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IgSF8: A developmentally and functionally regulated cell adhesion molecule in olfactory sensory neuron axons and synapses

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ABSTRACT

Here, we investigated an Immunoglobulin (Ig) superfamily protein IgSF8 which is abundantly expressed in olfactory sensory neuron (OSN) axons and their developing synapses. We demonstrate that expression of IgSF8 within synaptic neuropil is transitory, limited to the period of glomerular formation. Glomerular expression decreases after synaptic maturation and compartmental glomerular organization is achieved, although expression is maintained at high levels within the olfactory nerve layer (ONL). Immunoprecipitations indicate that IgSF8 interacts with tetraspanin CD9 in the olfactory bulb (OB). CD9 is a component of tetraspanin-enriched microdomains (TEMs), specialized microdomains of the plasma membrane known to regulate cell morphology, motility, invasion, fusion and signaling, in both the nervous and immune systems, as well as in tumors. In vitro, both IgSF8 and CD9 localize to puncta within axons and growth cones of OSNs, consistent with TEM localization. When the olfactory epithelium (OE) was lesioned, forcing OSN regeneration *en masse*, IgSF8 was once again able to be detected in OSN axon terminals as synapses were reestablished. Finally, we halted synaptic maturation within glomeruli by unilaterally blocking functional activity and found that IgSF8 did not undergo exclusion from this subcellular compartment and instead continued to be detected in adult glomeruli. These data support the hypothesis that IgSF8 facilitates OSN synapse formation.

Introduction

The coding of olfactory information is crucial for olfactory function, and underlies behaviors such as feeding, mating, aggression and predation. Unlike other sensory systems which employ point-to-point mapping of their receptive fields onto cortical targets, olfactory projections display a convergent topography from the olfactory epithelium (OE) to the olfactory bulb (OB). It is well established that each olfactory sensory neuron (OSN) in the epithelium expresses a single odorant receptor (OR) and all OSNs expressing the same OR target stereotypic glomeruli in the OB (Mombaerts, 2006).

The development of this pathway is precocious, and is necessary for sensory function. In a screen of candidate neurogenic and stem cell genes in developing olfactory sensory neurons, we found high expression of IgSF8, a member of the Ig super family. In the immune system and carcinoma cells, IgSF8 regulates cell motility and polarity via interactions with both direct binding partners (tetraspanins) and indirect partners present within tetraspanin (Tspan) webs (Yanez-Mo et al., 2009). The function of IgSF8 in the nervous system, however, is unknown. Cell adhesion molecules (CAMs) that belong to the Ig superfamily (IgSF) have documented roles in axon outgrowth and navigation (Maness and Schachner, 2007; Plachez and Richards, 2005). In the olfactory system Ig-containing CAMs such as neural cell adhesion molecule (NCAM), olfactory cell adhesion molecule (OCAM) and L1 have been implicated in regulating olfactory development (Miragall et al., 1989; Treloar et al., 1997; Yoshihara et al., 1997). Therefore we selected IgSF8 for further study to test the hypothesis that it has a role in the development of the olfactory pathway.

Here, we characterize IgSF8 expression during the formation of glomeruli, as the OSN axons arrive at the OB. Consistent with a role in OSN development, we describe the differential distribution of IgSF8 along OSN axons and enrichment in newly formed glomeruli. IgSF8 is initially highly expressed in axon terminals, but is down-regulated in adult glomeruli after synapses have been established. When OSN replacement is induced by a chemical lesion, IgSF8 is re-expressed in axon termini. Moreover, expression of IgSF8 is maintained at high levels at later stages of development if functional activity is blocked with unilateral naris occlusions. Furthermore, we demonstrate that IgSF8 interacts with the Tspan CD9 and is present in OB synaptosomes, consistent with the hypothesis that IgSF8 is present in Tspan-enriched microdomains (TEMs) in OSNs. Collectively, these data suggest that IgSF8 has a role in OSN maturation and synaptogenesis.

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Results

Identification of IgSF8 in OE screening

During a targeted microarray screen (OMM-404; SABiosciences, Fredrick MD) of candidate neurogenic and stem cell genes in the developing olfactory epithelium (OE), we selected IgSF8, a member of the Ig superfamily for further study (Fig. 1, and Supplementary Table 1). These arrays identified several known developmentally regulated genes in the OE such as Fmr1, Lhx2, Npn1, Nlgn1, and Nogo (Fig. 1A) and a number of candidate genes (e.g. IgSF8, Jag1 and Pcdhb16; Fig. 1A). A factor in our selection of IgSF8 was the observation that CD9, a known binding partner of IgSF8 was also present in the OE (Fig. 1A). In immune and tumor cells CD9 and IgSF8 modulate integrin-dependent cell motility and/or spreading. A primary reason that we focused our analyses on IgSF8 is the well established roles other members of the Ig superfamily play in olfactory pathway development. For example, OSNs express the cell adhesion molecules NCAM and N-cadherin which are important for olfactory pathway formation (Akins and Greer, 2006; Treloar et al., 1997). Similarly, they express many cell surface receptors which are members of the Ig superfamily, such as DCC, Neuropilin-1, DSCAM and Robos1-2 (Agarwala et al., 2001; Astic et al., 2002; Cho et al., 2007; Nagao et al., 2000).

