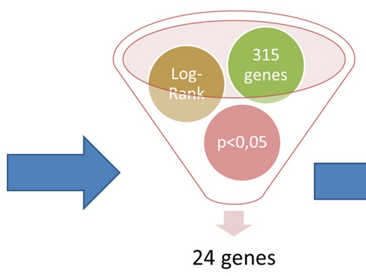
METHODOLOGY

- Transcriptome analysis was performed in 14 LSCC and 12-matched nonmalignant adjacent mucosa samples using Affymetrix microarray Human Exon 1.0 ST
- Log-rank analyses were carried out in looping across all LSCC-overexpressed genes. DEG with Log-rank p value < 0.05 were selected for validation in an independent sample set, TCGA provisional Data, using clinic and pathological data for Cox Regression Model.
- TCGA data were used to evaluate the impact of gene expression on the patients survival rate and in the correlation analyses between gene expression and DNA methylation levels.

RESULTS

Bayesian hierarchical clustering of LSCC and nonmalignant samples according to DEG expression

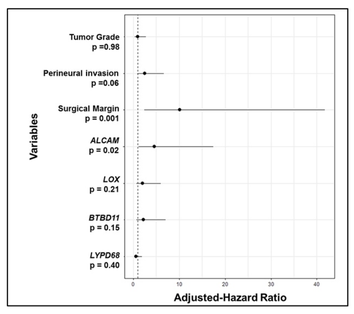




Univariate survival analysis using independent set of samples (TCGA Data)

		Univariate analysis		
		HR	(95% CI)	*P* value
TCGA Provisional Data				
Age at diagnosis (years	>62 vs <62	1,19	(0,67 – 2,12)	0,54
Tumor Stage	III-IV vs I-II	0,74	(0,31 – 1,77)	0,51
Tumor Differentiation	G3 vs G2 vs G1	0,69	(0,43 – 1,12)	0,14
Perineural Invasion	Yes vs No	3,97	(1,67 – 9,47)	**0,001**
Surgical Margins	Positive/Close vs Negative	4,20	(1,79 – 9,83)	**0,0009**
ACOX1	High vs Low	1,04	(0,59 – 1,85)	0,86
ACVR1	High vs Low	1,26	(0,70 – 2,25)	0,43
ADH7	High vs Low	1,05	(0,59 – 1,87)	0,84
AGFG2	High vs Low	0,75	(0,42 – 1,33)	0,33
ALCAM	High vs Low	2,05	(1,13 – 3,69)	**0,01**
BTBD11	High vs Low	1,44	(0,81 – 2,54)	0,20
C12ORF75	High vs Low	1,04	(0,59 – 1,86)	0,87
CDK14	High vs Low	0,92	(0,51 – 1,66)	0,78
CYP2C19	High vs Low	1,23	(0,70 – 2,18)	0,45
GBP6	High vs Low	1,09	(0,61 – 1,94)	0,75
GLTP	High vs Low	0,90	(0,51 – 1,61)	0,74
GNG4	High vs Low	1,44	(0,81 – 2,54)	0,20
LOX	High vs Low	1,81	(1,01 – 3,24)	**0,04**
LYPD6B	High vs Low	0,65	(0,36 – 1,18)	0,16
ME1	High vs Low	1,14	(0,64 – 2,02)	0,64
NPEPPS	High vs Low	1,16	(0,65 – 2,05)	0,60
ODC1	High vs Low	1,38	(0,78 – 2,46)	0,26
PMM1	High vs Low	1,21	(0,68 – 2,15)	0,49
PTGR1	High vs Low	1,31	(0,74 – 2,33)	0,34
SERPINA3	High vs Low	1,44	(0,39 – 1,23)	0,21
ST3GLA4	High vs Low	0,80	(0,45 – 1,43)	0,46
TP052L1	High vs Low	0,85	(0,48 – 1,51)	0,59
ZDHHC13	High vs Low	0,97	(0,55 – 1,71)	0,91
ZNF750	High vs Low	0,90	(0,51 – 1,60)	0,73

