Expression of microRNAs in HPV negative tonsil cancers and their regulation of PDCD4

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A B S T R A C T

Global rates of tonsil cancer have been increasing since the turn of the millennia, however we still have a limited understanding of the genes and pathways which control this disease. This array dataset which is linked to our publication (Zhang et al., 2015) describes the profiling of human miRNAs in tonsil and normal adjacent tissues. With this dataset, we identified a list of microRNA (miRNA) which were highly over represented in tonsil cancers and showed that several miRNAs were able to regulate the tumour suppressor PDCD4 in a temporal manner. The dataset has been deposited into Gene Expression Omnibus (GSE75630).

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1. Direct link to deposited data


2. Experimental design, materials and methods

2.1. Patient cohort

The cohort consisted of 43 patients (39 males, 4 females) treated for tonsillar cancer at Royal Prince Alfred Hospital Sydney, Australia between 2002 and 2006. The mean age was 57 years (range 39–80). Seventeen Tonsil Squamous Cell Carcinomas (SCCs) and matched microscopically normal adjacent (2 cm outside the surgical margin) tissues proved suitable for the profiling analyses (see Fig. 1 for experimental overview).
2.4. LNA Microarrays

A commercial LNA-modified oligonucleotides library (Exiqon, Denmark) based on miRBase release 7.1, covering 371 human and mouse miRNA was utilized for expression profiling. Features were deposited onto GAPS II slides (Amersham, USA) at a concentration of 10 μM (Australian Genome Research Facility, Australia). Individual miRNA LNA probes were printed four times on each array. In addition,
all samples were arrayed in technical duplicate. Pre-hybridization array slide was performed in 3 × SSC, 0.1% SDS, 0.2% BSA at 60 °C for 1 h. Each slide was then rinsed in full DEPC treated water, followed by 100% ethanol. Hybridization was then performed in disposable reaction chambers (ABGene, USA). The combined hybridization mixture of 330 μL was injected into the chamber, and then incubated in a hybridization oven with constant rotation at 5 rpm for 3 h at 52 °C. Slides were then washed briefly in 4 × SSC, twice in 2 × SSC plus 0.1% SDS, twice in 0.2% SSC and twice in 0.1% SSC. These were then scanned with a GenePix 4000B Scanner (Axon Instruments, USA). Scanning wavelengths were set between 600 and 700 for the red channel (wavelength 635) and 500–700 for the green channel (wavelength 532). Measures of intensity were assigned to each spot on the array using the software GenePix® Pro Version 5.0 (Axon Instruments, USA).

2.5. Microarray data analysis

Raw data manipulation and downstream statistical analyses were performed using the TM4 suite (http://www.tm4.org) [4]. The first step involved the GenePix Results GPR File conversion using Express Converter Version 1.7 (Institute for Genomic Research, USA). The Microarray Data Analysis System (MIDAS) version 2.19 (Institute for Genomic Research, USA) program was used to normalise raw experimental array data [4]. In brief, three types of normalisation were performed. Firstly, total intensity normalisation was performed, followed by Lowess normalisation and finally, in-slide replicate normalisation. The Multi-experimental Viewer (MeV) Version 4.0 (Institute for Genomic Research, USA) program was used to identify genes and expression patterns of interest. Normalised expression data was loaded onto the program and percentage cut-offs were used to remove genes with invalid expression values, such as buffers. The following operations were performed on the data to extract gene expression information:

1) % cutoff filtering
2) HCL
3) T-test K-Means Clustering
4) Significance Analysis of Microarrays

Fig. 2. Data processing pipeline using the TM4 suite of tools.

Fig. 3. Unsupervised HCL incorporating miRNA signatures from our previous study in salivary gland tumours [3] and other oral cancers [1]. HCL was able to separate the various head and neck tumours into distinct clusters. Red bars denote tumour tissue, and green bars represent adjacent normal tissue.
hierarchical clustering, K-means clustering (KMC), t-test and Significance Analysis for Microarrays (SAM). Refer to Fig. 2.

3. Discussion

Using this dataset we identified eleven differentially expressed miRNAs (>2.0 fold) using the statistical analysis of microarrays (SAM) algorithm [5]. Of these, nine miRNAs were up-regulated and two down-regulated in cancers relative to normal tissue. Of particular note, miR-499, miR-372, miR-18a, miR-21, and miR-30d were up-regulated while let-7c and miR-198 down-regulated. Expression data for selected miRNAs were then validated using two independent patient cohorts which strongly supported the array data. This analysis was then extended to determine if these tonsil miRNAs signatures could be used as potential diagnostic markers. HCL incorporating miRNA signatures from the previous study in salivary gland tumours [3] and other oral cancers was performed. This analysis was able to define the different types of head and neck tumours and cancers (Fig. 3). Clustering was extremely robust, clearly separating tumour from normal groups and separating the different head and neck tumour types.

References