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Novel procedure for N-glycan analysis and detection of endo H-like activity in human tumor specimens

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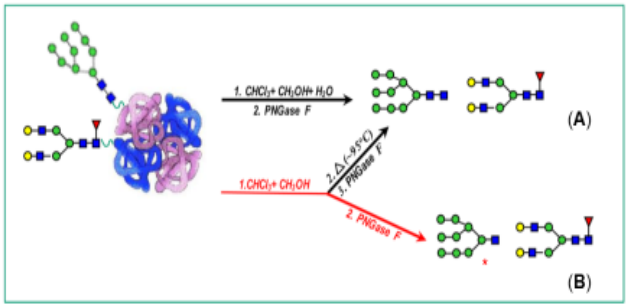
Statement of the problem: Post translational modification of proteins takes place via glycosylation, and changes in glycan structure, which can be associated with biological function, are also seen in malignancies. Efficient assessment of glycans, particularly in clinical settings, can be hampered by specimen size, and lengthy sample preparation. The aim of this study was to develop a method applicable for N-glycan profiling of different specimens: glycoproteins, tumor tissues, and cultured cancer cells requiring low cell numbers.

Method: We have developed an effective procedure for N-glycan analyses using chloroform-methanol (CM) extraction of specimens in the absence or presence of water (CMW), prior to enzymatic cleavage with PNGase F.3-5 Following purification and labeling, the released N-glycan samples were subjected to MS profiling. This procedure was used to determine glycan profiles in cancer cells propagated in vitro, tumor tissues (xenotransplants) and biopsies from lung cancer.

Findings: The method was successfully applied to investigation of N-glycans from small numbers of in vitro cultured cells (≤1x105) and to tumor tissues, including patient biopsies of small size. MALDI-MS analysis confirmed the efficient release of all N-glycan types, including complex forms with poly-N-acetyllactosamine chains. To demonstrate applicability of the method for a broad histological spectrum of cancer cells, N-glycan profiles were also determined in cells of Hodgkin B-lymphoma, choriocarcinoma and histiocytoma. Importantly, in patient biopsy and a number of other specimens from tumors, the non-aqueous CM extraction yielded high-mannose glycans with one GlcNAc moiety termed truncated forms, suggesting preservation of an Endo-H like enzyme activity.

Conclusion & Significance: This method enables practical application of glycan profiling of small-sized clinical specimens, as well as detection of Endo H-like enzymatic activities in cancer cells, which is a previously unrecognized phenomenon. Scheme of extraction conditions for the cleavage of high-mannose glycans and their role in generating Endo H-like activity in analyzed specimens. Note (A) absence of truncated forms with one GlcNAc moiety (red star) following CM extraction of specimens in presence of H20 or exposure to +950C prior to PNGase F treatment; (B) generation of truncated forms by CM extraction.

Influence of Extraction Conditions on the Cleavage of High-Mannose Glycans and Its Role in Endo H-Like Activity Detection in Cancer Specimens



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