Kidney morphogenesis and primary considerate neurons

A) GFR and Ret51 both are receptors, GDNF is found to promote PNS development and kidney morphogenesis through the receptor complex consisting of GDNFfamilyreceptor 1 (GFR1) and the other receptor tyrosine kinase (Ret).

Ret signal transduction is increased by translocation of GFR. GFR-mediated Ret activation is essential too for the kidney morphogenesis and for various other functions of abdominal precursors that form abdominal nervous system. Also, GFR has many lipid rafts because its GPI anchorage, but Ret is expelled from lipid rafts.

In this paper, the gene replacement for GFR in mice results GDNF resulting in Ret activation but prevented its translocation into lipid rafts. These mice showed renal agenesis, and other disorders including loss of the enteric nervous system, and defects in motor neuron axon path similar to GFR mice that was knocked out, all this provided evidence along with lipid rafts GFR is also needed for neurotrophic factor signaling.

B) Primary considerate neurons secluded from Gfr1and Gfr1TM/TM mice were maintained in vitro for some days. Then they treated the neurons with GDNF or medium for exact time of 15 minutes. The Detergent-resistant membranes quarantined from the neurons were examined by immunoblotting for Ret51.

The comparative purity of detergent resistant and detergent soluble fractions was confirmed by using immunoblotting for caveolin and transferrin receptor, respectively B, the experiments shown in A, were computed and graphed. Otherwise, Substantial decline in the amount of Ret51 was

recorded statistically that translocated into lipid rafts while GDNF stimulation in Gfr1TM/TM neurons compared with Gfr1 neurons.

Similar Results were obtained after performing the experiment four times. Moreover, Lipid raft translocation experiments were performed to prove the concept that GDNF/GFR1/Ret complex does not translocate into lipid rafts in Gfr1TM/TM mice. Primary sympathetic neurons from Gfr1/ and Gfr1TM/TM mice were used to extract detergent-resistant membranes. Upon stimulation of Gfr1/ neurons with GDNF, Ret translocated quickly into lipid rafts.

This was a contrast to Gfr1TM/TM neurons that an evident reduced movement of Ret into the detergent-resistant was recorded because of GDNF exposure. A small portion of Ret that did translocate into lipid rafts while stimulation may be owing to Ret kinase-dependent translocation of Ret into rafts that occurs with slower movements.

There was a significant, 75% reduction in the kinetics of the Ret receptor complex into lipid rafts during GDNF exposure in Gfr1TM/TM neurons according to computation made by these experiments.

C) The negative control design here for confirming the results that Ret doesn't translocate into lipid rafts during GFL activation in Gfr1TM/TM neurons, the primary sympathetic neurons isolated from Gfr1/and Gfr1TM/TM mice will be grown in the same way as test ones, with the only difference that there will be no treatment with GDNF or medium for 15 minutes, and the impact of this will confirm the result to much greater extent upon immunoblotting.