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ORIGINAL PAPER

Increased expression of cellular retinol-binding protein 1 in laryngeal squamous cell carcinoma

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Abstract
Purpose To investigate the genomic alterations in larynx carcinomas (LaCa) tissues and its prognostics values in predicting survival.
Methods To analyse the aberrations in the genome of LaCa patients, we used array comparative genomic hybridization in 19 human laryngeal tumour samples. DNA samples were also subjected to detect human papillomavirus (HPV) sequences by polymerase chain reaction (PCR). Copy number gain was confirmed by real-time PCR. The cellular retinol-binding protein 1 (*CRBP-1*) gene expression was also confirmed by immunohistochemistry assay on LaCa tissues. To identify prognostic feature, *CRBP-1* gene gain was correlated to patient survival.
Results The most common gains were detected for *CRBP-1* and *EGFR* genes, while DNA lost in *RAF-1* gene. Immunohistochemistry assay was revealed strong expression of CRBP1 protein in those cases with *CRBP-1* gene gain. The *CRBP-1* gene gain and its expression correlated significantly with survival ($P = 0.003$). Cox regression analysis indicated that *CRBP-1* expression level was a factor of survival ($P = 0.008$). HPV sequences were detected in 42% of the samples, and did not show any relationship with specific gene alterations.
Conclusion Our data shows that *CRBP-1* gene gain can be determined by immunohistochemistry on routinely processed tissue specimens, and could support as a potential novel marker for long-term survival in laryngeal squamous cell carcinoma.

Keywords Array CGH · HPV · *CRBP-1* · Larynx carcinoma · Expression · Prognosis

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Introduction

Larynx cancer (LaCa) represents an important public health problem affecting mainly the population over 50 years old in worldwide. The epithelium of the larynx is quite similar to other epithelia to be susceptible to human papillomavirus (HPV) infection. It has been reported that high risk HPV is present at a range of 30–90% of the larynx tumours, suggesting that besides HPV infection other carcinogenic factors could be also participating in this tumoural process.

(McKaig et al. 1998; Pérez-Ayala et al. 1990; Almadori et al. 1996; Mineta et al. 1998; Atula et al. 1999; Mutiu et al. 2001) Several researches have showed that the infection of HPV is related to laryngeal tumours with differences in the frequencies (Munger and Howley 2002; Torrente et al. 2005; Syrjäden 2005; Chai et al. 2004).

Comparative genomic hybridization (CGH) on metaphase has been widely used in cancer research (Kujawski et al. 2002a, b; Struski et al. 2002; Solinas-Toldo et al. 1997; Albertson and Pinkel 2003; Pinkel et al. 1998; Lichter et al. 2000; Pollack et al. 1999; Huang et al. 2004) detecting specific patterns of chromosomal imbalances, e.g. the loss of chromosome 13 in all squamous cell carcinoma of the larynx (Kujawski et al. 2002a, b; Struski et al. 2002). However, given the spatial resolution of CGH (Huang et al. 2004), not much is known about the identity of specific genes that might be the targets of regional chromosomal imbalances. Array CGH overcomes this problem by increasing the sensitivity for the detection of DNA copy number changes at specific loci, through the use of well-defined genomic DNA fragments whose mapping location is known, arrayed onto a solid surface, thereby achieving a resolution of copy number imbalances up to a single gene level (Pinkel et al. 1998; Pollack et al. 1999; Huang et al. 2004).

To refine the patterns of chromosomal imbalances present in larynx squamous carcinoma, and especially to identify specific genes that might be targets of copy number changes in this kind of tumour, we applied array CGH harbouring already known oncogenes, tumour suppressor genes and some other genes associated with cancer on 19 human LaCa tissues. Our results are showing that in more of the half of the LaCa, the cellular retinol-binding protein 1 (*CRBP-1*) gene gain followed by epidermal growth factor receptor (*EGFR*) gene amplification could be common genetic features in this tumour, and suggest that the *CRBP-1* gene or protein expression gain could be related with survival.

Materials and methods

Biological samples

Larynx cancer samples were collected from patients who attended the Head and Neck Service at Hospital de Oncologia, SXXI-IMSS, Mexico City in the period of 1 year (2005). The described procedures have been approved by the local committee of ethics of the Mexican Institute of Social Security (IMSS; CLICHO), and all samples were taken after informed consent from the patients. All the cases were not previously treated. The biopsies specimens were divided into three sections: the central part was used for genomic DNA extraction, and both extremes were fixed with 70% ethanol overnight and paraffin embedded. Haematoxylin–eosin stained sections from these biopsies were analysed to confirm the presence of at least 80% tumoural cells in the samples. LaCa samples were histological confirmed as squamous cell carcinomas and clinically invasive tumours as follows: six of T4N0M0 samples; three of T2N0M0, T3N0M0, T4N2M0 samples; two of T3N1M0, and one of T2N1M0, T2N3M0 samples according TNM classification. Two papillomatosis lesions were also included. High-molecular weight DNA was extracted by using the Wizard Genomic System (Promega Co., Madison, WI, USA). Genomic DNA was resolved in agarose gel ethidium bromide stained and quantified by a Perkin Elmer MBA 2000 spectrophotometer.

