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Dynamic changes in gene expression profiles of 22q11 and related orthologous genes during mouse development ☆

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Abstract

22q11 deletion syndrome (22q11DS) is a developmental anomaly caused by a microdeletion on human chromosome 22q11. Although mouse models indicate that *Tbx1* is the gene responsible for the syndrome, the phenotypic spectrum of del22q11 patients is complex suggesting that gene–gene and gene–environment interactions are operative in delineating the pathogenesis of 22q11DS. In order to study the regulatory effects of 22q11 haploinsufficiency during development, the expression pattern of the orthologous MM16 genes was analysed in total embryos at different stages (from 4.5 dpc to 14.5 dpc; corresponding to pharyngeal development) by using a low-density oligonucleotide microarray (the "22q11DS-chip"). This microarray consists of 39 mouse genes orthologous to the 22q11 human ones and 29 mouse target genes selected on the basis of their potential involvement in biological pathways regarding 22q11 gene products.

Expression level filtering and statistical analysis identified a set of genes that was consistently differentially expressed (FC>±2) during specific developmental stages. These genes show a similar profile in expression (overexpression or underexpression). Quantitative real-time PCR analyses showed an identical expression pattern to that found by microarrays. A bioinformatic screening of regulative sequence elements in the promoter region of these genes, revealed the existence of conserved transcription factor binding sites (TFBSs) in co-regulated genes which are functionally active at 4.5, 8.5 and 14.5 dpc.

These data are likely to be helpful in studying developmental anomalies detected in del22q11 patients.

Keywords: Microarray; Time-course analysis; Regulation of gene expression; 22q11DS

1. Introduction

Deletion of the 22q11.2 chromosomal region causes the most common microdeletion syndrome in humans with an incidence of approximately 1:4000 live births (Scambler, 2000). The major malformations include congenital heart defects such as truncus arteriosus (TA) and interrupted aortic arch type B (IAA-B), hypo/aplasia of the thymus gland and craniofacial dysmorphism. However more than 180 clinical symptoms are due to 22q11 microdeletion (Ryan et al., 1997).

Although it has been demonstrated that many such phenotypic traits are due to changes in gene regulation of a

Abbreviations: 22q11DS, 22q11 deletion syndrome; FC, fold change; TFBSs, trasncription factor binding sites; DGS, DiGeorge syndrome; RA, retinoic acid.

☆ The data reported in this publication have been deposited in the NCBIs Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) and are accessible through GEO Series accession number GSE5050.

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subset of genes mapping within the critical area (Lindsay and Baldini, 2001; Merscher et al., 2001; Jerome and Papaioannou, 2001; Epstein, 2001; Paylor and Lindsay, 2006), little is known concerning how this microdeletion acts on deregulating the expression of 22q11 genes. Identification of key genes involved in specific developmental processes requires an understanding of the patterns of gene expression in a specific tissue at a specific time. Microarray studies and transcriptional profiling experiments have revealed the involvement of newly discovered genes as well as providing a better understanding of the genetic pathways involved in embryo development in mouse models for the 22q11 deletion syndrome (Prescott et al., 2005; Ivins et al., 2005).

In this study, we evaluated the expression profile of the mouse orthologous genes in embryos at different developmental stages (from 4.5 dpc to 14.5 dpc), corresponding to the pharyngeal development. We used a low-density DNA microarray which includes both mouse genes orthologous to the human 22q11 region and genes considered to be putative "modifier genes" on the basis of their expression pattern and/or their interaction at a biochemical level with proteins encoded by 22q11 mapping genes.

2. Materials and methods

2.1. 22q11DS-chip

Microarrays are constructed by using oligonucleotide probes (50 mer, Ocimum Biosolutions, Indianapolis, USA), which are designed on the nucleotide sequence of the 3′ UTR of 68 genes. The 68 selected genes are 39 genes orthologous to human 22q11 ones and 29 genes mapping outside 22q11. These latter ones are genes involved in retinoic acid metabolism, in embryogenesis and genes already known to be "modifier" genes of the DGS phenotype (Table 1). We verified that each oligonucleotide probe was specific for the corresponding gene by using BLAST software (http://www.ncbi.nlm.nih.gov). Only for *Hira* gene, we decided to design two different oligonucleotide probes to recognize specifically the full-length cDNA and an alternative splicing form including exon 3a (Table 1). All the 68 oligonucleotides together with the positive controls were diluted in a spotting solution (Corning incorporated-Life Science, Acton, USA) at a final concentration of 50 pmol/μl. As positive controls of hybridization we used the oligonucleotides of Scorecard (Amersham Pharmacia Biotech, USA) and as negative controls we spotted only the spotting solution without oligonucleotides.

