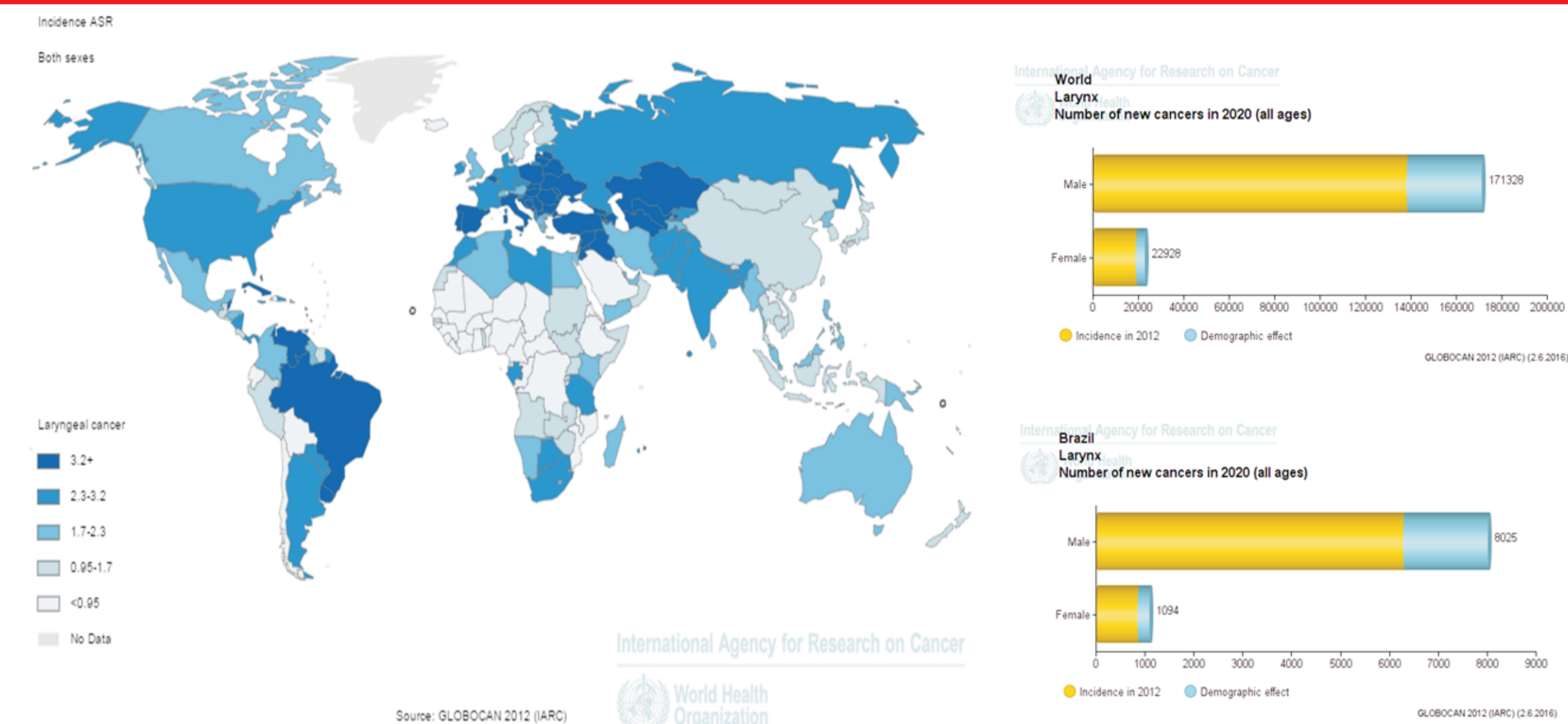


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INTRODUCTION

- 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 of 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
- Microarray data was normalized in Expression Console software applying RMA algorithm. Differentially expressed genes (DEG) were identified using R environment with *Limma* package. DEG determination cut-off was gene expression fold-change > |2| and adjusted-p value < 0.05
- 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.