HPV detection and typing

Human papillomavirus detection was carried out by polymerase chain reaction (PCR) using the consensus primers GP5+/GP6+ for L1 gene of HPV. After 5 min of denaturation at 94°C, 100 ng of DNA was subjected to 35 amplification cycles with the following parameters: 94°C for 1 min, 55°C for 2 min and 73°C for 3 min, with a final extension step of 7 min at 72°C. The amplified products were purified using the Wizard SV gel and PCR-clean-up System Kit (Promega Co., Madison WI, USA) and labelled using the Big Dye sequencing kit (Applied Biosystems, Foster City, CA, USA). The labelled products were then sequenced in an Applied Biosystems 373 automated DNA sequencer (Perkin Elmer, Applied Biosystems; in the Core Instrument Facility at IMSS-Mexico), and the obtained sequences were aligned and compared with the existing databases using the BLAST program via Internet.

Hybridization on GenoSensor Array

Microarray CGH was performed using the GenoSensor Array 300 system, following the manufacturer's instructions (ABBOT-VYSIS, Downers Grove, IL, USA). Each array contains 861 spots, representing 287 chromosomal regions that are commonly altered in human cancer, such as telomeres, regions involved in microdeletions, oncogenes, and tumour suppressor genes. Briefly, 500 ng of genomic DNA was labelled by a random primer reaction during 2 h. Tumour DNA was labelled with dATP Cy3 and the normal female reference DNA (ABBOT-VYSIS Co.) with dATP Cy5. After labelling reaction, the probes were digested with DNase at 15°C for 1 h, followed by two ethanol purifications; finally, the probe size was checked by gel electrophoresis. The hybridization mixture consisted of 2.5 µl of each of the DNAs differentially labelled plus 25 µl of hybridization buffer provided in the kit. This mixture was denatured at 80°C for 10 min, followed by incubation at 37°C for 1 h.

Then, the mixture was applied onto the spotted area of the array under a coverslip and hybridized in a humid chamber containing 50% formamide (FA)/2× SSC at 37°C for 72 h. After hybridization, the arrays were washed 3× in 50%FA/2× SSC at 40°C for 10 min/wash, followed by four 5-min washes in 1× SSC at room temperature. Finally, the arrays were briefly rinsed in distilled water, mounted and counterstained in the dark for 45 min with 4,6-diamino-2-phenylindole (ABBOT-VYSIS Co).

Image capture and analysis

Array analysis was performed immediately after counterstaining using the GenoSensor scanner and software. This system generates a "genomic analysis report", indicating which chromosome regions in the array are involved in copy number changes, as well as a spreadsheet containing the data from each. To compare all the experiments, a database was created using the normalised, bias corrected, tumour/normal ratio value of each experiment, since each spot in the array is present in triplicates, the median of the three spots was calculated and its $\log_2$ transformed value was used for further analysis. A fluorescence ratio >1.25 ($\log_2 = 0.32$) was considered as a DNA gain, while DNA losses were scored when the ration was <0.75 ($\log_2 = -0.41$). A ratio >2 ($\log_2 = 1$) was considered as a high copy number amplification. The microarray data were plotted by using the ISCN2matrix converter at http://www.progenetix.de. As essential part of this methodology, previous to study of LaCa samples, normal DNA from larynx epithelium revealed a well-balanced chromosomal composition (data not shown).

Real-time quantitative PCR

To validate *CRBP-1* gene imbalances detected by array CGH in this study, some DNA samples from the present series were analysed using real-time PCR. For relative quantitation, the reactions were performed using 2× Platinum Quantitative PCR SuperMix-UDG (Invitrogen Life Technology, Carlsbad, CA, USA). The PCR amplification were performed in a LightCycler® 2.0 Real-Time PCR System (Roche Diagnostics Co., Salt Lake City, UT, USA) with 100 ng of DNA for 35 cycles, each with 10 s at 94, 64 and 72°C, with initial UDG incubation at 50°C for 5 min and denaturation 94°C 5 min. The primers D-LUX used were designed in Invitrogen Technology webpage. *CRBP-1* oligonucleotides forward FAM 5′-cgtcccaaagagatcgtgcag gag-3′, and reverse 5′-tgcctgtcagatcctcctcaaa-3′; beta 2 microglobulin (B2M) gene was used as reference: forward FAM 5′-acataccttggttgatccactt-3′ and reverse 5′-catctgttgct ctatacgtggcagatg-3′; the relative genomic copy number was calculated using the comparative Ct methods.

Immunohistochemistry assay

Staining for detection of bound antibody was performed according to standard protocols using the DAKO Envision™ Doublestain system (DAKO Co., Carpinteria, CA, USA). For CRBP-1 protein, a 1:100 dilution of the monoclonal mouse antibody (AbD Serotec Inc., Raleigh, NC, USA; clone MCA2530) was used. According to immunostaining results, the evaluation was considered as positive or negative detection.

