Defining the interface between palladin and actin using crosslinking mass spectrometry

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Abstract: Palladin was discovered in 2000 and has been shown to play a significant role in actin growth, also known as polymerization, which is a key mechanism in cancer metastasis. A single immunoglobulin domain (Ig3) of palladin has been identified as the portion which binds directly to actin and there are several lysine amino acids which have been shown to be responsible for this interaction with actin. Ig domains have not been previously associated with actin binding and it is not yet known which amino acids on the surface of actin participate in its interactions with palladin. Currently we are conducting chemical crosslinking mass spectrometry (XL-MS) experiments to gain a better understanding of which residues are found at the interface between actin and palladin. For crosslinking experiments, actin is polymerized before addition of crosslinking reagents that will form covalent bonds between specific chemical groups on the surface of palladin and actin. We have used the crosslinkers DMTMM, DFDNB, EDC-DE and intend to conduct triplicate trials with DMTMM and BS3 to confirm our results. Future experiments to confirm the XL-MS results will entail the expression of beta and gamma actin in Pichia pastoris. We will use site directed mutagenesis of actin residues identified in XL-MS to determine exactly which residue on actin is responsible for its interactions with palladin. This approach, if successful, will be a powerful tool for identifying specific residues that are involved in the palladin interaction with F-actin, which would consequently allow us to examine the biological role of this interaction, both in vitro and in vivo, using actin-binding-deficient or cancer-associated palladin mutants and has application in the development of therapies to drastically slow pancreatic cancer metastasis.

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