IgSF8 mRNA expression in the developing olfactory system

Structurally, IgSF8 possesses a short signal sequence at the N terminus, four Ig-like domains, 3 putative N-glycosylation sites, a transmembrane domain and a short intracellular region (Yamada et al., 2006; Zhang et al., 2003) (Fig. 2A). Limited data exists about the role of IgSF8 in the nervous system. During embryogenesis, IgSF8 mRNA has been reported in the developing nervous system including the OE (Murdoch et al., 2003) and in a tissue wide northern screen highest expression was detected in whole human brain (Bonkobara et al., 2003). IgSF8 induces neurite outgrowth of Neuro-2a cells in vitro and therefore has a proposed role in the regulation and maintenance of neurite outgrowth (Yamada et al., 2006). In light of these previous reports, we hypothesized that IgSF8 may have a role in the regulation of OSN axon outgrowth and synaptogenesis. To test this hypothesis, we performed a series of experiments to characterize IgSF8 expression and function in the developing olfactory sensory neurons.

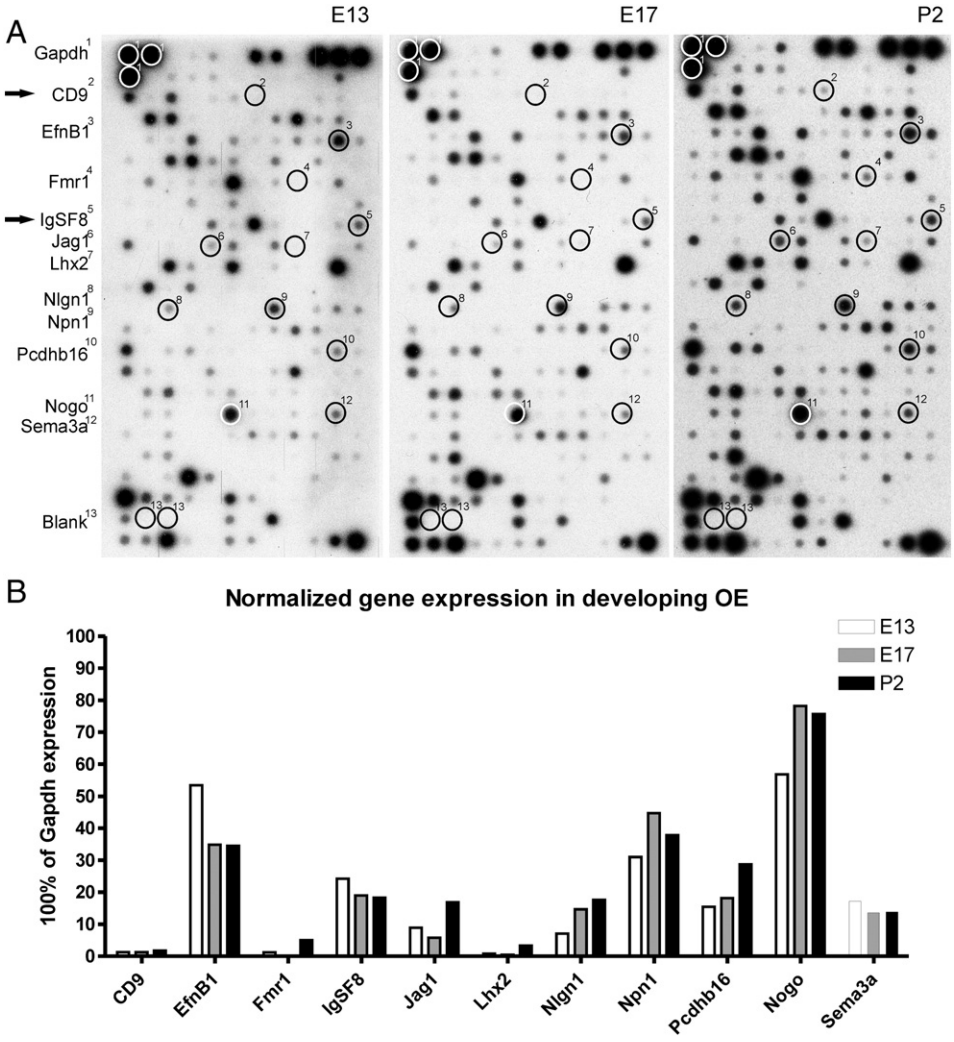

Fig. 1. Gene expression analyses from three different developmental ages (E13, E17 and P2) in the OE (A) using Oligo GE Arrays. (A) A selection of candidate developmentally regulated genes (IgSF8, Jag1 and Pcdhb16) as well as some known developmentally regulated genes (Fmr1, Lhx2, Npn1, Nlgn1 and Nogo) were identified in the OE from the Oligo GE array. (B) Relative expression of the identified genes across three developmental ages normalized to GAPDH gene expression from representative arrays. Spot intensity of genes expressed in the OE was normalized to spot intensity of GAPDH gene. Abbreviations: IgSF8: Immunoglobulin superfamily, member 8; Jag1: Jagged1; Pcdhb 16: Protocadherin beta 16; Fmr1: Fragile X mental retardation syndrome 1 homolog; Lhx2: LIM homeobox protein 2; Npn1: Neuropilin1; Nlgn1: Neuroligin1.