TARGETING LUNG TUMOR ANGIOGENESIS: TRANSLATIONAL POTENTIAL FOR LKB1 TUMOR SUPPRESSOR GENE

Presenter: Imoh Okon, PhD

1Imoh Okon, 1Kathleen Coughlan, 1Cate Moriasi, and 1,2Ming-Hui Zou
1Section of Molecular Medicine, 2Department of Biochemistry and Molecular Biology, College of Medicine, University of Oklahoma Health Sciences Center.

Introduction: Tumor angiogenesis and metastasis remain the leading causes of cancer-related mortality. Although anti-tumor properties of Liver Kinase B1 (LKB1) have been previously described in human neoplasm, a mechanistic link between LKB1 and tumor progression has yet to be fully explored. The prevalence of frequent LKB1 mutations in lung tumors (~30%) prompted investigation of its tumor suppressor functions in the disease. Here, we demonstrate the attenuation of tumor-promoting processes, including aberrant cell growth, angiogenesis and metastasis by LKB1 in vitro and in vivo.

Methods: Recombinant LKB1 protein or transient LKB1-vector was transfected into LKB1-deficient A549 lung cancer cells, while endogenous LKB1 expression was stably silenced in H1792, a stage IV, highly metastatic lung cancer cell line. Endogenous LKB1-expressing H1299 and H1703 lung cancer cell lines were also utilized. Angiogenesis was assessed by chorioallantoic membrane (CAM) assay which involved subcutaneous implantation of LKB1 null or positive cells into CAMs of 10-day old chick embryos, while tumor development and growth was investigated in nude mice. Cell proliferation and tumor metastasis, as a function of invasive and migratory potentials of LKB1-deficient or -expressing cancer cells was measured using the xCELLigence label-free, real-time system from Roche (Indianapolis, IN). Caspase-3 activity was determined by the EnzChek Caspase-3 Assay Kit #2 from Molecular Probes (Eugene, OR).

Results: LKB1-deficient A549 cells demonstrated strong angiogenic potential compared with LKB1-expressing H1299 or H1703 cell lines, as measured by vessel density in the CAM assay. Increased migration and invasion was also evident in A549 cells, indicative of a stronger metastatic potential compared with H1299 or H1703 cells. Ectopic gain-offunction experiments employing LKB1-expression vectors or recombinant LKB1 proteins correlated with decreased cell growth in A549 cells. Attenuation of AKT and caspase-3 signaling pathways contributed to the observed growth inhibition demonstrated by LKB1-expressing cells. Conversely, loss-of-function experiments utilizing stable LKB1 knock-down (shRNA) in H1792 cells correlated with tumor development and growth in nude mice, as well as increased angiogenesis in chick CAM assay. Enhanced migration and invasion was evident in LKB1-null cells compared with control (scramble shRNA) group.

Conclusion: LKB1-mediated repression of tumor growth, angiogenesis and metastasis was determined in vitro and in vivo. Recombinant LKB1 protein demonstrated strong anti-tumor growth properties suggesting potential development of an LKB1 mimic for therapeutic applications. Furthermore, LKB1 or the loss of its expression could provide potential biomarker application in a subset of metastatic lung tumors.

In addition to the regulation of AMPK-mTOR pathway, and NF-kB transcription factor, novel mechanistic insights of anti-tumor LKB1 functions is currently under investigation.