

Chemical Biology (Poster Abstracts)

Quantitative Phosphoproteomics predicts Kinase Activities involved in E-cadherin signaling.

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518 human protein kinases and 107 protein phosphatases form an essential part of the cellular signaling network. Recent progress in proteomics allows the identification and quantitative analysis of phosphopeptides by LC-MS/MS and offers a new strategy to systematically re-analyze signaling pathways. E-cadherin is decisive for the formation of cell-cell contacts and tightly regulated during any kind of tissue morphogenesis. It shares downstream components with other pathways such as the Wnt or the HGFR signaling. However, the connectivity and the flux of information between these fundamentally important pathways remain incompletely characterized. Furthermore, the E-cadherin pathway can be modulated and subverted by the human pathogen *Listeria monocytogenes*. Direct interaction between the virulence factor Internalin A and E-cadherin induces the uptake of the bacteria into epithelial cells. Here we report a strategy to reveal network components that are part of the E-cadherin signaling pathway, which also can be applied to analyze crosstalks between different pathways and cellular processes: (i) We induced E-cadherin signaling in two different cell lines and identified regulated phosphoproteins by combining iTRAQ™, LC-MS/MS and an exhaustive statistical approach for the evaluation of regulatory peptide data. (ii) Kinase activities that might be responsible for the experimentally observed phosphorylation events were predicted based on validated relations between kinases and substrates. We presume 7 new kinases that might specifically contribute to the E-cadherin pathway. Outlook: Noteworthy, recent progress in proteomics (Wissing et al, 2006, MCP) will allow to monitor the phosphorylation events directly at the human kinases in order to reveal their activity states. About 30% of the human kinome was covered by affinity chromatography based on immobilized small kinase inhibitors and give access to known and novel regulatory phosphorylation sites.