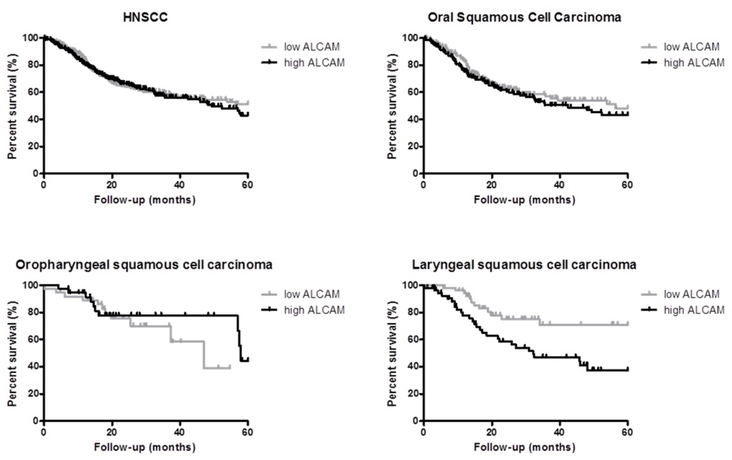
Forest plot showing the result of multivariate survival analysis (Cox Regression Model)



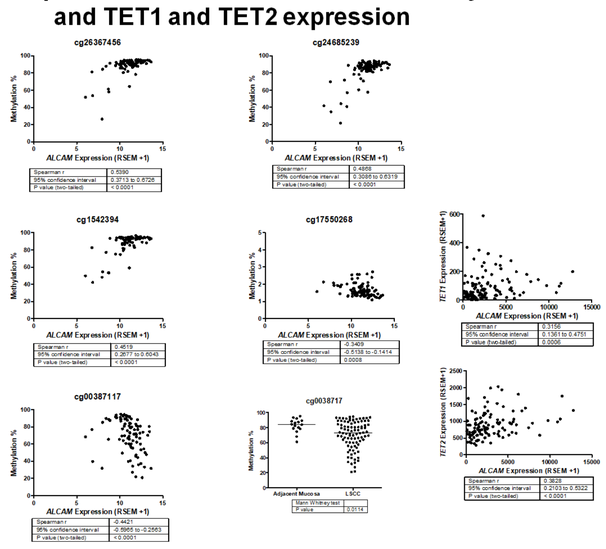
Association between *ALCAM* expression and clinical and pathological features

Clinical Feature		*ALCAM*
Perineural Invasion	No	2161.0 (114.3-11225.0)
	Yes	2139.0 (63.23-7897.0)
	p	0.974
Surgical Margins	Negative	2111.0 (63.23-10881.0)
	Positive	2346.0 (107.5-8759.0)
	p	0.275
Tumor Grade	G1	535.8 (430.7-875.8)
	G2	2502 (107.5-8759.0)
	G3	1354.0 (63.23-3915.0)
	p	**0.002**
Tumor Stage	I / II	3359.0 (421.5-12776.0)
	III / IV	2166.0 (63.23-11225.0)
	p	0.080
Lymph node metastasis	No	2456.0 (114.3-12776.0)
	Yes	1886.0 (63.23-11225.0)
	p	**0.032**

ALCAM expression was only related to LSCC patients outcome



ALCAM Expression was correlated to DNA methylation and TET1 and TET2 expression



CONCLUSION

With these results, we can conclude that ALCAM expression might be an independent prognosis biomarker to LSCC patients and it's expression regulation could be related to DNA methylation

REFERENCES

Barnes L et al (2005). Pathology & Genetics - Head and neck Tumours. Lyon: IARC Press; 168p.
INCA. Estimativa 2014: Incidência de Câncer no Brasil. Instituto Nacional de Câncer José Alencar Gomes da Silva, Coordenação de Prevenção e Vigilância. 124p.
Parkin DM et al. (2005). CA Cancer J Clin. 55: 74-108.
Chuang SC et al. (2008). Cancer Epidemiol Biomarkers Prev. 17(6):1543-9.
Chung CS et al. (2013). BMC Gastroenterol. 13:154.

Funding source: Ministério da Saúde, FAPERJ, Swiss Bridge.

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA