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Research article

Expression and identification of olfactory receptors in sciatic nerve and dorsal root ganglia of rats

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HIGHLIGHTS

- Olfactory receptors (ORs) are present in peripheral nerves.
- ORs are differentially expressed in sciatic nerve and DRGs after sciatic nerve injury.
- ORs are up-regulated in primary culture of Schwann cells under H_2O_2 stimulation.

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ABSTRACT

The olfactory receptor (OR) genes are expressed mainly in the cell membrane of olfactory sensory neurons of the nasal epithelium, and the binding of specific odorant ligands to OR proteins leads to odor detection. ORs are also expressed in non-olfactory tissues and cells, but their functions are often elusive. In this study, microarray analysis was used to detect the presence of ORs in peripheral nerves. We found that a number of ORs were differentially expressed in sciatic nerve and dorsal root ganglia (DRGs) following sciatic nerve injury. The expression and expression profile of several ORs in sciatic nerve were verified by in situ hybridization and real time quantitative RT-PCR. We also observed that the expression of some ORs in primary culture of Schwann cells was up-regulated under H_2O_2 stimulation. Overall, all the results suggest that there may be a possible relationship between the differential expression of ORs in injured peripheral nerves and peripheral nerve regeneration.

1. Introduction

Olfactory receptor (OR) genes were first identified from rats by Buck and Axel in 1991 [2]. ORs belong to the g-protein–coupled receptor superfamily, which is characterized by seven hydrophobic transmembrane domains [17]. It is through the binding of odorous ligands to OR proteins that the mammalian olfactory system can recognize and discriminate a large number of different odorant molecules [2]. To date, OR gene sequences have been cloned from different species including mammals [2,11,12,23], birds [18], amphibians [7], and fish [4]. ORs are expressed mainly in the cell membrane of olfactory sensory neurons of the nasal epithelium, and they are responsible for odor detection by binding specific odorant ligands. However, there have been reports that ORs are also expressed in non-olfactory tissues and cells, such as taste tissue [8], prostate [26], heart [5], spine [19], red blood cells [6] and male germ cells [24]. Although the functions of some of these ORs are known, for example, a human testicular OR mediates human sperm chemotaxis and may be a critical component of the fertilization process [24], not all of them are functional [12], or the functions of most of them are yet unclear.

In this study, we aimed to investigate the expression of ORs in peripheral nerves. The results showed that a number of ORs were differentially expressed in proximal sciatic nerve and dorsal root ganglia (DRGs) following rat sciatic nerve injury. It was further found that the expression of several ORs was up-regulated in primary culture of Schwann cells under H_2O_2 stimulation.

2. Materials and methods

2.1. Tissue collection and microarray analysis

The experiments in this subsection were performed exactly as described previously [13]. In brief, adult male Sprague–Dawley (SD)

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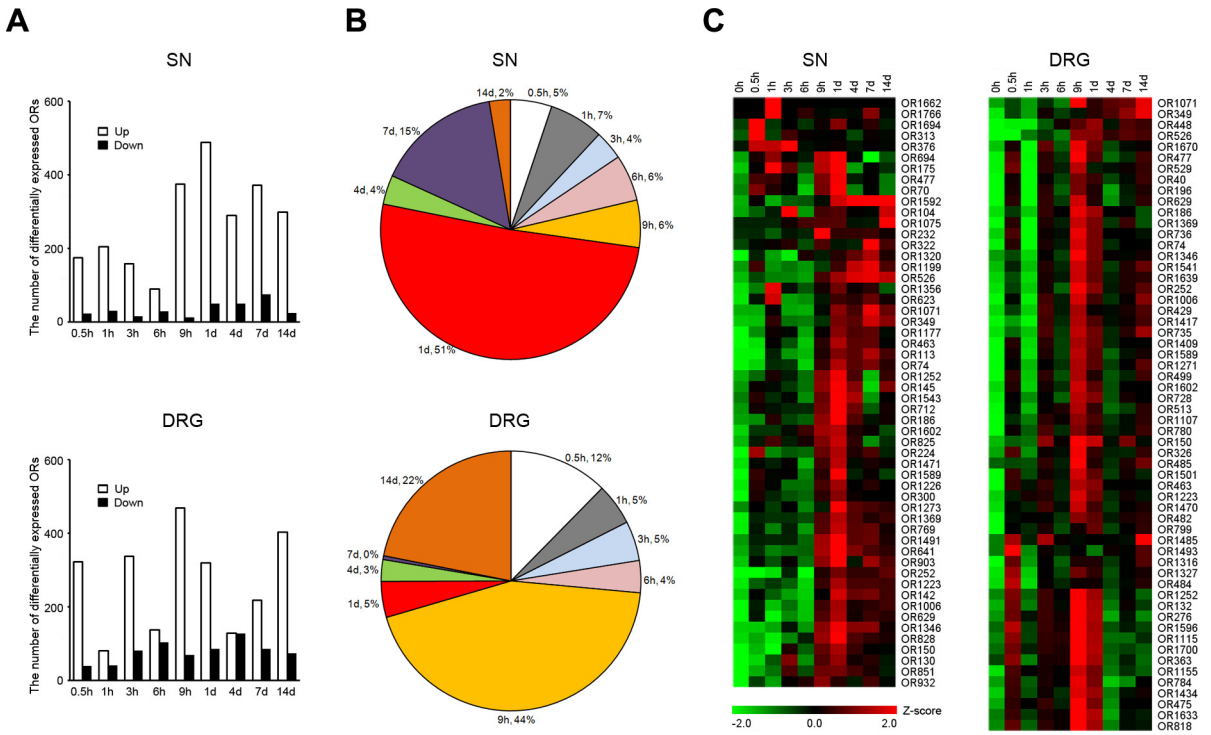

Fig. 1. The expression of ORs in proximal sciatic nerve (SN) and DRGs following sciatic nerve transection. (A) The number of differentially expressed ORs in proximal sciatic nerve and DRGs following sciatic nerve transection. (B) The number percentage of differentially expressed ORs that demonstrated the maximum change (up- and down-regulation) in their respective expression at different times following sciatic nerve transection. (C) Heatmap and cluster dendrogram of differentially expressed ORs that had more than 8 fold changes in their expression at least one time point following sciatic nerve transection as compared to their expression at 0 h following sciatic nerve transection.

rats (180–220 g) were subjected to surgery for sciatic nerve transection, and then proximal sciatic nerve and L4-6 DRGs were collected at different times (0.5, 1, 3, 6, and 9 h, and 1, 4, 7, and 14 days) after surgery. Total RNA was extracted using Trizol (Life technologies, Carlsbad, CA) according to the manufacturer's instructions. Total RNA was amplified and labeled using a Low Input Quick Amp Labeling Kit (Agilent Technologies, Santa Clara, CA). The cDNA was hybridized using a Gene Expression Hybridization Kit (Agilent Technologies) at 60 °C for 17 h in Hybridization oven. Microarrays were scanned by Agilent Microarray Scanner (Agilent Technologies) and the data were compiled with Agilent feature extraction software. All steps from RNA amplification to the final scanner output were conducted by National Engineering Center for Biochip at Shanghai (China).

The raw data were deposited in a MIAME compliant database (NCBI Accession number: Series GSE33175, GSE30165, GSE65053). The differentially expressed ORs were identified via screening when the fold change was greater than 2. Hierarchical clustering was performed on the expression profile of ORs, and the fold change was set at greater than 8.

2.2. Primary culture of Schwann cells and treatment

Schwann cells were isolated from the sciatic nerve of 1-day-old SD rats, and purified by removing fibroblasts with anti-Thy1.1 antibody and rabbit complement (Sigma, St Louis, MO) as described previously [14]. The cell preparation contained 98% of Schwann cells, as assessed by immunocytochemistry with anti-S100 (DAKO, Carpinteria, CA). Primary Schwann cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS, both from Gibco Life Technologies, Grand Island, NY) at 37 °C in humidified 5% CO_2 in air. H_2O_2 was added to cell culture at the final concentration of 0.2 mM to allow incubation for 16 h.

2.3. Quantitative real time polymerase chain reaction (qPCR) and semi-quantitative RT-PCR

Total RNA was isolated from the proximal sciatic nerve using Trizol, and the RNA was reversely transcribed to cDNA by using a Prime-Script reagent Kit (TaKaRa Dalian, China) according to manufacturer's instructions. qPCR was performed using SYBR Green Premix Ex Taq (TaKaRa) on an Applied Biosystems Stepone real-time PCR System. All reactions were run in triplicate. The PCR program was as follows: 95 °C for 10 min; 40 cycles of 95 °C for 15 s, 64 °C for 30 s, and 72 °C for 1 min; and a dissociation cycle consisting of 95 °C for 10 s, 60 °C for 1 min, and 95 °C for 15 s (ramp rate, 1%). The relative expression was calculated using the comparative $2^{-\triangle\triangle Ct}$ method.

For semi-quantitative RT-PCR, the generated cDNA was used as a template for PCR reaction. The thermocycler program was as follows: 5 min at 94 °C; 30 cycles of 30 s at 94 °C; 45 s at 58 °C; 30 s at 72 °C; and 5 min at 72 °C. The sequences of all primers is listed in Supplementary table 1.1.

2.4. RNA probes and in situ hybridization (ISH)

The fragment of ORs was amplified by PCR from total cDNA derived from Schwann cells, and PCR product was subcloned into pGEM-T vector. The orientation and sequence of the fragment were confirmed by sequencing. RNA labeling with digoxigenin-UTP by in vitro transcription with SP6 and T7 RNA polymerase from linearized plasmid containing OR fragment using the DIG RNA Labeling Kit (SP6/T7) (Roche, Mannheim, Germany) according to the manufacturer's instructions.