Genomics Data 5 (2015) 272–274

Contents lists available at ScienceDirect

Genomics Data

journal homepage: http://www.journals.elsevier.com/genomics-data/

Data in Brief

Global gene expression profiling reveals a suppressed immune response pathway associated with 3q amplification in squamous carcinoma of the lung

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ARTICLE INFO

Article history:
Received 27 May 2015
Accepted 1 June 2015
Available online 14 June 2015

Keywords:
Squamous carcinoma of the lung
3q amplification
Immune response

ABSTRACT

Chromosome 3q26–28 is a critical region of genomic amplification in non-small cell lung cancer (NSCLC), particularly lung squamous cell carcinomas (SCCs). No molecular therapeutic target has shown clinical utility for SCC, in contrast with adenocarcinomas of the lung. To identify novel candidate drivers in this region, we performed both Array Comparative Genomic Hybridization (array CGH, Agilent Human Genome CGH 244A oligo-microarrays) and Gene Expression Microarray (Agilent Human Gene Expression 4 × 44 K microarray) on 24 untreated lung SCC specimens. Using our previously published integrative genomics approach, we identified 12 top amplified driver genes within this region that are highly correlated and overexpressed in lung SCC. We further demonstrated one of the 12 top amplified driver Fragile X mental retardation-related protein 1 (FXR1) as a novel cancer gene in NSCLC and FXR1 executes its regulatory function by forming a novel complex with two other oncogenes, protein kinase C, iota (PRKCI) and epithelial cell transforming 2 (ECT2) within the same amplicon in lung cancer cell. Here we report that immune response pathways are significantly suppressed in lung SCC and negatively associated with 3q driver gene expression, implying a potential role of 3q drivers in cancer immune-surveillance. In light of the attractive immunotherapy strategy using blockade of negative regulators of T cell function for multiple human cancer including lung SCC, our findings may provide a rationale for targeting 3q drivers in combination of immunotherapies for human tumors harboring the 3q amplicon. The data have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE40089.

Specifications	
Organism/cell line/tissue	*Homo sapiens*
Sex	Male and female
Sequencer or array type	Agilent Human Genome CGH 244A oligo-microarrays and Agilent Human Gene Expression 4 × 44 K microarray
Data format	Raw and normalized data were provided
Experimental factors	24 untreated primary lung squamous tumors were fresh-frozen, with efforts made to use samples with tumor content >70%.
Experimental features	Both aCGH and expression microarrays were performed on the same tumors to identify novel amplified driver genes in 3q26–29.
Consent	All patients consented before starting the study in written form
Sample source location	Thoracic Program, Vanderbilt Ingram Cancer Center, Nashville, TN 37232, USA

1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE40089.

2. Experimental design, materials and methods

Frozen samples from 24 resected lung squamous carcinomas were collected from surgical specimens through the Specialized Program of Research Excellence (SPORE) in the lung at Vanderbilt University

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http://dx.doi.org/10.1016/j.gdata.2015.06.014

Table 1
Top significant functional enrichment of GO biological processes among 194 genes negatively correlated with 3q meta gene expression.

Biological process (GO term)	Number of genes	Adjusted p
Immune response	37	7.28E−08
Immune effector process	22	1.35E−06
Immune system process	45	6.09E−06
Regulation of immune response	20	3.00E−04
Activation of immune response	14	5.00E−04
Regulation of immune system process	25	7.00E−04
Defense response	29	7.00E−04
Positive regulation of immune response	15	8.00E−04
Response to stimulus	98	1.20E−03
Leukocyte mediated immunity	11	1.20E−03

Medical Center and the Department of Veterans Affairs (VA) Medical Center in Nashville, Tennessee. Total DNA or RNA was isolated using the Qiagen DNeasy Blood & Tissue Kit or RNeasy Kit according to the manufacturer's instructions (Qiagen, Valencia, CA). Genomic alterations were determined by aCGH using 244 K CGH oligonucleotide microarrays (Hu-244A, Agilent Technologies). Digestion, labeling, and hybridization were performed at the Vanderbilt Genome Sciences Resource Core by following Agilent's protocol version 4.0 for Agilent Human Genome CGH 244A oligo microarrays. The hybridized arrays were washed and scanned using an Agilent scanner. The array images were then analyzed using Agilent Feature Extraction Software (version 9.5.3.1), which also performs dye normalization for the data. Then Array CGH data were analyzed using Agilent DNA Analytics Software (version 4.0) with ADM^{-2} algorithm, a minimum of three consecutive probes per region, and a minimum absolute average log2 ratio of 0.25 for any given region. The average log2 ratio of 0.8 was defined as the cut-off for amplification or 0.3 for low level gain. Genomic positions are mapped to the hg18 genome build. Evaluation of gene expression was performed at Vanderbilt Genome Sciences Resource Core using the Agilent Human Gene Expression 4 × 44 K Microarray platform using manufacturer-recommended procedures for microarray-based one-color assay. The array was scanned and then analyzed using Agilent Feature Extraction Software (version 10.7.1.1). The raw data and associated sample information were loaded and processed by GeneSpring11 (Agilent Technologies).