Statistical analysis

The estimation of the survival distribution of patients with LaCa was done according to the methods of Kaplan and Meier. For pairwise comparisons of survival time distributions, the log-rank test was used (Harrell Jr and Shih 2001). Cox proportional-hazard analysis was used for univariate analysis to explore the effect of clinicopathological variables and *CRBP-1* gain or *EGFR* amplification on survival. The statistical analysis was performed using the statistical software SPSS 15.

Results

To know the presence of HPV infection, all the DNA samples were subjected to PCR to detect any HPV sequence. The HPV-positive cases were then sequenced.

As expected, both benign lesions were positive for low-risk HPV11 infection. For squamous cell carcinomas, eight were positive for HPV16 and the remaining cases (11/19) were HPV negative. This observation is based on the absence of HPV sequences screened by means of GPs, MYs, L1C1/L1C2 PCR primers (data not shown).

On the other hand, to identify specific gene alterations that could be related to human LaCa, array CGH on squamous cell carcinomas laryngeal tumours was applied. In this particular array, it comprises 287 chromosomal regions that are commonly altered in human cancer amongst them were oncogenes and tumour suppressor genes. After HPV detection, all DNA samples were subjected to labelling and hybridization to array CGH. In general, all the samples in the hybridization array analysis exhibited gene alterations (gains or losses) ranking from 3 to 50/287 (alterations/total targets in the array). It was observed that the number of gains was approximately more than 0.5× that the losses (252 vs. 119) and the average number of copy number alterations (total number of alterations in the sample collective/total number of cases) was 17.6 per case. Interestingly, only a reduced number of amplified genes were detected and mapped on the chromosomes 2, 3, 9, 11 and 17; whereas the main deletion was observed in a clone mapped

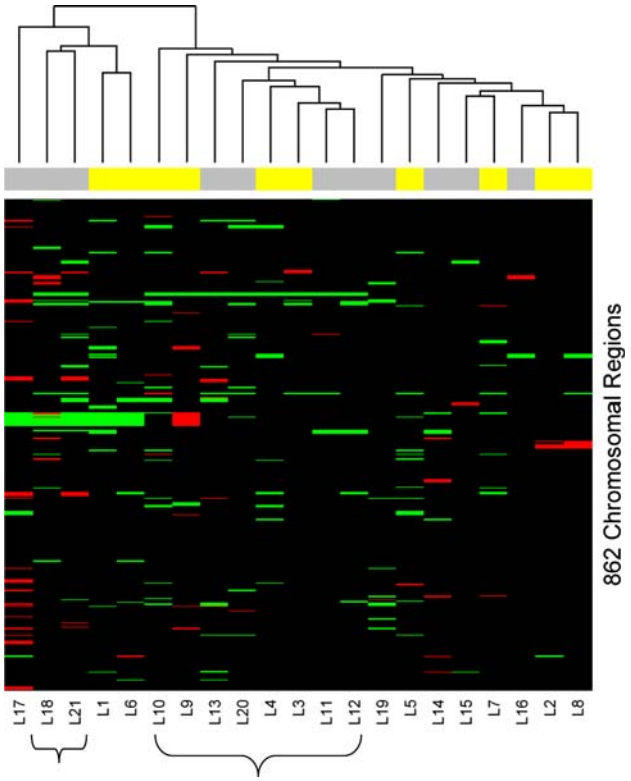

Fig. 1 Ideogram of gene alterations in laryngeal squamous cell carcinoma. The data were analysed and visualised by ISCN2 matrix. The *lanes* represent each sample, the *row* are each clone printed on the array starting from up to down from chromosome 1, 2, etc. The *yellow strip* represents the HPV-positive cases. *L1*, *L2*, etc, represent each larynx cancer sample. L1 and L2 were the benign lesions, the remaining were the invasive lesions. The keys are grouping the *CRBP-1* gene gain samples

Table 1 Clinical data and molecular findings in larynx cancer samples

Sample	Gender	Age	TNM	HPV status	*CRBP-1*[a]	*EGFR*[b]
L1	M	69	T4N2M0	–	+	+
L2	M	66	T2N0M0	+	+	–
L3	M	72	T2N1M0	+	–	+
L4	M	60	T4N2M0	–	–	+
L5	F	75	T2N3M0	–	–	+
L6	M	52	T2N0M0	–	+	–
L7	M	57	T4N2M0	–	+	–
L8	M	60	T2N0M0	+	+	+
L9	M	62	T3N0M0	–	+	–
L10	M	53	T4N0M0	–	+	+
L11	M	57	T3N1M0	+	–	–
L12	M	62	T4N0M0	+	–	–
L13	M	63	T3N0M0	+	–	–
L14	F	58	T4N0M0	–	–	–
L15	M	63	T3N1M0	+	+	+
L16	M	74	T3N0M0	–	+	+
L17	M	61	T4N0M0	–	+	+
L18	F	58	T4N0M0	+	–	–
L19	M	62	T4N0M0	–	–	–

