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Case Report

Array CGH analysis of the rare laryngeal basaloid squamous cell carcinoma – a case report

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Abstract: The aim of this study was to define copy number alterations in a rare laryngeal type basaloid squamous cell carcinoma (laryngeal BSCC) using high throughput array comparative genomic hybridization. This is the first genome wide screening of a laryngeal BSCC describing the unique events of DNA copy number changes. By NimbleGen Whole Genome Tiling Array CGH (consisting of 72,000 probes) we were able to identify 3,777 genes altered by copy number changes (1,726 genes with copy number gains and 2,051 genes with copy number with losses). The resolution of the array allowed us to identify a new alteration at the 17q21.31 region covering the *DUSP3* gene which encodes the dual-specific protein phosphatase. Functional studies of the altered genes (Database for Annotation, Visualization and Integrated Discovery v6.7 analysis) highlighted molecular pathways including chemokine signaling, cell cycle, adherent junction-, VEGF- and TGF-beta signaling pathways that might be disrupted by copy number alterations in laryngeal BSCC.

Keywords: Laryngeal BSCC, copy number alteration, array CGH

Introduction

The incidence of head and neck squamous cell carcinoma (HNSCC) has been increasing and the disease has one of the lowest survival rates [1]. Among HNSCCs, laryngeal basaloid type squamous cell carcinoma (laryngeal BSCC), which is an aggressive variant of HNSCC, has an extremely low incidence rate (0.66%) [2]. Less than 200 cases have been published till now and only 48 BSCCs were localized in the larynx. Majority of these studies were mainly focusing on the histology of the tumors, while others aimed to identify biological markers of BSCCs [3-5].

Although it is widely accepted that HNSCCs exhibit a large number of chromosomal abnormalities [5, 6], chromosome alterations in laryngeal BSCCs have not been published yet. The aim of the present study was to assess copy number changes by array CGH in a case of laryngeal BSCC.

Materials and methods

Case history

A 60-year old male patient was diagnosed with dysphagia, huskiness and GERD-like symptoms which began few months before he was diagnosed with laryngeal BSCC. Clinical examinations showed hypopharyngeal medial wall lesion involving the right larynx. Laryngomicroscopy showed tumor in the hypopharynx, which occupied the medial wall of the pyriform sinus, the aryepiglottic fold and the pseudovocal chord. Histological investigations revealed basaloid squamous cell carcinoma (**Figure 1**). Ultrasonography showed lymph node of 15mm in size located parajugular on the right side of the neck. After the right functional neck dissection and the lateral pharyngotomy the tumor was removed by partial pharyngectomy in intratracheal narcosis. Tissue samples that obtained from the neck were tumor-free. The patient received 50 Gy irradiation postoperatively (2 Gy

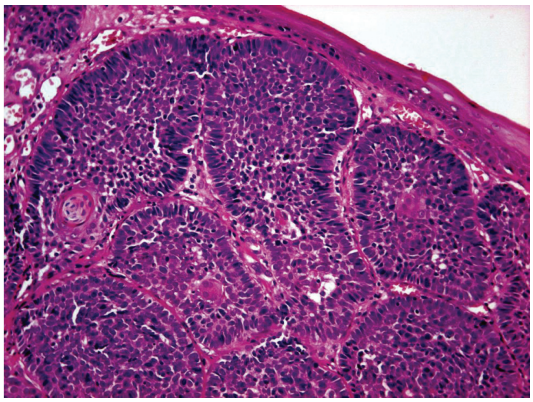

Figure 1. Histopathological characteristics of laryngeal BSCC: invasive basaloid formations embedded in stromal background (H&E staining, x200).

per day). The control MRI tests showed scar tissue mass on the right side of the neck but no signs of tumor recurrence were diagnosed either by physical examination nor CT and MRI. The recurrence of the lesion was excluded by fine needle aspiration biopsy. A written informed consent was obtained from the patient to perform the array CGH analysis.

Genomic DNA extraction and whole genome tiling array CGH

A G-spin™ Genomic DNA Extraction Kit (Intron, Korea) was used to isolate high-molecular DNA from the tumor according to the protocol provided by the manufacturer. The quantity of DNA was determined by NanoDrop ND-1000 UV-Vis Spectrophotometer. DNA integrity was controlled by 1.2% agarose gel electrophoresis.

