Data in Brief

Gene expression analysis of laryngeal squamous cell carcinoma

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1. Direct link to deposited data
   http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE59102.

2. Experimental design, materials and methods

2.1. Study population and clinical data

All the samples were collected from patients undergoing surgical ablation of larynx squamous cell carcinoma at the Head and Neck Surgery Division of the Department of Ophthalmology, Otorhinolaryngology and Head & Neck of Ribeirao Preto Medical School, USP (Brazil). Inclusion criteria were: histopathological diagnosis of LSCC, elective surgeries for LSCC in patients without previous treatments and patients’ allowance to donate part of their tumor for genetic studies. Consent: Informed consent was obtained from all patients included in the study.

A total of twenty nine LSCC tumor samples and thirteen adjacent non-neoplastic tissues proceed for the transcriptome analysis. Clinical information and TNM staging classification of the LSCC patients can be found in Table 1 and Supplemental Table 1. In our study most of the patients were male (96.8%), all of them were smokers and alcoholic, including both current and former. Thirty five percent of the tumors were originated from glottis, followed by larynx (29.0%) and supraglottis (22.6%). Patients were further classified according to the TNM system: 48.4% of the patients

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were in early staging and 51.6% in late staging. Both staging groups showed similar proportions of tumor relapse, cured and dead patients. This study was approved by the Ethics Committee of Ribeirao Preto Medical School, University of Sao Paulo (USP) (Proc. No. 9371/2003) and signed informed consent was obtained from all patients.

2.2. Microarray experiments

After LSCC histopathological confirmation and microdissection of the tumors from their non-neoplastic adjacent tissue, the samples were stored in liquid nitrogen. Total RNA was extracted with TRIzol (Life Technologies, USA). After extraction, the RNA was purified with RNeasy Kit (Qiagen, USA) and quantified with Nanodrop spectrophotometer (Thermo Scientific, USA). Its quality was evaluated by 1.5% agarose gel electrophoresis (28S and 18S ribosomal RNA detection).

For the microarray analysis it was used the Whole Human Genome Oligo Microarray kit 4 × 44K (Agilent, G4112F, USA). cDNA was hybridized to the microarray chip in the Fluidics Station 450 system (Affymetrix, USA), using the Quick Amp Labeling one-color kit (Agilent, USA). As an internal mRNA control, the One Color RNA Spike-In Kit was used (Agilent, USA). The arrays were scanned by the Agilent Scanner G2505C. Agilent Feature Extraction software (version 9.5.3.1) was used to analyze acquired array images.

2.3. Quality control and signal pre-processing

Plain text files were loaded and processed into R with the bioconductor package Agi4 × 44Pre-Process [5]. The annotation package hgu4112a.db [6] was used to assign gene information to each probe. The CV.rep.probes function was used to estimate the percent of coefficient of variation (% CV) for replicated non-control probes, inferring the reproducibility of the arrays (Fig. 1). The gProcessedSignal and the gBGUsed values of each array were loaded as foreground and background signals, respectively into an RGLList object. Next, those arrays were renamed according to each sample clinical information. The following flags were used to filter probes: Control, Well Above BG, Is Found, Well Above NEG CTRLs, Is Saturated, Population Outlier, and Non Uniform Outlier. Additionally probe signals with at least 90% of good features in an experimental condition were selected. Logarithmic transformation of base 2 was applied for high quality probes. Quality control was accessed with package arrayQualityMetrics [7]. Outlier detection did not identify experimental problems. It was performed by looking for arrays (a and b), which the sum (∑) of the distances (d) to all other arrays, ∑dab was exceptionally large; calculating the Kolmogorov–Smirnov statistic (Kₐ) between each array and the pooled data distribution;
and computing Hoeffding’s statistic \( D_n \) on the joint distribution of \( A \) and \( M \) for each array \( (M = \log_2(I_1) - \log_2(I_2); A = 1/2 \left( \log_2(I_1) + \log_2(I_2) \right) \), where \( I_1 \) is the intensity of the array studied, and \( I_2 \) is the intensity of a “pseudo”-array that consists of the median across arrays).

2.4. Normalization

Stage-wise normalization is recommended when data include technical and biological replicates producing a better median chip [8]. Normalization steps were done with the R bioconductor package limma [9]. Technical replicates were normalized between arrays using the smooth function “cyclicloess”. Then, quantile normalization methods were applied, within groups and subsequently between all arrays. Each set of replicated non-control probes has been collapsed into a single value computed as the median of the probes intensities belonging to the same set.

2.5. Differentially expressed genes and pathways analysis

Principal component analysis (PCA) was applied to classify samples by tumor and adjacent non-neoplastic tissue as demonstrated on Fig. 2. We compared the gene expression between tumor and adjacent non-neoplastic tissue using a non-paired-T test analysis. False discovery rate (FDR) adjusted p-value \( < 1e-07 \) and fold change (FC) > 10 were accepted to consider genes to be differentially expressed, identifying a total of 81 probes above this cut-off (Fig. 3). Then we investigated the biological process of upregulated and downregulated genes in tumor compared to normal samples with Web-based Gene Set Analysis Toolkit (WebGestalt) [10]. P-value was calculated using hypergeometric distribution and FDR as multiple hypothesis test correction method. The cut-off was set at \(<0.05\). Tissue morphogenesis (GO: 0009888) and differentiation (GO: 0032332) are the main biological process in upregulated genes while cytokines and growth factor genes are highly expressed in adjacent non-neoplastic patients, suggesting a role on LSCC tumorigenesis.

3. Discussion

We describe detailed technical methods to reproduce the analysis of Whole Human Genome Oligo Microarray kit (Agilent, G4112F, USA) from twenty-nine LSCC tumor samples and thirteen adjacent non-neoplastic tissues. LSCC is the second most common malignancies of the head and neck tumors [1], and this high frequency may be associated with the lifestyle of patients [2–4]. The high quality gene expression data are concordant with important clinical parameters, demonstrating the relevant expression profile signature of differentially expressed genes. This data can contribute to future investigations examining molecular changes that promote LSCC tumorigenesis.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gdata.2015.04.024.

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