Malwina Maria Michalak
Dr. sc. hum.

Galectin-4 structure and functions in colorectal cancer cells using mass spectrometry-based proteomics and phosphoproteomics

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Doktorvater: Prof. Dr. rer. nat. Jürgen Kopitz

Galectins, a group of structurally related carbohydrate binding proteins (lectins), have the capacity to translate glycan-encoded information on the cell surface into effects on cell growth and differentiation. Expression of several galectins and their serum levels have been found altered in cancer patients compared with healthy controls. Correspondingly, binding of galectins to the cell surface of tumor cells was suggested as modulator of the cells growth behavior and differentiation. In particular, for galectin-4 a growth inhibitory effect on tumor cells was recently described. However, the molecular base of its action on tumor cell growth is still largely unexplored. In order to screen for gal-4 induced changes in the molecular phenotype of colorectal cancer (CRC) cells in depth analysis of proteome changes was applied in gal-4 treated CRC cells as compared to untreated controls. These studies were supplemented by phosphoproteomic analysis in order to get hints about the signaling pathways that are involved in the gal-4-induced effects.

Since even subtle deviations or modifications of galectins structure can have considerable impact on their glycan binding and thereby their functions, first of all comprehensive MALDI-based mass spectrometric methodology has been established to check primary structure of recombinant galectins that are used in studies of their physiological function. In this way also the wild-type structure of recombinant galectin-4 was confirmed.

Growth inhibitory effects of cell surface binding of gal-4 were confirmed in 5 human colon carcinoma cell lines: LS 180, Vaco 4323, Colo 205, CX 1 and HCT 116. All tested cell lines responded with differentiation and reduced proliferation to gal-4 treatment. In the next step the cell line LS 180 was chosen as a model for detailed examination of proteome changes and phosphorylation alterations associated with tumor growth inhibition induced by cell surface binding of gal-4. Due to its robustness, easy implementation and quantitative reliability SILAC was chosen as the best method available for MS-based quantification and applied

together with high-throughput nanoLC-ESI-LTQ-Orbitrap for global analysis galectin-4-induced proteomic and phosphoproteomic changes in the CRC cells.

In proteomic analysis, 2654 individual proteins were quantified: 190 were found down- and 115 were up-regulated >2-fold. 1D annotation analysis of the results indicated down-regulation of DNA replication-associated processes, while protein presence for secretory and transport functions appeared increased. The strongest induction was found for CALB2 (calretinin; ~24-fold), TGM2 (protein-glutamine γ-glutamyltransferase 2; ~11-fold), S100A3 (~10-fold) and GSN (gelsolin; 9.5-fold), most pronounced decreases were seen for CDKN2A (tumor suppressor ARF; ~6-fold), EPCAM (epithelial cell adhesion molecule; ~6-fold), UBE2C (ubiquitin-conjugating enzyme E2C; ~5-fold), KIF2C (kinesin-like protein KIF2C; -fold) and LMNB1 (lamin-B1; ~5-fold). Remarkably, presence of the common cell proliferation marker Ki-67 was diminished by about a factor of 4. Further phosphoproteomic analysis at 2 time points revealed early and sustained effects induced by gal-4. Early reaction indicated modulation of membrane organization, vesicle-mediated transport as well as microtubule-kinetochore attachment by hypophosphorylation of BET1 (~3-fold) and hyperphosphorylation of CENPF (centromere protein F; ~2-fold), among others. Sustained phosphorylation changes provided additional information to the observed proteomic alterations, suggesting role of cytoskeleton organization in gal-4-induced phenotype shift by phosphorylation of CFL1 (cofilin; ~5-fold) – a downstream effector of Rho-signaling, and thereby its inactivation. Enrichment analysis of motifs modulated upon long-term treatment with gal-4 indicates activity of Akt kinase with hyperphosphorylation of AHNAK (~2-fold) – a protein involved in cell proliferation and differentiation, being one of the potential substrate.

Gal-4, as a part of interactive galectin network with additive functionalities, caused increase of cellular levels of growth regulatory gal-1 (~4-fold) and gal-3 (almost 2-fold). Moreover, established multiplex bead-based immunoassay for simultaneous galectin profiling revealed also higher levels of extracellular gal-3 in cultures of gal-4 treated cells.

Taken together, tracing significant alterations of protein expression and phosphorylation likely relevant for the observed phenotypic effects reveals the capacity of galectin-4 to affect signaling of human colon cancer cells at multiple sites, thereby defining promising starting points for further characterization of the role of gal-4 in CRC development and progression.