Genetic aberrations were screened by NimbleGen CGH Whole Genome Tiling Array (HG18 CGH 4x72K WG Tiling v2.0) consisting of 72,000 probes (Core Facility of the Roche NimbleGen, Reykjavik, Iceland). Array CGH data analysis was performed using a NimbleScan software package. Normalization and further statistical analyses were achieved by the Nexus Copy Number 5.1 software. After the exclusion of all data points derived from sex chromosomes, normalization was made by combining replicates within the array by their median. Statistical analysis of the CGH data was performed by FASST2 Segmentation method. In order to adjust the sensitivity of the segmentation algorithm we determined the significant threshold at 1.0E-6, specified 1,000 kb being the maximum spacing between the adjacent probes. The minimum number of probes per segment required to eliminate small CNVs was 5. Gains and losses were defined at ± 3 x SD of all probes and the threshold was adjusted at ± 0.4 for both.

The gains detected by array CGH on chromosome 8 was validated using dual color FISH probes specific for the *C-MYC* (8q24.21) and the for the *LPL* gene (8p22). Hybridization was carried out according to the manufacturer's instruction (Abott Molecular, IL, USA). For functional representation and annotation of genes with copy number alterations we used the Database for Annotation, Visualization and Integrated Discovery v6.7 (freely available Bioinformatics Resources at http://david.abcc.ncifcrf.gov website). Enriched Gene Ontology (GO) groups were ranked according to statistical significance measured by EASE score, a modified Fisher's exact p-value.

Results and discussion

Copy number changes of the laryngeal BSCC tumor sample showed diverse DNA copy number alterations including large and regional gains and losses (**Figure 2**). The full list of copy number changes is summarized in **Table 1**. By detailed analysis of the array CGH data, we found 3,777 coding genes that were affected by copy number changes. Among these genes, 1,726 exhibited copy number gains, while as many as 2,051 genes were involved in copy number losses. Although several genomic regions were affected (1p22.2-p21.2, 2p14-p12, 4q12-q23, 6p25.3, 8p11.22, 14q32.33, 15p11.1-q11.2, 15q11.2-q26.2, 16p13.3-p11.1, 16q11.2-q24.3) by copy number losses, clear monosomy of full chromosomes was not detected on any chromosomes.

Gains involved both arms of chromosome 8, 18 and 20. Using the Nexus software we could detect a small deleted (158 kb) region at 8p11.22 covering 2 genes (*ADAM3A* and *ADAM18*) (**Figure 3B**). In order to validate the polisomy of chromosome 8 we used dual color FISH probes specific for 8p22 (*LPL gene*) and 8q24.21 (*C-MYC gene*) (**Figure 3C**). Both genes were present in three copies in majority of the tumor cells.

