GALECTIN-3 LIGANDS OF TOTAL PROTEIN EXTRACTS FROM HUMAN NEUTROPHILS, A PROTEOMIC APPROACH

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Galectin-3, a member of the galectin family of carbohydrate binding proteins, is widely expressed, particularly in cells involved in the immune response. The expression and release of galectin-3 is increased in various situations of infection/inflammation. It binds specifically to oligosaccharides present in many cell surface and cytoplasmatic glycoproteins. Through this cross-linking, galectin-3 induces several reactions involved in innate immunity, such as neutrophil-endothelial interaction and chemoattraction of monocytes and macrophages during inflammation. In order to understand the biological role of galectin-3 and to study how these complexes of galectin-3/ligands modulate these processes, the objective of the present research was to identify these ligands present in total protein extracts of neutrophils. Neutrophils (greater than 95% purity) were obtained from peripheric human blood using a percoll gradient and characterized by flow cytometry and optical microscopy. Proteins were extracted with a buffer containing 8% Urea, 2% Thiourea, 4% CHAPS and protease inhibitors and submmited to 10% SDS-PAGE gels. Ligands of galectin-3 were detected using lectin blot procedure with galectin-3/alkaline phosphatase and revealed with NBT/BCIP. Five galectin-3 immunopositive bands from 45 to 220kDa were observed and trypical hidrolysates were submitted to mass spectrometry for protein identification.

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