RESULTS

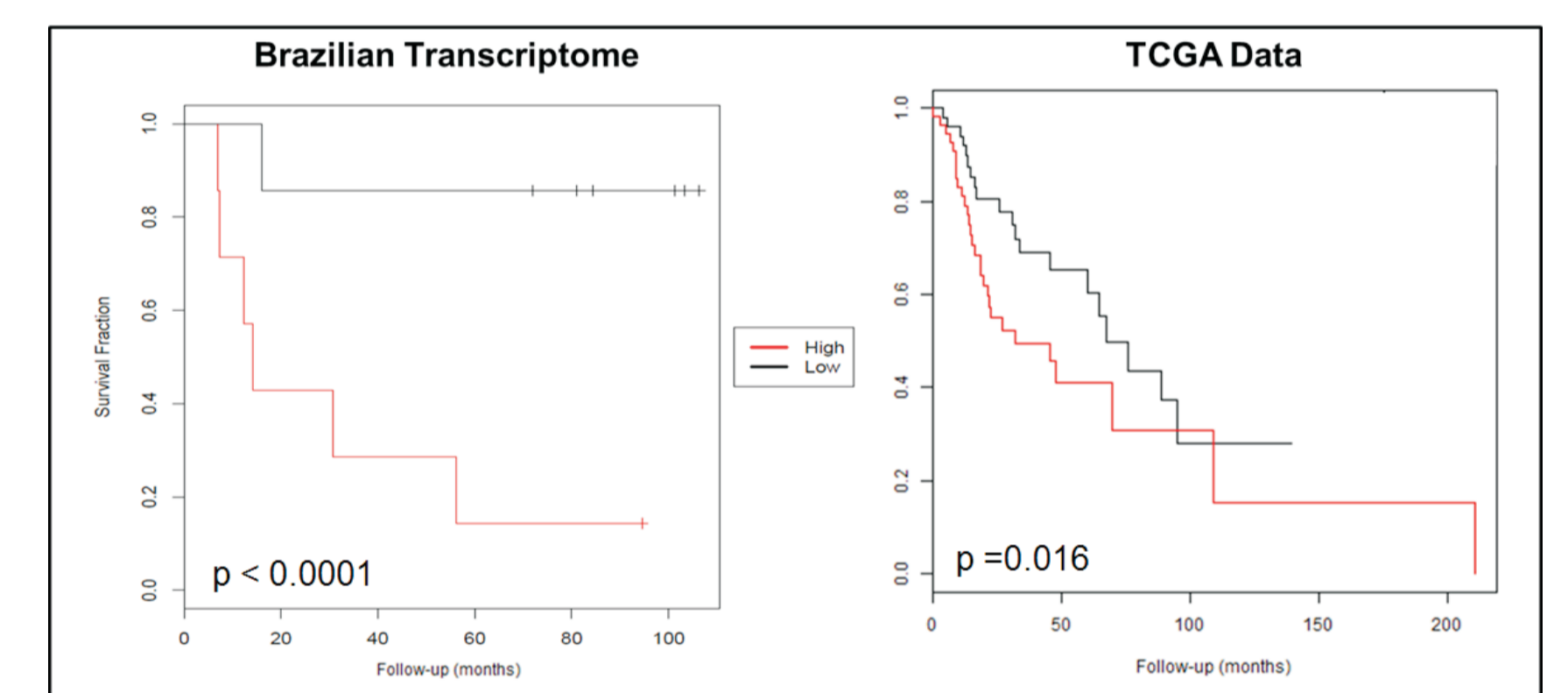
Clinic and pathologic features of LSCC patients enrolled in this study

		Investigation Set		Validation Set	
		Transcriptome INCA	%	TCGA, Provisional Data	%
Age (years)	Median	58		62	
	Interval	45 – 77		38 – 83	
Gender	Female	1	7,1%	19	17,3%
	Male	13	92,9%	91	82,7%
	NA	0		0	
Smoking History	Never	1	7,1%	6	5,5%
	Current	5	35,7%	55	50,0%
	Former	4	28,6%	46	41,8%
	NA	4	28,6%	3	2,7%
Alcohol Consumption History	Never	4	28,6%	38	34,5%
	Current	4	28,6%	70	63,6%
	Former	2	14,3%		
	NA	4	28,6%	2	1,8%
Tumor Differentiation	Well	1	7,1%	7	6,4%
	Moderately	12	85,7%	70	63,6%
	Poor	1	7,1%	29	26,4%
	NA	0	0,0%	4	3,6%
Tumor Stage	I	1	7,1%	2	1,8%
	II	2	14,3%	9	8,2%
	III	1	7,1%	22	20,0%
	IV	5	35,7%	77	70,0%
	NA	5	35,7%	0	0,0%
Perineural Invasion Status	No	7	50,0%	45	40,9%
	Yes	4	28,6%	24	21,8%
	NA	3	21,4%	41	37,3%
Surgical Margin Status	Negative	11	78,6%	81	73,6%
	Positive	3	21,4%	9	8,2%
	Close	0	0,0%	4	3,6%
	NA	0		16	14,5%

