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Functional and quantitative proteomics using SILAC in cancer research

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In recent years, mass spectrometry-based proteomics has emerged as a powerful tool in cancer research and the importance of quantitation strategies has become appreciated. Several methods based on stable isotope coding and MS have been established and have become increasingly popular as alternatives to 2D-PAGE-based methods (Gorg *et al*., 2005). Among these methods, stable-isotope labeling by amino acids in cell culture (SILAC) has shown great promise for the simultaneous identification and quantitation of complex protein mixtures (Mann, 2006). Here we present two applications of functional and quantitative proteomics in cancer research. In the first approach, the discovery of novel FGFR3-related effectors and signaling mechanisms is being addressed. FGFR3 is one of the four receptor-tyrosine kinases that respond to fibroblast growth factor (FGF) and its overexpression has been related to bladder cancer, cervical carcinomas and multiple myeloma. In our experiments a bladder cancer cell line (RT112) and the HEK293 cell line stably transfected with FGFR3 are being used together with the SILAC strategy to compare protein expression levels in pY IPs from cells stimulated or unstimulated with FGF9/heparine with the aim to propose a signaling network for this receptor. This would help to understand the role of FGFR3 in cancer and the pathways that confer the oncogenic potential and how they differ from the “healthy” situation. In our second approach, two colorectal cancer cell lines (KM12C and KM12SM) representing two stages of cancer progression (metastatic vs. non-metastatic) are being used to compare differences in protein expression. The study has been focused on the membrane proteome since 2/3 of the current protein targets for drugs are membrane proteins. Differentially expressed proteins are providing us clues for the discovery of novel potential biomarkers and drug targets for colorectal cancer and to better understand the mechanisms involved in cancer progression.

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