[a] *CRBP-1* gene gain

[b] *EGFR* gene amplification

on chromosome 9. In the case of the benign lesions, both samples exhibited small number of molecular alterations mainly DNA gains but none of those alterations were shared between all samples. Only a loss in the testicular haploid expressed gene in subtelomeric region of the long arm of chromosome 19 (Mannan et al. 2003) was shared between these two samples. For larynx tumours, the most common DNA gain (10/19 samples, 52.6%) was found in the spot corresponding to *CRBP-1* gene followed by *EGFR* gene (9/19 samples, 47.3%). *EGFR* gene presented copy number amplification in four samples ($\log_2 > 2$). It is worth noting that the most frequent DNA gain (for *CRBP-1* and *EGFR* genes) in HPV-positive samples was quite similar in HPV-negative samples. The most common losses were found at the clones corresponding to *RAF-1* oncogene present in 5/19 (26%) followed by ribosomal protein S6 kinase gene, and the subtelomeric 9ptel. These last two genes were present in 3/19 samples (15.7%). To visualize the array CGH results, the data were plotted using the ISCN2matrix converter that allows the online conversion from an aberration list in ISCN format to a band-specific aberration matrix, with optional generation of ideograms, clustering and XML output (Fig. 1). Table 1 shows the clinical data and molecular findings in all the patients. The analysis of correlation between *CRBP-1* gene in clinicopathologic variables are listed in Table 2. It is clear that no statistically significant differences between *CRBP-1* and the variables were found.

To verify our finding array CGH data about *CRBP-1* gene, real-time PCR assay for larynx tumours samples was performed. The fold change in *CRBP-1* gene of some tumour samples was measured in comparison with the B2M gene reference. As expected, *CRBP-1* was amplified in samples which presented amplification in the array CGH with variability in the copy numbers (2.5–4.5 times the degree of amplification). In contrast, no fold change was observed in normal DNA (control) and in larynx tumour without evidence of gene alteration by array CGH (Fig. 2).

The strong correlation between array CGH results for gain of *CRBP-1* gene and DNA copy numbers by real-time PCR lead us to investigate the CRBP1 protein expression in laryngeal cancer samples with or without *CRBP-1* gene gain. Protein expression was determined by immunohistochemistry assay. The positive CRBP-1 immunostaining was quite homogeneous in the transformed cells with an intense cytoplasmic immunoreaction (Fig. 3). As expected,

Table 2 Correlation between *CRBP-1* gene gain and clinicopathological variables in larynx cancer samples

Clinicopathologic variables	*n*	*CRBP-1*		*P* value
		Positive	Negative	
All cases	19	10	9	
Age				
≤60	8	4	4	0.605
>60	11	6	5	
Gender				
Male	16	10	6	0.087
Female	3	3	0	
Clinical stage				
T2	5	3	2	0.556
T3/T4	14	7	7	
Lymph node				
Positive	7	3	4	0.430
Negative	12	7	5	
Histological differentiation				
Well	9	5	4	0.586
Moderate/poor	10	5	5	
EGFR status				
Amplification	9	6	3	0.242
No amplification	10	4	6	
HPV status				
Positive	8	3	5	0.255
Negative	11	7	4	

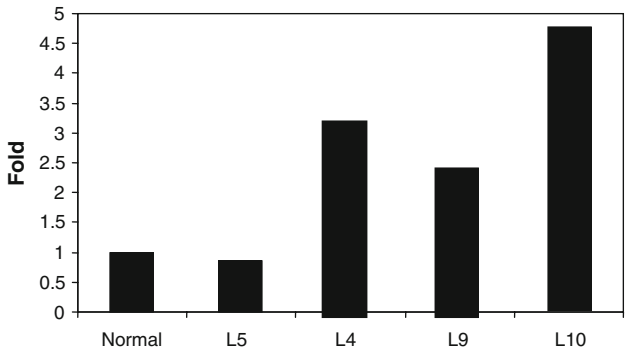

Fig. 2 DNA copy number quantitation of *CRBP-1* gene in laryngeal tumours by using quantitative real-time PCR. *CRBP-1* showed a significant increase in gene copy number above normal in L4, L9, L10 laryngeal cancer samples. For L5 sample no copy number gain was detected. Values above the cut-off line, being assigned as increased gene copy number compared with normal laryngeal epithelium. L5 tumour harboured normal *CRBP-1* gene, in contrast L4, L9 and L10 presented *CRBP-1* gene gain by array CGH

cytoplasmic immunoreactivity was observed in those cancer samples harbouring *CRBP-1* gene gain.

When log-rank tests were used, *CRBP-1* gene gain was found to be related significantly to good overall survival ($P = 0.003$) in the patients included in the array CGH study (Fig. 4), while LaCa samples with *CRBP-1* normal gene (non-alteration) were associated significantly with poor clinical outcome. In contrast, the *EGFR* gene amplification cases showed a poor survival ($P = 0.627$). Univariate Cox regression analysis also identified that clinical variables including clinical stage (TNM), age, histological differentiation, HPV status and *EGFR* amplification were not significantly associated with overall survival (Table 3). Patients showing *CRBP-1* gene gain or expression had a significantly longer survival ($P = 0.008$) than those with normal *CRBP-1* gene.

