

HPLC-MS METHOD FOR DESCRIBING HEPARAN-SULPHATE COMPOSITION OF TISSUE MICROARRAYS

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Cancer research is a dynamically developing area of science and prostate cancer (PCa) is one of the most common types of cancer among men. Tissue biopsies are often used in mass spectrometry based biomarker research and have a great potential understanding biochemical mechanisms underlying diseases such as cancer. Tissue microarrays (TMA) consist of several biopsies (generally 1.5 mm in diameter) of different patients fixed on a microscope slide.

The aim of our work was to develop and apply an advanced nanoLC-MS-based workflow to characterize glycosaminoglycans, in particular the degree of sulphation of heparane sulphate (HS) polysaccharide chains, occurring in tissues (PCa TMA⁺ cores).

The first step of analysis is tissue surface digestion. HS chains were digested into disaccharides with bacterial lyase enzymes prior to the nanoLC-MS analysis. Thus the development and optimization of a chromatographic and MS method for the investigation of the HS disaccharides was performed with the mixture of the commercial standards of HS disaccharides. The separation method is based on the use of novel self-packed HILIC-WAX capillary columns; the effects of the ionic strength, the pH and the eluent strength were investigated and an isocratic separation method was found to be suitable for separating the HS disaccharides. The MS was operated in negative mode, and conditions were also optimized, eliminating fragmentation of the sulfate groups in the ion source. Relative ionization efficiency of the positional isomers was also determined. The limit of detection was ca. 1 fmol for each disaccharide and the limit of quantitation was between 10-50 fmol.

The ratio of the different HS disaccharides was found to be a potentially useful indicator of PCa progression. A good correlation occurred between the cancer grade and sulphation patterns of heparan-sulphate chains; the ratio of doubly and triply sulfated disaccharides increased with cancer progression.

Acknowledgements

Lilla Turiák acknowledges support of the National Research Development and Innovation Office (OTKA PD 121187); Lilla Turiák and Ágnes Révész the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

MS-SPECTROMETR

A high-throughput metabolomics, for the discovery of biomarkers, a group is building such a metabolomic fingerprinting. Mass spectrometry provide same metabolites. Like other fields of data. Handling, processing, specialized mathematical, bioinformatics span through metabolomics standards and mathematical modeling of examples of using this platform metabolic biomarkers in human response will be presented.