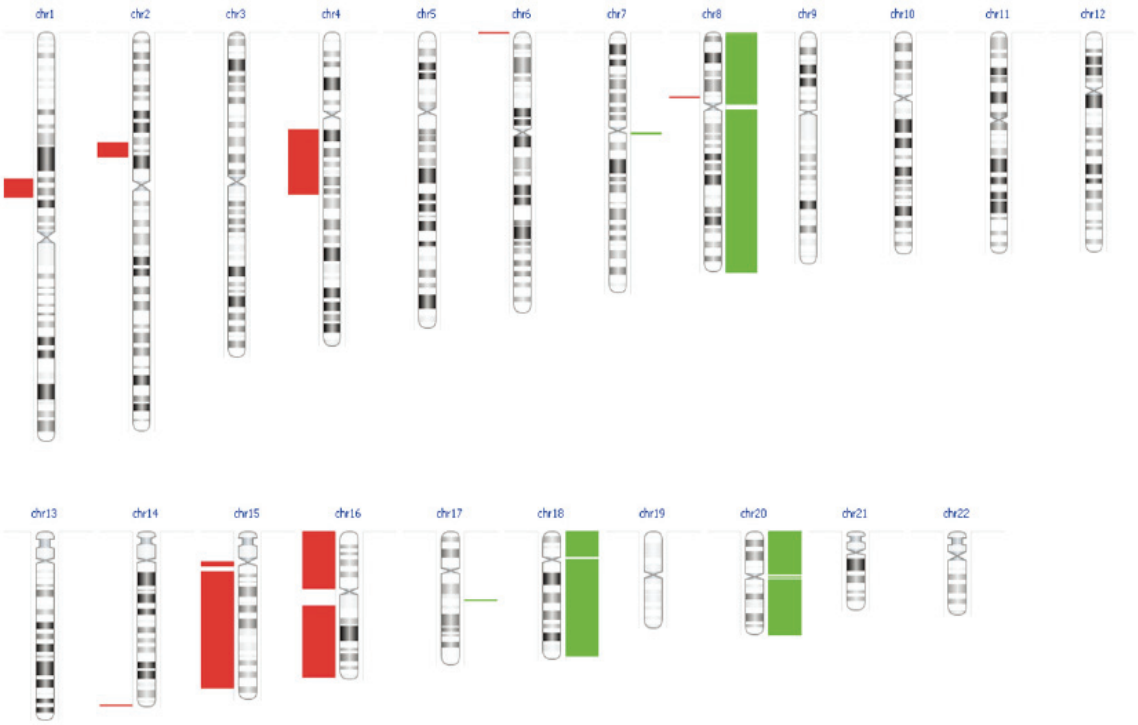

Figure 2. Summary of chromosome copy number alterations of laryngeal BSCC detected by tiling array CGH. Green color on the right side of each chromosome represents copy number gains and red color on the left sides indicates copy number losses.

Table 1. Copy number alterations in laryngeal basaloid squamous cell carcinoma

(A) Copy number gains

Chromosome	Cytoband Location	Region Length (bp)	*Number of genes involved	**CNV overlap (%)
7	q11.1 - q11.21	941,545	0	97
8	p23.3 - p11.22	37,347,557	284	21
8	p11.22 - p11.1	4,365,687	31	22
8	q11.1 - q24.3	99,305,964	480	3
17	q21.31	558,792	15	0
18	p11.32 - p11.21	15,366,783	79	5
18	p11.1 - q23	59,323,179	217	8
20	p13 - p11.1	26,202,881	207	7
20	q11.1 - q13.33	33,728,458	413	5

(B) Copy number losses

Chromosome	Cytoband Location	Region Length (bp)	*Number of genes involved	**CNV overlap (%)
1	p22.2 - p21.2	11,435,375	77	1
2	p14 - p12	9,059,016	104	4
4	q12 - q23	39,861,512	192	5
6	p25.3	193,981	1	83
8	p11.22	158,476	2	78
14	q32.33	780,408	11	82
15	p11.1 - q11.2	2,623,664	5	36
15	q11.2 - q26.2	71,054,501	757	13
16	p13.3 – p11.1	35,042,190	537	21
16	q11.2 – q24.3	43,770,189	365	4

*Analyses were performed using the Nexus software; **CNV overlap is the occurrence of copy number events that exist in healthy donors according to the Copy Number Project database (Wellcome Trust Sanger Institute, Hinxton, UK).