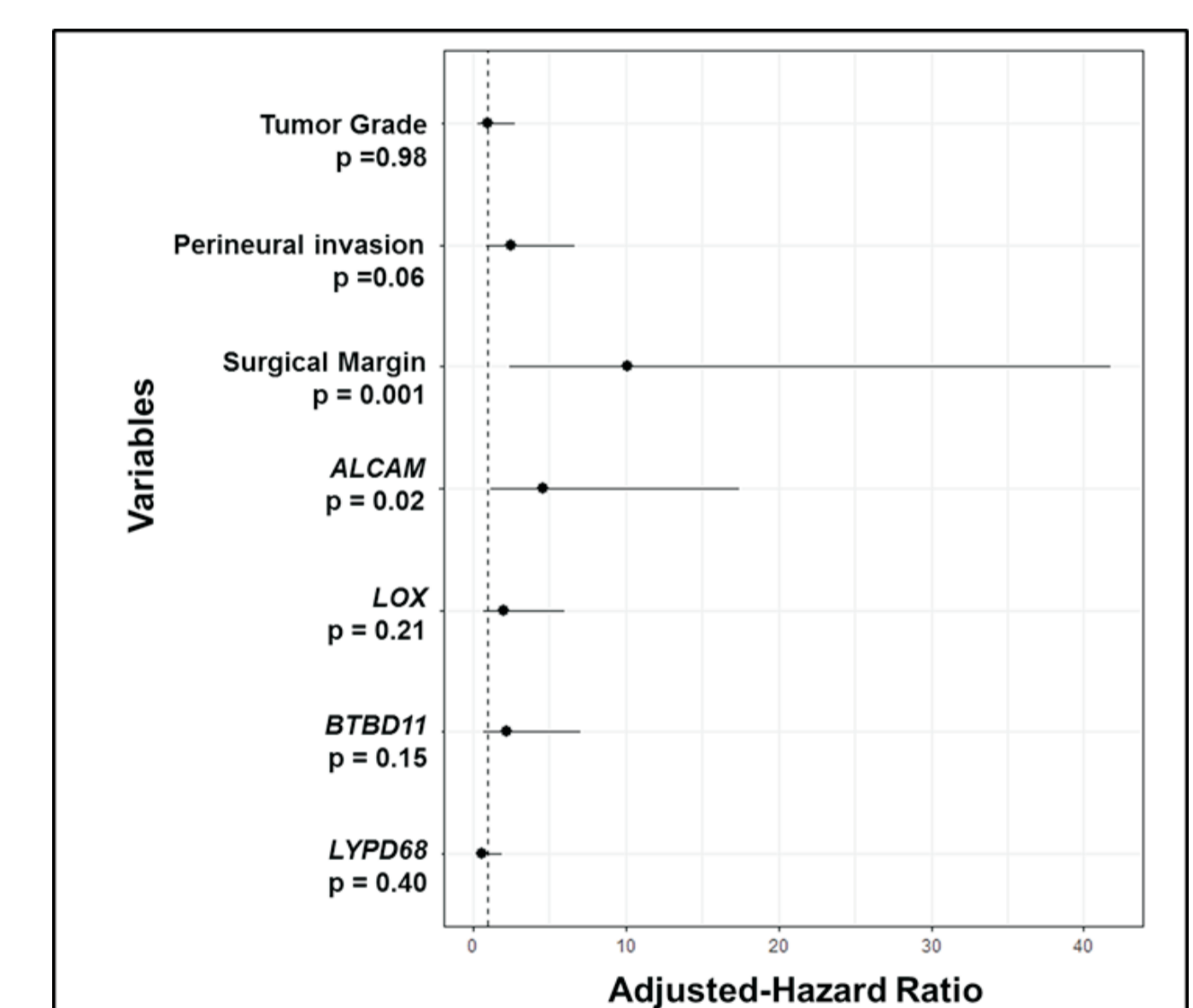
Transcriptome analysis: LSCC-overexpressed genes associated to patients outcome

