Pierchala Lab Research Description:

Development and refinement of peripheral neural circuits
During development of the nervous system, axonal projections and synaptic contacts are supported by target-derived neurotrophic factors, such as the neurotrophins and the glial cell line-derived (GDNF) family ligands (GFLs). During this period of target innervation, neurons often make excessive projections into their targets that are later pruned back. Development of the neuromuscular junction (NMJ), for example, undergoes a process in which NMJs are initially innervated by several axons, known as polynervous innervation. Then, through a process known as synaptic elimination, weaker connections are eliminated resulting in a 1:1 pairing between the presynaptic motor neuron and the postsynaptic receptor clusters in the muscle. This competitive “pushing out” of weaker axonal connections occurs by the release of inhibitory factors by the successful axons. The delicate balance between growth and survival-promoting neurotrophic factors, and inhibitory competitive factors, is thought to ultimately sculpt the architecture of mature circuits. Peripheral neurons also promote the differentiation and maintenance of their targets. Motor neurons, for example, promote the differentiation of muscle fibers into slow and fast fiber types, and gustatory nerves induce the continual renewal and maintenance of taste buds. My laboratory is interested in understanding the ligand and receptor mechanisms responsible for growth and survival promotion, synaptic elimination and the neural control of target phenotype. We utilize biochemical and cell biological methods for the analysis of transgenic mice in which these receptors and ligands are deleted at specific developmental times. The determination of these mechanisms of survival, cell death and circuit maintenance will enable a more rational approach for the development of therapeutic strategies for diseases and injuries of the nervous system. Some current topics of investigation are:

A p75-Ret Signaling Complex Mediates Neuronal Survival and Death in the Developing Peripheral Nervous System
In the PNS, GFLs signal through their common receptor tyrosine kinase, Ret, to produce signals critical for neuronal survival, migration, and axonal growth. Recently, our lab has discovered that GFL activation of Ret induces the association of Ret with p75NTR in sympathetic and sensory neurons. This interaction is surprising, as p75NTR is a member of the tumor necrosis factor (TNF) family of death receptors, and is a critical mediator of apoptosis in sympathetic and sensory neurons in the PNS. We have also found that Ret interacts with p75NTR upon brain-derived neurotrophic factor (BDNF) stimulation of neurons as well as NGF deprivation, both of which lead to apoptosis mediated by the p75NTR. Moreover, we have identified a functional role of Ret in p75NTR pro-apoptotic signaling, and conversely, a functional role of the p75NTR in pro-growth/pro-survival Ret signaling. Thus, we propose that the newly identified Ret-p75 receptor complex has dual, opposing signals and functions, which depend upon which ligand promotes the assembly of this complex. Current experiments are aimed at determining of the developmental functions of this p75-Ret complex and the mechanisms that dictate Ret-mediated apoptosis.
Functions of Novel Ret Isoforms in the Peripheral Nervous System

Ret encodes a transmembrane receptor that is 20 exons long and encodes two known protein isoforms differing in C-terminal amino acid composition, referred to as RET9 and RET51. Studies of human pheochromocytomas have identified three novel splice variants involving alternative splicing of the 5’-region of Ret. These splice variants involve the skipping of exon 3, exons 3 and 4, or exons 3, 4, and 5 and are referred to as Ret3-, Ret34-, and Ret345-, respectively. Further analysis revealed that these 5’-Ret splice variants are also expressed in normal kidney tissue in both mice and humans, but their functional significance has not been established. The RET3- and RET345- isoforms are predicted to encode Ret proteins, but with deletions in the extracellular domain, while the RET34- isoform is expected to encode a soluble form of Ret, which is secreted from the cell. Our RT-PCR analysis has shown that all three 5’-Ret splice variants are expressed in a wide array of tissues and at different ages in mice, and our preliminary experiments have confirmed the stable expression of these Ret isoforms in transfected NIH/3T3 cells. Interestingly, RET3- functions as a GFL receptor, RET34- can augment full-length Ret activation, and RET345- may act as an inhibitor of full-length Ret activation. We are continuing experiments to determine the expression patterns and functions of these isoforms in vivo, particularly during development.