Discussion

Previous reports of CGH data of larynx tumoural tissues have showed a delimited and specific chromosomal imbalances pattern. However, not much is known about the identity of specific genes that might be the targets of these and other regional chromosomal imbalances. This fact makes the array CGH an attractive strategy to define particular gene targets. Performing array CGH in DNA samples of laryngeal tissues, we were able to detect alterations at particular genes that might be related to the transformation process in the laryngeal epithelium.

Invasive head and neck squamous carcinomas are amongst the cytogenetically most complex tumours, and at present there is not much consensus on the prognostic value of specific chromosomal aberrations. For example, in a recent work of CGH on metaphase analysis of larynx and pharynx carcinomas showed major gains in chromosomes 3q26, 7, 8q24, 5p, 11q13, 17q and 18p11, and losses occurred at 3p, 11qter, 4p, 18q and 5q (Hermsen et al. 2001, 2005). In other study, have also reported different and significant involvement of chromosomes gains and losses in progression of LaCa, detected by CGH, where the most common sites of losses were 13q, 1p and Xp, while the gains in 1p, 9q, 16q. This heterogeneity of results is not consistent with data obtained in our study, this may be due to the technique used and also the samples studied (Schlade-Bartusiak et al. 2005).

In general, our results showed heterogeneity in the hybridization signal in all clones spotted on array without a clear profile, being the *CRBP-1* and *EGFR* genes mapped on chromosome regions 3q21 or 7p12, respectively, were consistently gained. Thus, our results could support a genomic complexity in this type of lesions or simply intrinsic characteristics of the samples (Gebhart et al. 2004; Huang et al. 2002).

CRBP-1 is a protein involved in the transport of retinol from its liver storage sites to the peripheral tissues. Vitamin A plays an important role in a variety of cellular events related to proliferation and epithelial tissue differentiation.

