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Proteomic Method Development for the Analysis of Tissue Transglutaminases Role in Cancer Progression

Tissue transglutaminase (tTG), the most significant and diverse member of the transglutaminase family, is an enzyme best known for its ability to covalently cross-link proteins. tTG plays an important role in the progression of many diseases, including cancer. However, the mechanism of tTG's role in cancer is unknown. This research develops the tools to investigate the cross-linking activity of tTG, and future research will study the role of tTG-mediated cross-links in the progression of diseases like cancer. Cross-linking activity in proteins occurs at specific sites, and to analyze cross-linking, these substrate sites must be identified; a three-tiered approach was used to develop the necessary tools. First, small molecules (MEA and CADV) were utilized to identify glutamine sites targeted by transglutaminase for cross-linking. Mercaptoethylamine (MEA) resulted in tTG-modified peptides more readily identifiable by mass spectrometers. Also, over 30 unique glutamine substrate sites on fibronectin were identified in addition to the two previously reported in Transdab Wiki, the tTG cross-linking substrate database. Second, a synthetic peptide, a realistic tTG substrate, was used to cross-link proteins to identify new lysine substrate sites. Six novel lysine cross-linking sites were identified and mapped onto tTG. The third tier consisted of analytical, heuristic methods that identified cross-linked peptides analyzed by tandem mass spectrometry. The methods developed in steps one and two to identify active glutamine and lysine sites were tested using cornified envelope, a sample previously reported to contain a large amount of transglutaminase cross-links. Finally, the method will be applied to study cross-linking in cancerous tissue. Using this three step approach, improvements were made to the data analysis methods for finding potential cross-linked peptides. The tools proposed should allow for the investigation of transglutaminase-mediated cross-linking's role in cancer progression.