Endogenous Phosphotyrosine Signaling in Zebrafish Embryos

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Study Design
Here we explored whether LC-MS-based analysis of the proteins trypic digests can be complemented with kinase activity based on PamChip arrays for global analysis of in vivo signaling processes involved in zebrafish development.

Key Findings
Tyrosine phosphorylated proteins were immunoaffinity-purified from zebrafish embryos at 3 and 5 days postfertilization (dpf) and identified by multidimensional liquid chromatography mass-spectrometry (LC-MS). Among the identified proteins were tyrosine kinases, including Src family kinases, Eph receptor kinases, and focal adhesion kinases, as well as the adaptors proteins paxillin, p130Cas, and Crk. The LC-MS identified kinases, were confirmed by incubation of the zebrafish lysates on PamChip<sup>®</sup> microarray containing peptide substrates (figure 1). Immunoprecipitation studies were used to confirm the findings. In short, anti-Tyr(P) immunoprecipitates were blotted and probed with antibodies specific for proteins identified by mass spectrometry, notably paxillin, Src, and Eph receptor, with actin as control (figure 2, see also Lemeer et al 2007).

“Author Quote”
Our experiments are the first to show that global tyrosine phosphorylation-mediated signaling can be studied at endogenous levels in complex multicellular organisms.

Background
Embryonic development is tightly regulated, and numerous developmental processes like cell growth, differentiation, and migration are controlled by phosphotyrosine signaling, which is mediated by protein-tyrosine kinases and protein-tyrosine phosphatases. Zebrafish is an established model organism for vertebrate development and human disease. Zebrafish embryos are optically transparent, fertilization is external, and after 5 days of development most organs are formed, making it an ideal system to study development of organisms.

Conclusion
In vivo observed tyrosine phosphorylation by mass spectrometry, in vitro kinase activity by PamChip<sup>®</sup> arrays and Western blot show the value of confirmatory use of three technologies in the field of functional proteomics.

References: