Chem. Senses 30 (suppl 1): i12–i13, 2005 doi:10.1093/chemse/bjh088

cDNA Microarray Screening for Taste-bud-specific Genes

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Key words: annotation, expression profile, genetic information, *in situ* hybridization, screening

Introduction

Information about taste signaling molecules is still insufficient to understand the mechanisms of taste signal transductions, while novel taste receptors have been found recently. There are two strategies to discover novel taste signaling related genes; one strategy is homology based cloning and another strategy is global gene screening. Homology based cloning has been mainly used to find out the taste related genes, but we cannot expect the discovery of unexpected genes with critical roles in this strategy. Global gene screening can lead to the discovery of unexpected expressed genes. At present, new techniques for global gene screening are developed, for example, differential display-PCR, SAGE (serial analysis of gene expression), DNA array technology and so on. We tried here gene screening by DNA array technology to find taste-bud-specific genes, then predicted their function using gene expression pattern and genetic information.

Results and discussion

Fabrication of cDNA microarray

To obtain the cDNA microarray containing taste-bud-specific genes at high rate, the mRNA from the epithelium of mouse circumvallate papillae was used for the cDNA library. As described previously (Bonaldo *et al.*, 1996), the cDNA library was normalized and subtracted with cDNA library from the tongue epithelium without taste buds to increase the rate of taste-bud-specific genes in the library (Bonaldo *et al.*, 1996). Using the subtracted library, each of the cDNA clones in the subtracted library was printed on slide glass by the arrayer. Our cDNA microarray contained 3500 clones which were from the cDNA library and known taste related genes.

Probe preparation

Two kinds of fluorescent probes were synthesized from a single taste bud of circumvallate papillae and from the tongue epithelium without taste cells to compare the expression profile between these two tissues. The amount of mRNA from one taste bud is too small to make first strand cDNA (fs-cDNA) probes by standard methods, so we carried out global amplification of fs-cDNA from the single taste bud by the PCR-based method (Brady and Iscove, 1993). We confirmed that the amplified cDNA contained taste-related genes, then labeled it Cy3 or Cy5. The probes from the tongue epithelium were prepared by the same method.

Microarray image analyses

To identify novel taste cell-specific genes, we carried out subtraction-coupled cDNA microarray analyses using the cDNA microarray and the probes from a single taste bud (TB) and epithelium without taste cells (EP) (Figure 1). The method for standard cDNA micro-array image analyses was not suitable for ours, because we used amplified cDNA probe which did not reflect the level of expression so accurately. Therefore, it was determined by our original selection criteria, described below, whether each clone was taste-bud-specific or not. The signal intensity of each clone for the TB or EP probe was plotted as relative intensity (*RI*) to that of the liver-specific gene, apoA1. We confirmed that apoA1 was expressed neither in taste buds nor tongue epithelium, so the signal intensity of apoA1 was treated as background noise ($RI = 1$). The plot area was divided into three sub-areas on the basis of RI (Figure 2). $RI_{tb} > 1$ and $RI_{ep} \leq 1$ was defined as Area 1. (RI_{tb}, *RI* for TB probe; RI_{ep}, *RI* for EP probe). $RI_{tb} > 1$, $RI_{ep} > 1$ and $RI_{tb} > 2 \times RI_{ep}$ was defined as Area 2. The remaining area was defined as 'rejection area'. Using a pilot array with 1172 cDNA fragments, we examined that known taste-bud-specific genes are located in Area 1 or Area 2. Almost all tested genes in the array (e.g. endoA, $G\alpha_{gust}$, T1r3) were plotted in area 1 and 2, indicating that our selection method is useful to select taste-bud-specific genes.

Gene expression pattern analysis

We considered the cDNA clones in Areas 1 and 2 as the candidates of taste-bud-specific genes. Then, *in situ* hybridization analyses were carried out using the probes of the clones to examine their regional expression patterns (Figure 3). Thirty-seven genes were identified to be expressed selectively in taste bud and divided into two groups depending on their expression pattern in taste buds. One group was comprised of the genes expressed in almost all taste cells in taste

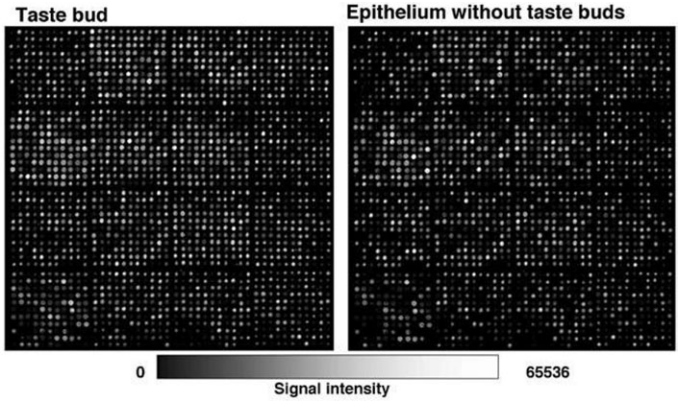

Figure 1 Images from a cDNA microarray after hybridization with fluorescence-labeled probes. Both images are acquired from the same microarray. Left panel: the signals were from the spots hybridized with the probe for single taste bud. Right panel: the signals were from the spots hybridized with the probe for tongue epithelium without taste buds.

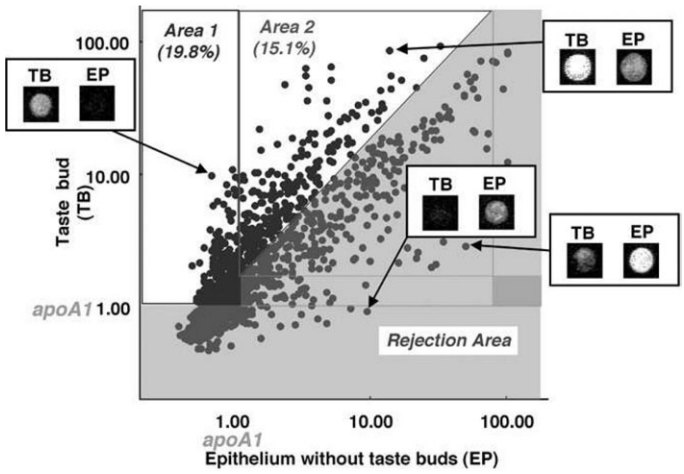

Figure 2 Comparison of spot intensities from images from a cDNA microarray. Relative intensity means the intensity of each spot to the intensity of the apoA1 spot. Longitudinal axis: relative signal intensity of each spot hybridized with the probe for one taste bud. Horizontal axis: the signal intensity of each spot hybridized with the probe for the tongue epithelium without taste buds.

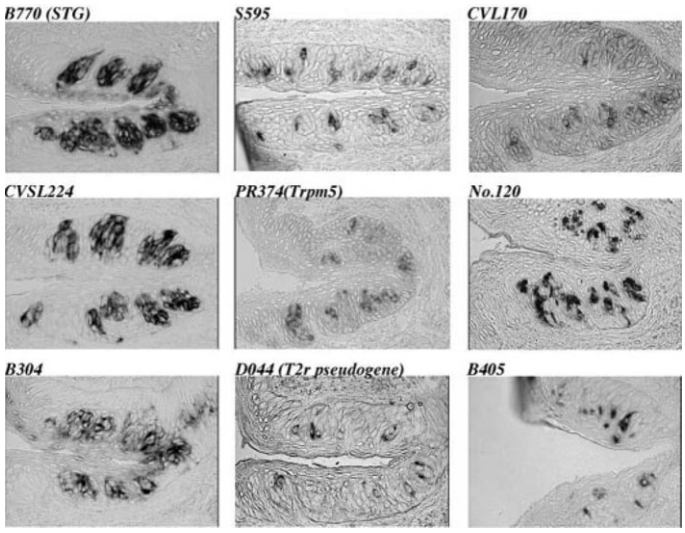

Figure 3 The expression patterns of taste-bud-specific genes in Area 1 and Area 2. Each image represents sections from mouse circumvallate papilla.

buds, e.g. specific taste bud gene (STG) (Neira *et al.*, 2001). Another was comprised of the genes expressed in a subset of taste cells in taste buds, e.g. the T1r family (Hoon *et al.*, 1999; Kitagawa *et al.*, 2001). By comparing the co-expression pattern of the genes expressed in a subset of taste cells with known taste-related genes, we can predict which kind of taste the genes related to. For example, the gene co-expressed with T1r2 and T1r3 might be related to sweet taste. We carried out double-labeled *in situ* hybridization to compare the expression patterns of the genes expressed in a subset of taste cells with known taste signal transduction-related genes. Two genes were co-expressed with T1r2 and T1r3, suggesting that they might be related to sweet taste. One gene was co-expressed with Trpm5, suggesting that it might be related to sweet, bitter and umami (Zhang *et al.*, 2003). And another gene was co-expressed with Mash1, a candidate for a marker of immature taste receptor cells (Kusakabe *et al.*, 2002). Therefore, this result suggested that it might be related to taste cell differentiation.

Genetic information analysis

All the cDNA clones in Areas 1 and 2 were sequenced. Homology search against Unigene revealed that the cDNA clones contained known the taste-related gene, STG, Trpm5, Gβ3 and a pseudogene for bitter taste receptor T2r. From these results, it was confirmed again that Areas 1 and 2 contained taste related genes. About 90% of the clones were annotated by the homology search and all of the genes expressed in a subset of taste cells were annotated. Taken together, these annotations and the information about the gene expression by double *in situ* hybridization, enabled the prediction of the functions of the genes in taste cells in more detail. These predictions suggested the possibility that the product of one clone (No. 120) might interact with IP_3R3, which is the taste signal transduction-related gene. *In vitro* studies of interacting No. 120 and IP_3R3 and a role in taste signal transduction are under investigation.

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