Schematic of N-terminal Ret isoforms. The Ret3- isoform deletes 43 amino acids of CLD1, a 9 amino acid spacer, and 44 amino acids of CLD2. The Ret 345- isoform also deletes 43 amino acids of CLD1 and a 9 amino acid spacer, but deletes 190 amino acids of CLD2 and CLD3, which abolishes the calcium-binding domain. Unlike Ret3- and Ret345-, the Ret 34- isoform does not maintain its open reading frame (ORF), which causes exon 5 to be translated in another reading frame. This frame shift causes the creation of a stop codon in exon 6, which truncates the protein before the transmembrane domain. Thus, RET34- is expected to encode a soluble form of RET that could be secreted from the cell.
Ligand-receptor mechanisms responsible for development, maintenance and regeneration of the NMJ

We are utilizing molecular genetic methods to determine which neurotrophic factors are responsible for the establishment of NMJs, and which competition factors are necessary for their refinement. Functional reinnervation of the adult neuromuscular junction (NMJ) following injury is a highly regulated process that involves the coordinated effort of motor axon terminals, postsynaptic acetylcholine receptors (AChRs), and terminal Schwann cells (TSCs). Utilizing both nerve crush and cardiotoxin injury paradigms, we are investigating how these same ligand/receptor complexes impact functional reinnervation of NMJs, with the long-term goal of elucidating mechanisms by which enhancement of muscle reinnervation can be achieved.

Role of p75 in the pathogenesis of Huntington’s Disease

Huntington’s Disease (HD) is a dominantly inherited neurodegenerative disorder, currently affecting approximately 30,000 people in the US. Despite the identification of the gene, huntingtin (HTT), responsible for HD in 1993, the pathogenesis of this disorder remains elusive. Htt protein is expressed ubiquitously throughout the brain, and yet degeneration occurs more rapidly and more severely in specific brain regions, of which the striatum is one of the earliest affected. Alterations in neurotrophin signaling have been proposed as a potential mechanism of striatal degeneration in HD. Striatal medium spiney neurons are dependent on brain-derived neurotropic factor (BDNF) from the cortex for development and maintenance. Levels of BDNF and its high-affinity receptor, TrkB, are both reduced in the striatum of HD-like animals as well as in post-mortem striata from human HD patients. Additionally, recent studies reported that expression of p75 is higher in post-mortem striata of HD patients than control striata, suggesting a potential role for an altered balance between TrkB and p75 signaling in HD pathogenesis. We are currently investigating whether germline and conditional p75 knockout mice are altered in their sensitivity to neurodegeneration in a mouse model of HD, and whether changes in p75 signaling coincide with HD pathogenesis.

Immunofluorescence image of a mature NMJ

The presynaptic terminals and axons are labeled in green, and the postsynaptic acetylcholine receptors are labeled in red.
GDNF/Ret signaling complexes in the development of the peripheral taste system

The lingual sense of taste is initially communicated by taste buds, clusters of sensory cells located in highly organized taste papillae, to sensory neurons whose cell bodies are located in the geniculate and petrosal ganglia. The specialized chemosensory cells in taste buds continuously turn over and are replaced throughout life. The molecular mechanisms responsible for the development and maintenance of taste buds are incompletely understood, but chemosensory innervation is critical for the maintenance of taste buds throughout the lifespan. The GFLs are expressed in the tongue during the period of chemosensory innervation, but it is not known whether gustatory chemosensory neurons require GFL/Ret signaling for survival, innervation or phenotypic maintenance. Furthermore, the function of the GFL/Ret pathway in the development and maintenance of taste buds is unexplored. We have recently discovered that Ret is expressed in chemosensory neurons that innervate fungiform taste buds, and that this innervation is impaired in Ret-/- mice. We are currently examining to what extent the GFL/Ret pathway in necessary for chemosensory innervation and maintenance of taste buds, and whether Ret has an early function in the formation of taste papillae. These experiments may aid in the rational design of therapeutic interventions for diseases and injuries of peripheral sensory neurons, potentially including abnormal or impaired taste sensation, conditions known as dysgeusias and hypogeusias.

Chemosensory neurons innervating taste buds express Ret. Immunolabeling with the taste bud marker K8 (blue) and Ret (Red, RFP) of a P9 fungiform papillae from a Ret<sup>dsRed<sup>reporter line. This image demonstrates robust Ret+ intragemmal fibers.