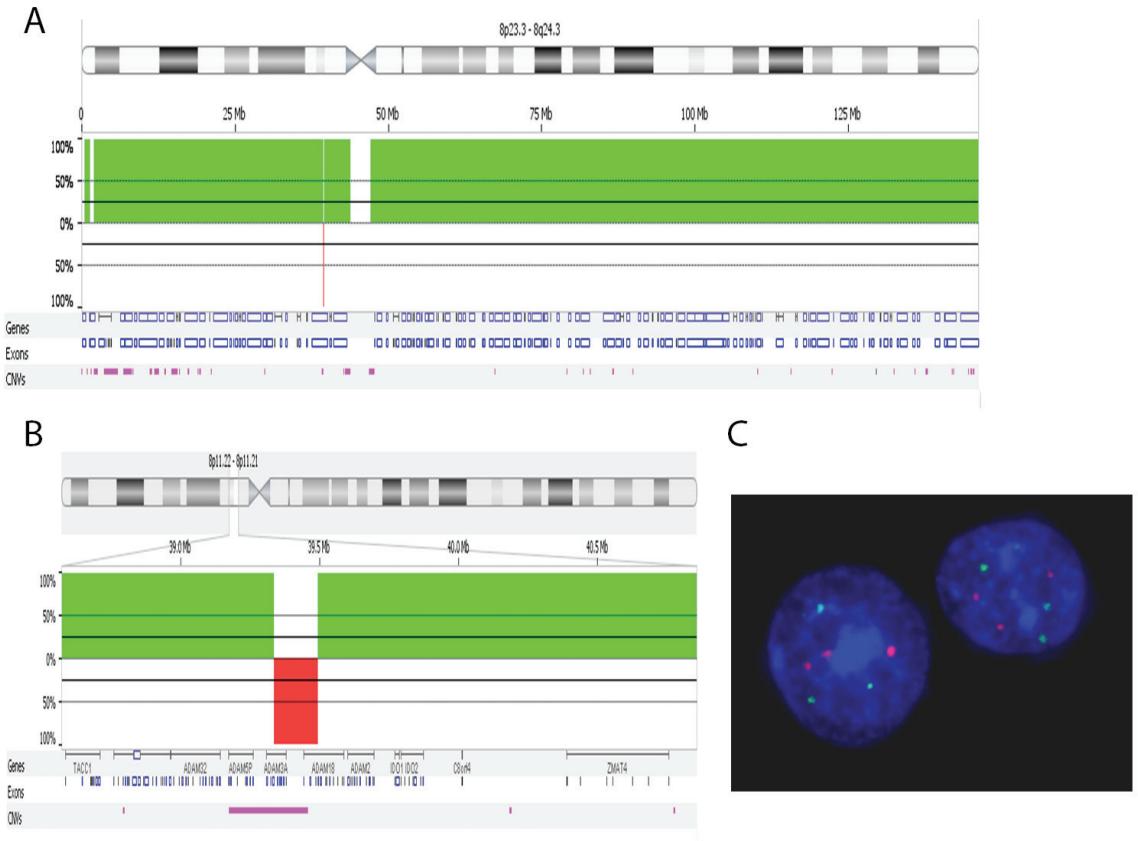

Figure 3. Summary of copy number alterations of chromosome 8 detected by tiling array CGH and FISH. A: Copy number gains were found on both arms of chromosome 8 while a small region at 8p11.22 exhibited loss. B: Enlargement of the 8p11.22 identifies *ADAM3A* and *ADAM18* genes coded by this region. C: Representative FISH images were obtained by dual color FISH: green fluorescence (spectrum green) indicates *LPL* gene (8p22) and red fluorescence (spectrum orange) indicates *C-MYC* gene (8q24.21) confirming the existence of extra copies of both chromosome arms.

A large, 942 kb copy number gain was seen on 7q11.1-q11.21 without any coding region, while there was a 559 kb gain on the 17q21.31 sequence coding 15 different transcripts. The increased copy number of 17q is a frequent event in various tumors [4, 6], but gain on the small 17q21.31 sequence was not described yet. The Whole Genome Tiling Array CGH analysis allowed to map the *DUSP3* gene to this region. Previous studies indicated that upregulation of *DUSP3* could enhance the signaling of ErbB receptors. Its direct role in the inhibition of JNK-dependent apoptosis was also described in epithelioid cancers such as prostate, cervical and non-small cell lung cancer [4].

In order to describe the characteristics and obtain biological relevance of the functions of the copy number altered genes, we determined the Gene Ontology (GO) groups. Based on this analysis 946 out of the 3,777 copy number altered genes had known biological functions. **Figure 4** illustrates the 16 most relevant pathways that can be modified by DNA copy number changes, considering only molecular pathways that exhibit statistically significant EASE score ($p \leq 0.05$) and involve at least 10 genes. It is important to note that beside chemokine signaling, the cell cycle and the adherent junction pathway, VEGF and TGF-beta signaling pathways can also be influenced by DNA copy number changes.