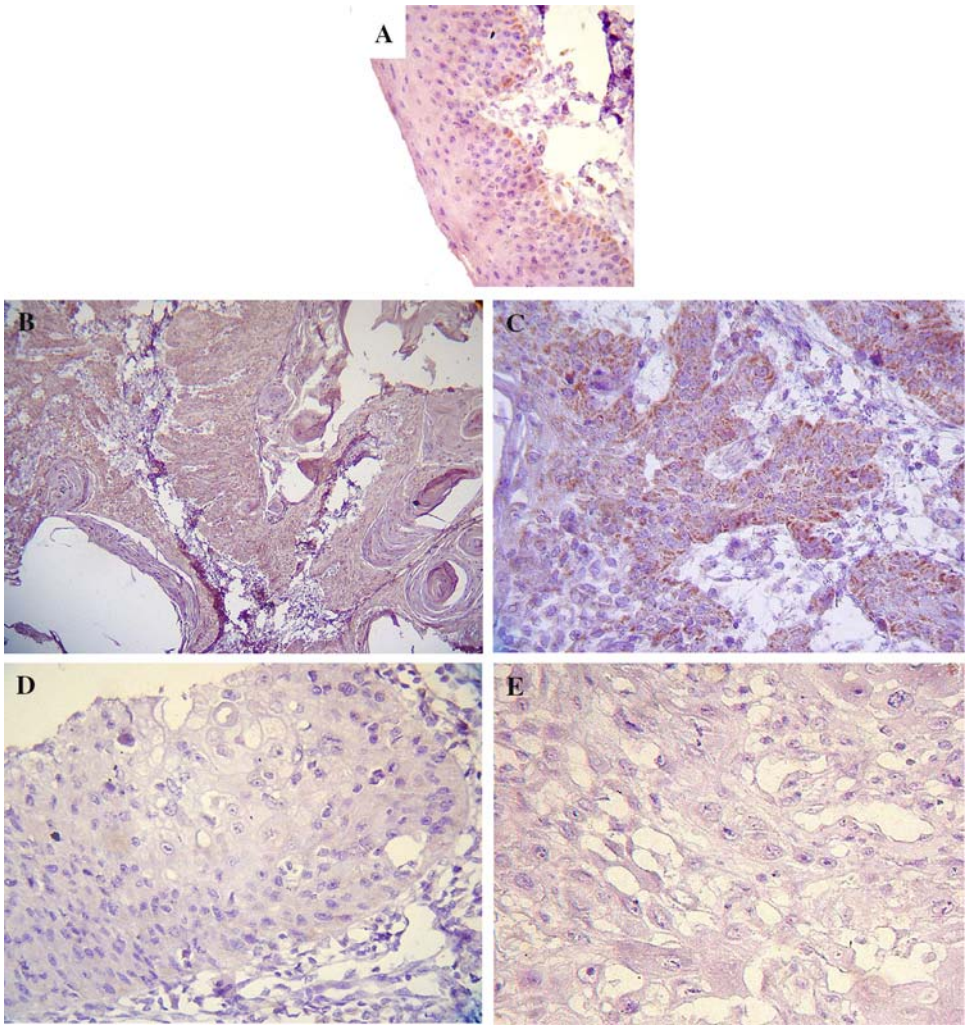

Fig. 3 CRBP1 protein immunodetection in the human larynx tissues. *CRBP-1* expression was observed in the basal cell layer of the normal larynx tissue (**a**), as well as, the immunodetection in the transformed cells of larynx carcinoma tissue harbouring the *CRBP-1* gene gain (L5 and L10). The brownish reaction was detected in the cellular cytoplasm (**b**, **c**). Some larynx carcinoma samples negative (L5 and L15) for *CRBP-1* gene gain did not show any reaction (**d**, **e**). All tissue sections were haematoxylin counterstained. **a**, **b**, **d**, original amplification ×200; **c**, **e**, original amplification ×400

It is known that a clear relationship exists between Vitamin A, CRBPs and cancer (Mrugacz et al. 2005; Baeten et al. 2004; Bellovino et al. 2003; Chen et al. 2002). Specifically, *CRBP-1* has been observed downregulated in breast, prostate and ovarian cancer (for review see Farias et al. 2005). In contrast, in squamous epithelium of the cervix, *CRBP-1* expression is increased with the presence of neoplasia (Esteller et al. 2002). Thus, according to our findings, both mechanisms (up and downregulation) could be present in the LaCa epithelium. An increased concentration of CRBP in squamous cell cancer of the head and neck region (Fex et al. 1986) was reported several years ago. Now, we can mention that the increased concentration could be due to a CRBP amplification result.

One of the main gene alterations observed in head and neck tumours are *EGFR* oncogene amplification (tyrosine kinase activity transmembrane protein receptor), which induce the basal cells differentiation (Baeten et al. 2004). Interestingly, previous reports involving *EGFR* with carcinogenesis process have been noted and associated with poor prognosis (Takeyama et al. 1999; Johnston et al. 1999; Almadori et al. 2001). Our findings could support an important role of these two genes in squamous LaCa.

With respect to lost genes, it was observed that RAF1 gene (Vairaktaris et al. 2007) (oncogene in 3p) was the most significant in 27% of the cases.

On the other hand, it is widely known the important role of HPV and cervical cancer (Walboomers et al. 1999) and some other anatomic sites. Larynx epithelium can also be susceptible to HPV infection. In this context, many reports are claiming two different mechanisms of carcinogenesis in LaCa, depending on this virus infection (Steinberg and DeLorenzo 1996; Gillison et al. 2000; Schwartz et al. 2001; Gillison and Shah 2003). We observed that almost half of the carcinoma samples studied was HPV positive, supporting that a group of the LaCa could be clinical identities associated with HPV infection. Owing to the most common gains detected in *CRBP-1* and *EGFR* genes were observed quite similar in both HPV-positive or HPV-negative larynx samples; our data could indicate that these gene alterations are related with the proper molecular alterations of this epithelium.

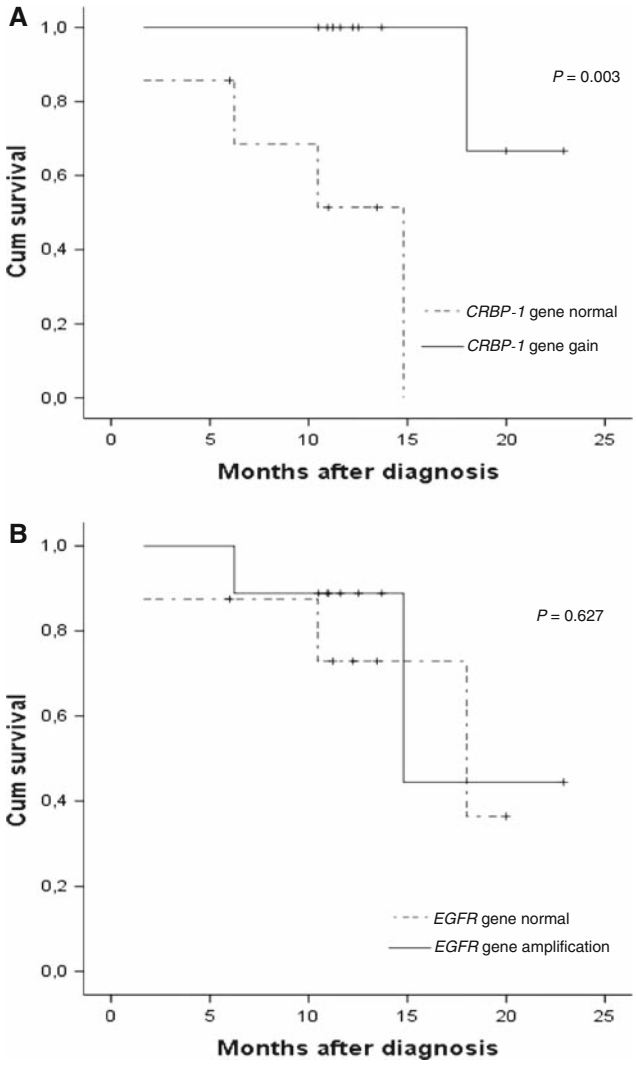

Fig. 4 Survival probabilities of larynx cancer patients with *CRBP-1* or *EGFR* gene alterations. **a** *CRBP-1* gene gain is significantly associated with survival probability ($P = 0.003$ at Kaplan–Meier analysis). **b** *EGFR* gene did not show any correlation with survival probability ($P = 0.627$)