3. Results

Consistent with the literature, our data confirmed that chromosome 3q is one genomic region that has most prevalent and significant copy number gain in lung SCC [8]. We further identified a region at 3q26–28 (182,013,954–186,351,959 bp) containing 370 probes representing 41 genes that have highest amplification score ($p = 3.12E-16$) across all 24 samples. Using an integrative genomics approach we previously published [11], we found that 12 out of 41 3q genes are significantly correlated in SCCs (ABCC5, ACTL6A, DCUN1D1, MFN1, ZNF639, DVL3, FXR1, ATP11B, NDUFB5, PIK3CA, DNAJC19 and YEATS2, FDR < 0.05). When we examined 12 gene mRNA levels in TCGA SCC dataset, all of them are significantly unregulated in lung SCC ($n = 502$) compared with normal lung tissues ($n = 51$). Notably, all but one novel amplified 3q gene YEATS2 were reported as candidate driver gene in our previous studies [7,11].

To identify molecular pathways associated with 3q amplicon in lung SCC, we performed Pearson correlation analysis using 12-gene signature on 3q chromosome against whole genome expression data obtained from 24 SCCs. In total we found that 194 gene expressions are significantly negatively correlated with 3q driver gene expression (FDR < 0.05) while the mRNA level of 215 genes is significantly positively correlated with 3q driver gene expression (FDR < 0.05). Gene ontology (GO) Enrichment Analysis using negatively correlated 194 genes revealed that top 10 enriched biological processes are immune response related (Table 1). In addition, we found that 24 out of 26 immune checkpoint genes including CD137, PDCD1 (known as PD-1),HVEM, LAG3, CD40 and CD27 [1] are also negatively associated with 3q gene expression (Fig. 1 and Supplementary Table 1).

4. Discussion

Recently, immunotherapy using blockade of negative regulators of T cell function is an especially attractive approach for multiple human cancer including lung SCC [6]. Yet there is no defined biomarkers that can be used as molecular determinants of response to immunotherapies such as anti–PD-1 therapy [9]. The 3q amplification is considered to be one of the underlying causes of response to the traditional therapy or targeted molecular therapy such as anti-PI3K [5]. Here we described a study combining aCGH and microarray expression on 24 lung SCCs of

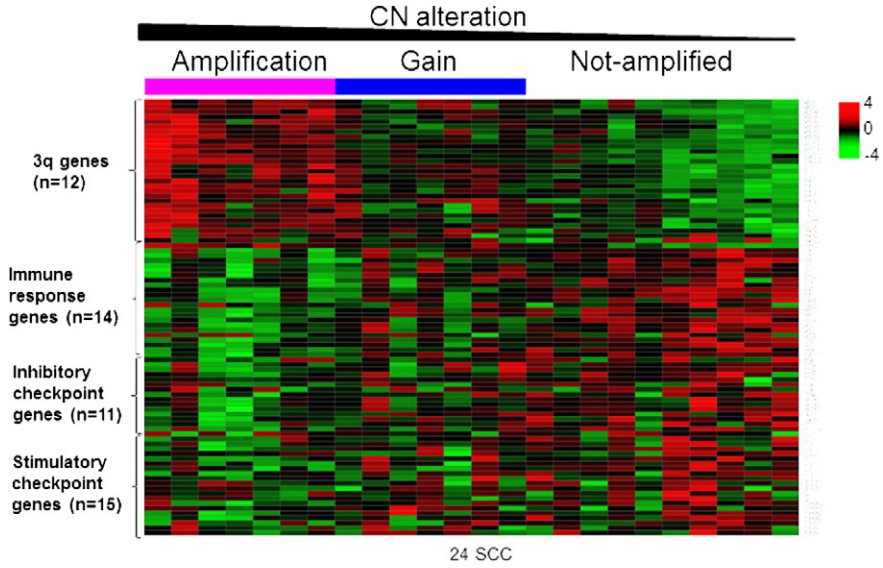

Fig. 1. mRNA level of the immune system related genes including 14 immune response genes, 11 inhibitory checkpoint genes and 15 stimulatory checkpoint genes is negatively associated with 12 3q drive gene expression in 24 lung SCCs. The correlation of indicated gene expression to 3q metagene expression and the corresponding p value was listed in Supplementary Table 1.

the lung that led to the identification of 12 candidate 3q driver genes including a novel driver gene YEATS2, encoding a scaffolding subunit of the ADA2A-containing (ATAC) histone acetyltransferase complex [12], raising the hypothesis that 3q drivers might be involved in the epigenetic regulation and metabolism [2]. Moreover, this study revealed a suppressed immune response pathway in SCC of the lung and its suppression is associated with the overexpression of 3q driver gene expression signature. Being a newly identified cancer gene in lung cancer [7], FXR1 encodes an RNA binding protein that controls the expression of tumor necrosis factor-alpha (TNF-α)[4,10], a key mediator of inflammation in host defense against infection and in autoimmune disease, as well as several T cell response associated cytokines or chemokines such as IL-6, CCL2, and CCL5 [3]. Taken together, this work indicates a potential role of 3q amplicon in the regulation of immune response and may provide a rationale for targeting 3q driver genes in tumors harboring the 3q amplicon who might benefit from immune therapy.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gdata.2015.06.014.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This work was supported by grants to PPM from the National Cancer Institute CA102353 and CA 090949, and from the DOD CDMRP LC090615P3.

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