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DNA Microarray Analysis of Cranial Sensory Ganglia Identifies Genes Involved in Somatosensation in Craniofacial Structures Including Oropharynx Related to Food Intake

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Introduction

Food intake causes various kinds of sensation such as sweet, bitter, hot, cool, soft, hard and so on. These sensations are categorized into two types: gustatory and somatic sensations. Gustatory information is transmitted by gustatory neurons innervating to the taste cells, which are derived from three cranial sensory ganglia: geniculate, petrosal and nodose ganglia (Saper, 2000). Somatosensory information is conveyed by somatosensory neurons projecting into the oropharynx, which are derived from two sensory ganglia, trigeminal and petrosal ganglia (Saper, 2000). Therefore, four cranial sensory ganglia are involved in the reception and/or transmission of sensory information brought about by food intake.

Each of cranial sensory ganglia involved in the food intake-related sensations contains multiple kinds of sensory neurons in terms of morphology, physiology and neurochemical properties. The rrigeminal ganglion (TG) contains ~30 000 neurons, in which at least three kinds of somatosensory neurons such as nociceptor, mechanoceptor and proprioceptor are included. Geniculate ganglion, GG, contains only ~1000 sensory neurons. A majority of GG neurons are gustatory, and somatosensory neurons are also included. The petrosal ganglion (PG) contains ~1300 sensory neurons, in which gustatory, somatosensory and general visceral sensory neurons are included. The nodose ganglion (NG) contains ~40 000 sensory neurons. Gustatory neurons are a minority and a majority are general visceral sensory neurons. Physiologically, gustatory neurons are classified into multiple types and the composition of GG, PG and NG is more complicated. Therefore, each of these four sensory ganglia contains highly heterogeneous types of neurons. neurons that receive or transmit food intake stimuli have not yet been identified in any ganglia. In order to understand neurons involved in the food intake-related sensations, it is necessary to obtain molecular information about neurons in sensory ganglia. We carried out comparative study of gene expression using DNA microarray (Matsumoto *et al.*, 2003). By analyzing DNA microarray data, one possiblity to identify genes involved in specific cellular functions is introduced.

Overall profile of gene expression in four cranial sensory ganglia involved in the food intake-related sensations

Four cranial sensory ganglia (TG, GG, PG and NG) were dissected from male Wistar rats, and total RNA was extracted from respective ganglia. A DNA microarray experiment was performed according to the manufacture's instructions using the Rat Genome U34A array, GeneChip system and software Microarray Suite 5.0 (Affymetrix). Gene expression data obtained were linearly normalized with the expression level of the GAPDH gene. Using expression data of all genes on the microarray, overall comparative properties of gene expression in the four sensory ganglia were investigated by scatter plot analysis. In all of six scatter plots obtained from gene expression data of four sensory ganglia, dots were distributed on or near the diagonal. This result showed similar overall profiles of gene expression among four sensory ganglia.

Genes differentially expressed in sensory ganglia

In order to identify genes showing differential expression, genes were classified and arranged on the basis of their expression patterns in sensory ganglia, using hierarchical cluster analysis, a statistical method that is very useful for analyzing the gene expression data (Eisen *et al.*, 1998; Quackenbush, 2001). At first, genes were divided into six grades based on the expression level and those in intermediate four grades were used for analysis. Genes with ganglion-dependent expression were sorted in both ends by hierarchical cluster analysis (Matsumoto *et al.*, 2003). In eight regions of four grades, 498 genes were contained as candidate genes showing differential expression. Proteins encoded by them are involved in signal transduction, cellular and tissue architecture, energy metabolism, growth and differentiation and so on. In addition, there are many genes related directly to neural properties such as neurotransmission and regulation of membrane potential.

Tissue trees and expression characteristics of gene clusters revealed by hierarchical cluster analysis

By the hierarchical cluster analysis, 498 genes showing ganglion-dependent expression patterns sorted in both ends were arranged on the basis of expression patterns. From gene trees showing the similarity of expression patterns among four sensory ganglia, 37 gene clusters were contained in 498 genes. Expression characteristics of gene clusters were represented as tissue trees, on which information about ganglia showing maximal and minimal level of expression was also shown. By the features of tissue trees of gene clusters such as the shapes of the trees and ganglia showing maximal and minimal expression, 37 tissue trees were divided into 18 groups (Matsumoto *et al.*, 2003).

Next, to investigate whether these features obtained from microarray data are consistent with the expression patterns at tissue and cell levels, *in situ* hybridization analysis was carried out. From 12 groups, one to five genes were selected and their expression analyzed. Among a total of 23 genes, 21 genes showed signals at least one ganglion, most of which are expressed in ganglion-dependent or neuron-type-dependent manner. For example, six genes are

expressed in all of the four sensory ganglia but not ubiquitous in any neurons and four genes are expressed in the three sensory ganglia other than GG. Based on the resemblance of cellular expression patterns like these, eight categories were found. Between cluster groups by tissue trees and categories by cellular expression patterns, significant correlation was observed in terms of 14 genes, although other seven genes did not show such significant correlation. Therefore, it can be concluded that tissue trees of gene clusters are related to cellular expression patterns. This means that using tissue trees as an index we can obtain genes putatively showing some characteristic cellular expression patterns.

Identification of characteristic genes putatively involved in specific neural function

As described above, the relationship between tissue trees and cellular expression patterns were analyzed using some genes in gene cluster groups. However, most of the genes in each of the gene clusters have not yet been analyzed by *in situ* hybridization. We selected three gene clusters showing the highest expression in either TG or PG and the significant relationship in-between in the characteristics of the tissue trees and *in situ* hybridization analysis was carried out for all of 11 genes contained in these gene clusters (Matsumoto *et al.*, 2004). Interestingly, all 11 genes showed similar expression patterns between TG and PG, although population of positive neurons varied depending on genes. They are rarely expressed in GG and NG. These features are consistent with the features found in the tissue trees. Remarkably, six genes are known to be associated with somatosensory function, nociception. Taken together, it is suggested that 11 genes are involved in somatosensory or specific function common to TG and PG.

Here we show one possible approach to identify genes involved in specific cellular functions starting from gene expression data obtained by DNA microarray experiment. During this process, we were able to obtain putative somatosensory genes from 498 genes showing differential expression among four sensory ganglia revealed by hierarchical cluster analysis. Among them, gustatory neural genes should be contained. Further analysis of gene clusters and respective genes will make us understand a molecular logic of sensory neurons, especially about gustatory neurons in these ganglia.

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