Table 3 Overall survival Cox regression analysis

Factor	RR (95% CI)	*P* value
Age (<60)	0.790 (0.126–4.943)	0.801
Clinical stage (T2)	0.376 (0.029–4.864)	0.454
Lymph node (positive)	6.268 (0.626–62.779)	0.118
Histological differentiation (well)	1.019 (0.141–7.343)	0.985
EGFR (amplification)	1.219 (0.200–7.425)	0.830
CRBP-1 (gain)	0.010 (0.001–39.664)	0.008[a]
HPV (positive)	0.016 (0.001–23.362)	0.266

Cox regression univariate analysis

RR relative risk

[a] Statistical significant

The frequent alteration of *CRBP-1* gene could support its central importance in LaCa pathogenesis. Interestingly, the *CRBP-1* gene gain correlates with its recurrent copy number gain, as well as an overexpression of *CRBP-1* protein and this with a good survival. In contrast, *EGFR* amplification could be associated with poor survival ($P = 0.627$). According to our results, *CRBP-1* gene gain could have a protector effect and then longer survival (Table 3).

In summary, our data suggest that *CRBP-1* gene alteration and its overexpression in larynx squamous cell carcinomas could provide potential prognostic information. More studies should be done and also other outcome measures need to be considered in elucidating the molecular mechanism role of *CRBP-1* gene in LaCa and its relation to other tumour types.

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References

Albertson D, Pinkel D (2003) Genomic microarrays in human genetic disease and cancer. Hum Mol Genet 12:R145–R152

Almadori G, Cadoni G, Cattani P et al (1996) Detection of human papillomavirus DNA in laryngeal squamous cell carcinoma by polymerase chain reaction. Eur J Cancer 32A:783–788

Almadori G, Cadoni G, Cattani P et al (2001) Human papillomavirus infection and epidermal growth factor receptor expression in primary laryngeal squamous cell carcinoma. Clin Cancer Res 7:3988–3993

Atula S, Grenman R, Kujari H et al (1999) Detection of human papillomavirus (HPV) in laryngeal carcinoma cell lines provides evidence for a heterogenic cell population. Eur J Cancer 35:825–832

Baeten JM, Richardson BA, Bankson DD et al (2004) Use of serum retinol-binding protein for prediction of vitamin A deficiency: effects of HIV-1 infection, protein malnutrition, and the acute phase response. Am J Clin Nutr 79:218–225

Bellovino D, Apreda M, Gragnoli S et al (2003) Vitamin A transport: in vitro models for the study of RBP secretion. Mol Aspects Med 24:411–420

Chai LP, Su ZZ, Lei WB, Liang WY (2004) Meta analysis of relationship between laryngeal tumor of Chinese and human papillomavirus infection. Ai Zheng 23:1400–1404

Chen MH, Antoni L, Tazi-Ahnini R et al (2002) Identification of known and novel genes whose expression is regulated by endogenous retinoic acid during early embryonic development of the mouse. Mech Dev 114:205–212

Esteller M, Guo M, Moreno V, Peinado MA, Capella G, Galm O, Baylin SB, Herman JG (2002) Hypermethylation-associated inactivation of the cellular retinol-binding-protein 1 gene in human cancer. Cancer Res 62:5902–5905

Farias EF, Ong DE, Ghyselinck NB, Nakajo S, Kuppumbatti YS, Mira y Lopez R (2005) Cellular retinol-binding protein I, a regulator of breast epithelial retinoic acid receptor activity, cell differentiation, and tumorigenicity. J Natl Cancer Inst 97:21–29

Fex G, Wahlberg P, Biörklund A, Wennerberg J, Willén R (1986) Studies of cellular retinol-binding protein (CRBP) in squamous-cell carcinomas of the head and neck region. Int J Cancer 37:217–221

Gebhart E, Ries J, Wiltfang J et al (2004) Genomic gain of the epidermal growth factor receptor harboring band 7p12 is part of a complex pattern of genomic imbalances in oral squamous cell carcinomas. Arch Med Res 35:385–394

Gillison ML, Shah KV (2003) Chapter 9: role of mucosal human papillomavirus in nongenital cancers. J Natl Cancer Inst Monogr 31:57–65

Gillison ML, Koch WM, Capone RB et al (2000) Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. J Natl Cancer Inst 92:709–720

Harrell FE Jr, Shih YC (2001) Using full probability models to compute probabilities of actual interest to decision makers. Int J Technol Assess Health Care 17:17–26