For array construction, each oligonucleotide probe is mechanically "spotted" onto UltraGAPS glass slides (Corning, USA) using The RoboArrayer™ (Microgrid Compact Plus, BioRobotics, Cambridge, UK). Each oligonucleotide probe is represented in triplicate per array. The printing conditions must be 50–55% humidity and a temperature of 25 °C. Printed slides are dried overnight and cross-linked with UV light at 600 mJ using a Strata-linker 2400 (Stratagene, Glenville, VA, USA). Finally, they are stored in a desiccator at room temperature. Before hybridization, each slide is incubated in a prehybridization buffer (5×SSC, 0.1% SDS, and 0.1 mg/ml BSA) at 42 °C for 45–60 min.

2.2. Preparation and hybridization of cDNA probes

Total RNA from mouse embryos at specific developmental stages is obtained by Seegene (Seegene Inc, Korea). Embryo samples from 4.5 dpc to 6.5 dpc include extra-embryonic tissues and maternal uterus, while samples from 7.5 dpc to 9.5 dpc are conceptuses, including embryo and extra-embryonic tissues. The samples from 10.5 dpc to 14.5 dpc are solely embryos. The RNA reference used in this study was obtained by pooling different CD1 mouse embryos at 18.5 dpc. The reference RNA was isolated by the TRIZOL standard protocol (Invitrogen Corporation, Carlsbad, USA). A small aliquot of RNA was then used for quantification and quality control by a spectrophotometer (Nanodrop, Wilmington, USA) and an agarose gel electrophoresis.

Synthesis of the labelled first strand cDNA was conducted using the Superscript Indirect cDNA labelling system (Invitrogen Corporation) with starting material of 10 μg of total RNA. The amino-allyl labelled dNTP mix was added to the reaction to generate amino-allyl labelled second strand cDNA. Following the hydrolysis reaction, single-stranded cDNA probes were purified using a Purification Module (Invitrogen Corporation). Probe mixtures where then evaporated in a vacuum centrifuge, and the cDNA pellet resuspended in 3 μl of water. The dye coupling reactions were performed by mixing the cDNA samples with AlexaFluor Dyes 555 or 647 and were incubated overnight in the dark. The reactions were purified with a Purification Module (Invitrogen Corporation) to remove the unincorporated/quenched dyes. After the purification, samples were combined for hybridization. The labelled cDNAs were co-hybridized to microarrays in duplicate, with one dye swap. The slides were scanned on the GenePix 4000B Microarray Scanner (Axon Instruments, Sunnyvale, USA) at the optimal wavelength for the Alexa555 (F532) and Alexa647 (F635) (Invitrogen Corporation) using lasers.

2.3. Image analysis and processing

The acquired images were analysed with GenePix Pro 5.0 (Axon Instruments). The oligonucleotide spots were automatically segmented; total intensities as well as the fluorescence ratios of the two dyes for each spot were then calculated. The spots were flagged when they exhibited poor hybridization signals, when they were saturated (F635 median = 65,535 or F532 = 65,535), or when their signal to background ratio was below two. We removed systematic bias in the data by applying the dye-swap normalization (Yang et al., 2001; Fang et al., 2003) in order to have the least possible information loss. Dye-swap normalization makes use of the reverse labelling in the two microarray replicates directly, and is particularly suitable for experiments in which a large number of the spotted genes are expected to change their expression level significantly. Correlations between raw intensity and background were computed for each spot; and background was not subtracted because of the low observed correlations, as suggested by Scharpf et al. (2005).

We compared the fluorescence for each gene at a specific developmental stage (i.e. 4.5 dpc) both against the reference