Official Gene Symbol	Official Full Name	Log-rank P Value
<i>ACOX1</i>	acyl-CoA oxidase 1	0,007
<i>ACVR1</i>	activin A receptor type 1	0,007
<i>ADH7</i>	alcohol dehydrogenase 7	0,005
<i>AGFG2</i>	ArfGAP with FG repeats 2	0,010
<i>ALCAM</i>	activated leukocyte cell adhesion molecule	0,007
<i>BTBD11</i>	BTB domain containing 11	0,003
<i>C12orf75</i>	chromosome 12 open reading frame 75	0,010
<i>CDK14</i>	cyclin dependent kinase 14	0,045
<i>CYP2C19</i>	cytochrome P450 family 2 subfamily C member 19	0,005
<i>GBP6</i>	guanylate binding protein family member 6	0,045
<i>GLTP</i>	glycolipid transfer protein	0,045
<i>GNG4</i>	G protein subunit gamma 4	0,010
<i>LOX</i>	lysyl oxidase	0,045
<i>LYPD6B</i>	LY6/PLAUR domain containing 6B	0,013
<i>ME1</i>	malic enzyme 1	0,045
<i>NPEPPS</i>	aminopeptidase puromycin sensitive	0,045
<i>ODC1</i>	ornithine decarboxylase 1	0,003
<i>PMM1</i>	Phosphomannomutase 1	0,016
<i>PTGR1</i>	prostaglandin reductase 1	0,000
<i>SERPINA3</i>	serpin family A member 3	0,045
<i>ST3GAL4</i>	ST3 beta-galactoside alpha-2,3-sialyltransferase 4	0,045
<i>TPD52L1</i>	tumor protein D52-like 1	0,045
<i>ZDHHC13</i>	zinc finger DHC-type containing 13	0,010
<i>ZNF750</i>	zinc finger protein 750	0,045

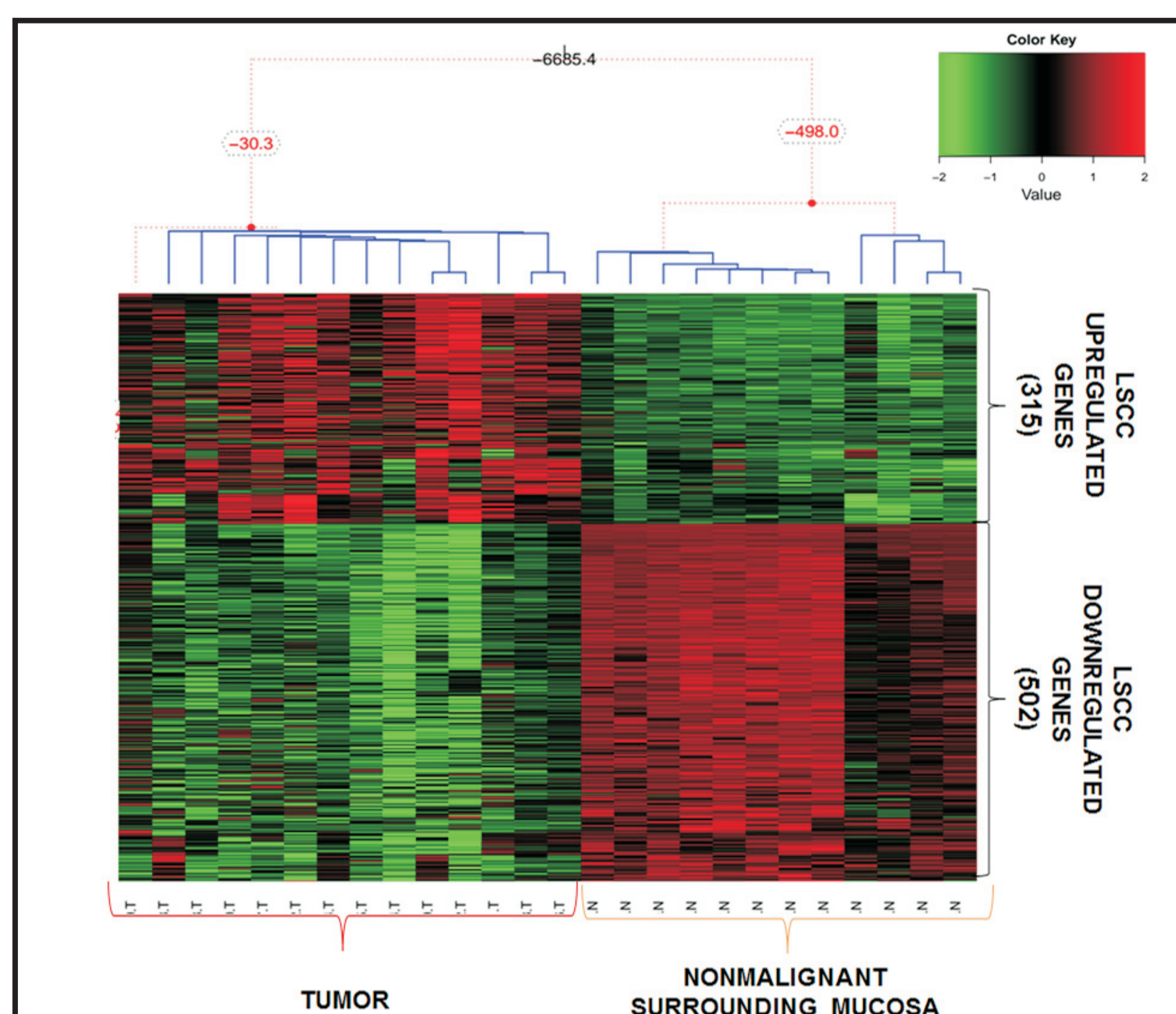
Kaplan-Meier Curves showing the impact of *ALCAM* expression in LSCC patients outcome



