Graduate Studies
Department of Physiology
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Protein Kinase Signaling, Proteomics, and Breast Cancer

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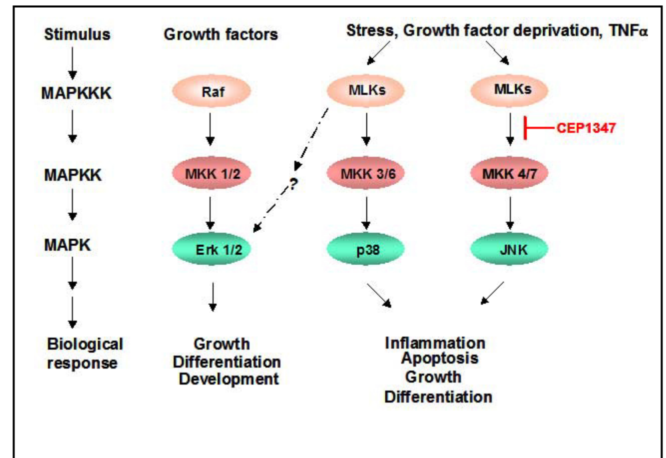
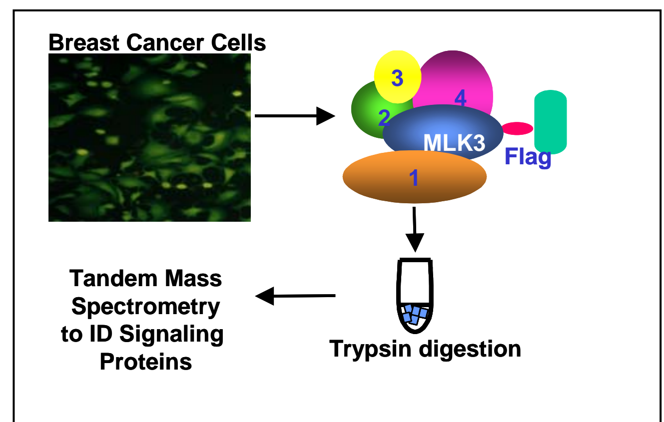
Protein phosphorylation is a dynamic and reversible event essential to the proper functioning of physiological processes, including cell proliferation and programmed cell death. Because protein phosphorylation is a regulatory event, it follows that the protein kinases that catalyze phosphorylation, should themselves be subject to regulation. The improper regulation of protein kinases has been implicated in many human pathologies, including cancer. The overall goal of the Gallo Laboratory is to understand the molecular basis by which protein kinases and their signaling pathways are regulated in normal cells and in cancer. The mixed-lineage kinases (MLKs) are a family of serine/threonine kinases that function as mitogen-activated protein kinase kinase kinases (MKKKs) to activate the JNK pathway. In some experimental settings, the MLKs may also activate the ERK, p38 MAPK and nuclear factor Kappa B (NF-kappa B) pathways. The MLKs have garnered attention as important mediators of apoptosis, particularly in neuronal cells.



Our lab is using MLK3 as a paradigm to study the mixed lineage family of protein kinases. MLK3 contains several potential protein-protein interactions domains that likely contribute to its regulation and signaling specificity, including an N-terminal SH3 domain, a centrally located zipper and a Cdc42/Rac Interactive Binding (CRIB) motif, and a C-terminal region with a preponderance of serine, threonine, and proline residues. Work in our lab indicates that MLK3 is autoinhibited through an interaction between its SH3 domain and a noncanonical SH3 binding motif that is situated between the zipper and CRIB motifs. We have also found that activated forms of the small GTPases Cdc42 and Rac increase MLK3's autophosphorylation and substrate phosphorylation activity, change the subcellular localization of MLK3, and are correlated with changes in MLK3's in vivo phosphorylation status.
The Gallo Laboratory uses biochemical, biophysical and cell biological approaches-including confocal microscopy and mass spectrometry- towards understanding the molecular mechanisms that regulate MLKs and their signaling pathways.



Protein levels of MLK3 are much higher in breast cancer cells than in human cell lines derived from other tissues. In order to learn more about MLK3 regulation and its signaling pathways in breast cancer cells, we engineered the estrogen-responsive human breast cancer cell line, MCF-7, to stably, inducibly express Flag epitope-tagged MLK3. We are isolating Flag-MLK3 complexes by affinity purification and identifying associated proteins by either in-gel or in-solution trypsin digests followed by tandem MS/MS. These studies are providing new information to understand MLK3 signaling in cancer cells.

Selected Publications

H. Zhang, W. Wu, Y. Du, S. J. Santos, S. E. Conrad, J. T. Watson, N. Grammatikakis, K. A. Gallo (2004) Hsp90/ p50^{cdc37} Is Required for Mixed Lineage Kinase 3 Signaling. J. Biol. Chem. 279(19):19457-63

Gallo, K. A. and Johnson, G.L. (2002) Signalling: Mixed-lineage kinase control of JNK and p38 MAPK pathways. Nature Reviews Molecular Cell Biology 3: 663-72

Vacratsis, P. O., Phinney, B. S., Gage, D. A., and Gallo, K. A. (2002) Identification of in vivo phosphorylation sites of MLK3 by mass spectrometry and phosphopeptide mapping. Biochemistry 41, 5613-24.

Zhang, H. and K. A. Gallo (2001) Autoinhibition of mixed lineage kinase 3 through its Src homology 3 domain. J. Biol. Chem. 276: 45598-603