Affinity Purification of Anti-Parkin Antibodies from Rabbit Serum for their Application in Immunohistochemistry

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Background:

Parkin is a mitochondrial autophagy protein that is associated with Parkinson's Disease (PD), as well as auditory function. Parkin dysfunction in the context of PD allows accumulation of protein aggregates, leading to dopaminergic neurotoxicity. Previous work has demonstrated that Parkin plays an essential role in normal auditory function, as *Prkn*$^{-/-}$ mice exhibit significantly reduced hearing sensitivity. However, *Prkn*$^{-/-}$ mice also experience a protective effect from cochlear hair cell death and hearing loss caused by aminoglycosides, which are commonly used antibiotics that can cause permanent hearing loss. Multiple antibodies marketed as suitable for immunohistochemistry (IHC) have been validated using *Prkn*$^{-/-}$ tissues; however, they exhibit non-specific activity. This project aims to generate a highly specific antibody for accurate and reliable detection of Parkin expression.

Project Methods:

Rabbits were immunized using two Parkin peptides, notated P1 and P2, and the resulting serum was collected. A two-step purification process was utilized in this project, first isolating the IgGs from serum via protein A/G columns, and secondly using affinity purification to obtain specific antibodies against antigens P1 and P2. Additionally, two different protocols for affinity purification were tested and compared. Purity of serum, IgG, and specific antibodies was assessed by Western blot and immunofluorescence.

Results:

Utilizing the first purification protocol, IHC antibodies were applied in Western blot, and all demonstrated a prominent ~50kD band specific to Parkin, with some degree of nonspecific binding. Immunostaining confirmed functionality of the IHC antibodies and revealed those against P2 exhibited higher binding specificity. The second purification protocol generated IHC antibodies of similar, if not slightly superior specific interaction.

Conclusion/Implications:

Further investigation of Parkin expression would facilitate a better understanding of how it may be associated with the development of PD, as well as auditory function, with the potential of utilizing Parkin as a therapeutic target in PD treatment and preventing aminoglycoside ototoxicity.