Enthusiastic efforts are being made to characterize the genomic changes of HNSCCs and studies have already shown that DNA copy number changes are important features of laryngeal squamous cell carcinomas [3, 4, 6]. However, the laryngeal type of BSCC represents a rare morphologic variant of HNSCC and this

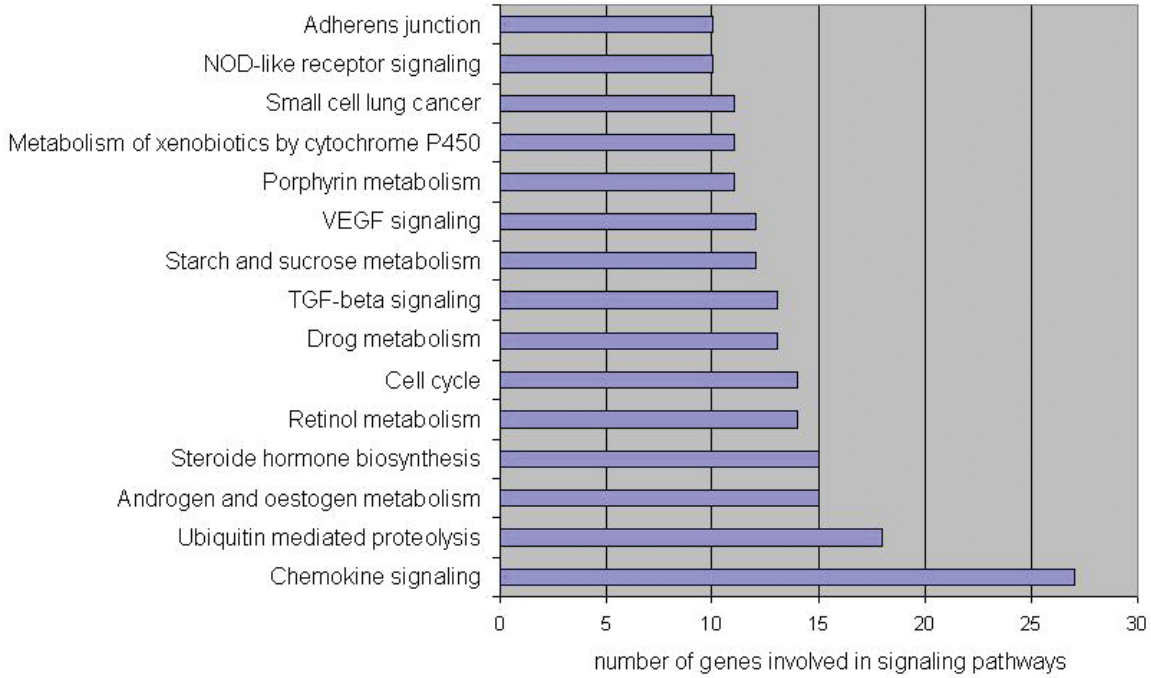

Figure 4. Gene Ontology groups enriched by genes with copy number losses and gains. Only significant groups containing at least 10 genes are mentioned here. Statistical analysis was performed by the modified Fisher's exact test implemented in the Database for Annotation, Visualization and Integrated Discovery v6.7 Software. The gene functional analysis revealed enrichments for otology terms related to diverse pathways, however, involved adherent junction, VEGF signaling, TGF-beta signaling, cell cycle and chemokine signaling pathways.

phenomenon might have a strong influence on the genetic characteristics of BSCCs. Other entities of BSCCs, e.g. oesophageal malignancy, were also investigated by array CGH [7]. Despite considerable progress in the genomic approach, the published data do not suggest any distinct genetic alteration of laryngeal BSCCs. Our study has revealed unique genomic changes and highlighted that the number of alterations were significantly higher in the BSCC than in other types of head and neck cancers.

While 8q gain is considered to be an important genetic alteration of laryngeal HNSCC [6-8], we have shown that both arms of chromosome 8 can be overrepresented in laryngeal BSCC. It is interesting that the only common genetic alteration of laryngeal BSCC with esophageal BSCC is the loss of 8p11.2 [6], however, this region is due to copy number variants in healthy individuals as well, therefore we assume that the 8p11.2 gain might not be a disease specific genetic marker, however, this assumption should be tested. Considering the biological relevance of the protein coding genes disturbed by copy number alterations, we performed GO enrichment analysis and identified several signaling pathways involving VEGF and TGF-beta signaling. The altered functions of these pathways have been previously suggested to play an important role in HNSCC and associated with poor prognosis. Furthermore, similarly to other epitheloid tumors, antisense therapy against VEGF is under development in HNSCC [4, 9, 10], and the downregulation of TGF-beta is also well-defined characteristic of HNSCC [11].

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