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



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



Univariate survival analysis using independent set of samples

TCGA Provisional Data	Univariate analysis	HR (95% CI)		P value
		HR	(95% CI)	
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
<i>ACOX1</i>	High vs Low	1,04	(0,59 – 1,85)	0,86
<i>ACVR1</i>	High vs Low	1,26	(0,70 – 2,25)	0,43
<i>ADH7</i>	High vs Low	1,05	(0,59 – 1,87)	0,84
<i>AGFG2</i>	High vs Low	0,75	(0,42 – 1,33)	0,33
<i>ALCAM</i>	High vs Low	2,05	(1,13 – 3,69)	0,01
<i>BTBD11</i>	High vs Low	1,44	(0,81 – 2,54)	0,20
<i>C12orf75</i>	High vs Low	1,04	(0,59 – 1,86)	0,87
<i>CDK14</i>	High vs Low	0,92	(0,51 – 1,66)	0,78
<i>CYP2C19</i>	High vs Low	1,23	(0,70 – 2,18)	0,45
<i>GBP6</i>	High vs Low	1,09	(0,61 – 1,94)	0,75
<i>GLTP</i>	High vs Low	0,90	(0,51 – 1,61)	0,74
<i>GNG4</i>	High vs Low	1,44	(0,81 – 2,54)	0,20
<i>LOX</i>	High vs Low	1,81	(1,01 – 3,24)	0,04
<i>LYPD6B</i>	High vs Low	0,65	(0,36 – 1,18)	0,16
<i>ME1</i>	High vs Low	1,14	(0,64 – 2,02)	0,64
<i>NPEPPS</i>	High vs Low	1,16	(0,65 – 2,05)	0,60
<i>ODC1</i>	High vs Low	1,38	(0,78 – 2,46)	0,26
<i>PMM1</i>	High vs Low	1,21	(0,68 – 2,15)	0,49
<i>PTGR1</i>	High vs Low	1,31	(0,74 – 2,33)	0,34
<i>SERPINA3</i>	High vs Low	1,44	(0,39 – 1,23)	0,21
<i>ST3GAL4</i>	High vs Low	0,80	(0,45 – 1,43)	0,46
<i>TPD52L1</i>	High vs Low	0,85	(0,48 – 1,51)	0,59
<i>ZDHHC13</i>	High vs Low	0,97	(0,55 – 1,71)	0,91
<i>ZNF750</i>	High vs Low	0,90	(0,51 – 1,60)	0,73

CONCLUSION

With these results, we can conclude that *ALCAM* expression might be an independent prognosis biomarker to LSCC patients.

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