Hermsen M, Guervos MA, Meijer G et al (2001) New chromosomal regions with high-level amplifications in squamous cell carcinomas of the larynx and pharynx, identified by comparative genomic hybridization. J Pathol 194:177–182

Hermsen M, Alonso Guervos M, Meijer G et al (2005) Chromosomal changes in relation to clinical outcome in larynx and pharynx squamous cell carcinoma. Cell Oncol 27:191–198

Huang Q, Yu GP, McCormick SA et al (2002) Genetic differences detected by comparative genomic hybridization in head and neck squamous cell carcinomas from different tumor sites: construction of oncogenetic trees for tumor progression. Genes Chromosomes Cancer 34:224–233

Huang J, Wei W, Zhang J et al (2004) Whole genome DNA copy number changes identified by high density oligonucleotide arrays. Hum Genomics 1:287–299

Johnston D, Hall H, DiLorenzo TP et al (1999) Elevation of the epidermal growth factor receptor and dependent signaling in human papillomavirus-infected laryngeal papillomas. Cancer Res 59:968–974

Kujawski M, Rydzanicz M, Sarlomo-Rikala M et al (2002a) Rearrangement of 13q chromosome arm committed to the progression of laryngeal squamous cell carcinoma. Cancer Genet Cytogenet 137:54–58

Kujawski M, Rydzanicz M, Sarlomo-Rikala M (2002b) Chromosome alterations reflect clonal evolution in squamous cell carcinoma of the larynx. Med Sci Monit 8:BR279–BR282

Lichter P, Joos S, Bentz M et al (2000) Comparative genomic hybridization: uses and limitations. Semin Hematol 37:348–357

Mannan AU, Nayernia K, Mueller C et al (2003) Male mice lacking the Theg (testicular haploid expressed gene) protein undergo normal spermatogenesis and are fertile. Biol Reprod 69:788–796

McKaig RG, Baric RS, Olshan AF (1998) Human papillomavirus and head and neck cancer: epidemiology and molecular biology. Head Neck 20:250–265

Mineta H, Ogino T, Amano HM et al (1998) Human papilloma virus (HPV) type 16 and 18 detected in head and neck squamous cell carcinoma. Anticancer Res 18:4765–4768

Mrugacz M, Tobolczyk J, Minarowska A (2005) Retinol binding protein status in relation to ocular surface changes in patients with cystic fibrosis treated with daily vitamin A supplements. Eur J Pediatr 164:202–206

Munger K, Howley P (2002) Human papillomavirus immortalization and transforming functions. Virus Res 89:213–228

Mutiu A, Alexiu I, Chivu M et al (2001) Detection of human papillomavirus gene sequences in cell lines derived from laryngeal tumors. J Cell Mol Med 5:49–59

Pérez-Ayala M, Ruiz-Cabello F, Esteban F et al (1990) Presence of HPV 16 sequences in laryngeal carcinomas. Int J Cancer 46:8–11

Pinkel D, Segraves R, Sudar D et al (1998) High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. Nat Genet 20:207–211

Pollack JR, Perou CM, Alizadeh AA et al (1999) Genome-wide analysis of DNA copy-number changes using cDNA microarrays. Nat Genet 23:41–46

Schlade-Bartusiak K, Stembalska A, Ramsey D (2005) Significant involvement of chromosome 13q deletions in progression of larynx cancer, detected by comparative genomic hybridization. J Appl Genet 46:407–413

Schwartz S, Yueh B, McDougall J et al (2001) Human papillomavirus infection and survival in oral squamous cell cancer: a population-based study. Otolaryngol Head Neck Surg 125:1–9

Solinas-Toldo S, Lampel S, Stilgenbauer S et al (1997) Matrix-based comparative genomic hybridization: biochips to screen for genomic imbalances. Genes Chromosomes Cancer 20:399–407

Steinberg B, DeLorenzo T (1996) A possible role for human papillomaviruses in head and neck cancer. Cancer Metastasis Rev 15:91–112

Struski S, Doco-Fenzy M, Cornillet-Lefebvre P (2002) Compilation of published comparative genomic hybridization studies. Cancer Genet Cytogenet 135:63–90

Syrjäden S (2005) Human papillomavirus (HPV) in head and neck cancer. J Clin Virol 32:S59–S66

Takeyama K, Dabbagh K, Lee HM et al (1999) Epidermal growth factor system regulates mucin production in airways. Proc Natl Acad Sci USA 96:3081–3086

Torrente MC, Ampuero S, Abud M, Ojeda JM (2005) Molecular detection and typing of human papillomavirus in laryngeal carcinoma specimens. Acta Otolaryngol 125:888–893

Vairaktaris E, Spyridonidou S, Goutzanis L et al (2007) Diabetes and oral oncogenesis. Anticancer Res 27:4185–4193

Walboomers J, Jacobs M, Manos M et al (1999) Human papilloma virus is a necessary cause of invasive cervical cancer worldwide. J Pathol 189:12–19