peared to mediate Akt activation in prostate tumor cells in vitro (Ghosh PM, Bedolla R, Mikhailova M, Kreisberg JI., Cancer Res 2002 May 1;62(9):2630-6). In this report, archival paraffin-embedded sections from 74 cases of resected prostate cancer were examined. We now demonstrate by immunohistochemistry decreased staining for membrane-associated RhoA small GTPase, indicative of RhoA inactivation, in high Gleason grade prostate cancer. Importantly, decreased membrane staining for RhoA was negatively correlated with Akt phosphorylation in prostate cancer (Pearson coefficient = -0.37, p = 0.0025). In addition, there was a strong correlation between expression of phospho-Akt (Ser 473) and the percentage of cells staining positively for the cell proliferation antigen Ki67, indicating that activation of Akt may mediate cell proliferation in prostate cancer (Pearson coefficient = 0.36, p = 0.0028). A similar comparison of phospho - ERK (Tyr 402/Thr 404) expression and Ki67 was not significant (Pearson coefficient = -0.2, p = 0.1173). Finally, loss of the tumor suppressor PTEN was thought to mediate increased Akt phosphorylation in prostate cancer. However, we found that PTEN was lost from a majority of all prostate cancers, including well-differentiated prostate cancers (Gleason grade 2-4), indicating that Akt phosphorylation may be mediated by another pathway. Our results suggest that RhoA inactivation may mediate Akt phosphorylation resulting in increased cell proliferation.

#2015 Cell cycle activation in lung carcinoma cells by a transforming growth factor α/ErbB3/Phosphatidylinositol 3 kinase/Akt pathway. Gunamani Sithanandam, Laura W. Fornwald, and Lucy M. Anderson. *Basic Research Program, SAIC, NCI-Frederick, Frederick, MD and National Cancer Institute at Frederick, Frederick, MD.*

An autocrine loop involving epidermal growth factor receptor and transforming growth factor α is implicated in the pathogenesis of human lung adenocarcinoma, and overexpression of transforming growth factor α together with ErbB2 or ErbB3 may be associated with poor prognosis. In mouse lung adenocarcinoma cell line E9 we found high expression of both transforming growth factor α and ErbB3, whereas both were absent from the non-transformed sister line E10. Both lines expressed epidermal growth factor receptor, ErbB2 and ErbB4. Exogenous transforming growth factor α treatment of E9 cells stimulated epidermal growth factor receptor phosphorylation, and formation of complexes containing phosphorylated ErbB2, phosphorylated ErbB3, and the p85 regulatory subunit of phosphoinositidyl 3 kinase, as indicated by co-immunoprecipitation experiments. Transforming growth factor α also stimulated phosphorylation of Akt and GSK3β, increase in cyclin D1, and cell cycle progression, and these events were blocked by the ErbB receptor inhibitor PD153035 and the Akt activation inhibitor LY294002. In human NSCLC cell lines H441 and H1373, pErbB3/p85 complexes were detected, and treatment with LY294002 blocked cell cycle progression. Direct evidence for participation of ErbB3 and its related pathway in proliferation of these human lung cancer cells included an antiproliferative effect of an ErbB3-specific antisense oligonucleotide. These results together suggest involvement of ErbB3 in growth of lung adenocarcinomas, through activation of phosphoinositidyl 3 kinase and Akt, inactivation of GSK3β, and stabilization of cyclin D1 for cell cycle maintenance. (Supported in part by NIH contract NO1-CO-12400.)

#2016 Role of PI3K/AKT/mTOR signaling in G1 cell cycle progression in human ovarian cancer cells. Bing-Hua Jiang, Ning Gao, Daniel Flynn, and Xianglin Shi. *West Virginia University, Morgantown, WV and NIOSH, Morgantown, WV.*

Ovarian cancer is one of the most common cancers among women. Recent studies demonstrated that the gene encoding the p110a catalytic subunit of phosphoinositide 3-kinase (PI3K) is frequently amplified in ovarian cancer cells. PI3K is involved in multiple cellular functions including proliferation, differentiation, anti-apoptosis, tumorigenesis, and angiogenesis. In this study, we demonstrate that the inhibition of PI3K activity by LY294002 inhibited ovarian cancer cell proliferation and induced the G1 cell cycle arrest. This effect was accompanied by the decreased expression of G1-associated proteins including cyclin D1, CDK4, CDC25A, and Rb phosphorylation at Ser780, Ser795, and Ser807/811. Whereas expression of CDK6 and b-actin was not affected by LY294002. Levels of cyclin kinase inhibitor, p16INK4 were induced by the PI3K inhibitor, while levels of p21CIP1/WAF1 were decreased in the same experiment. The inhibition of PI3K activity also inhibited the phosphorylation of AKT and p70S6K, but not MAPK. PI3K transmits a mitogenic signal through AKT, mTOR to p70S6K. The mTOR inhibitor rapamycin has similar inhibitory effects on G1 cell cycle progression and expression of cyclin D1, CDK4, CDC25A, and Rb phosphorylation. These results suggest that PI3K mediates G1 progression and cyclin expression through the activation of AKT/ mTOR/p70S6K signaling pathway in the ovarian cancer cells.

#2017 AKT regulation of MAPK signaling pathway provides a potential cross-talk mechanism for HIF-1 gene-expression in Hep3B cells. Yanira G. Figueroa, Yan Tang, Aline Scandurro, and Barbara S. Beckman. *Tulane University Health Center, New Orleans, LA.*

Hypoxia Inducible Factor-1 (HIF-1) is considered to be one of the master regulators of oxygen homeostasis. It regulates the expression of a variety of genes encoding proteins involved in diverse cellular adaptive mechanisms including, angiogenesis and anaerobic metabolism following hypoxic stress. Considerable attention has been focused in understanding the signaling pathways that are involved in HIF-1 activation under low oxygen (or hypoxia) conditions. Here we explored the potential cross-talk between the phosphatidylinositol 3-kinase (PI3K) and the mitogen activated protein kinase family (MAPK) pathway in hepatocellular carcinoma cells (Hep3B). Previously, we have shown that inhibition of the PI3K signaling pathway with LY294002 (30μM), specific inhibitor of PI3K, or transiently transfection of AKT dominant negative construct significantly decreased HIF-1 transactivation and mRNA gene expression under hypoxic condition. Interestingly, blockade of the P13K signaling pathway failed to alter HIF activation under normoxic conditions (21%O_2). Unexpectedly, this inhibition enhanced kinase activity and protein expression of members of the mitogen activated protein kinase family (MAPK, p42/44 and p38) in Hep3B cells in normoxia while LY294002 treatment under hypoxia significantly decreased their kinase activities and protein expression. To gain more insight into the potential role of the MAPK signaling pathway in HIF-1 activation, we pretreated Hep3B cells with a specific inhibitor of the mitogen extracellular kinase 1 (MEK1, PD098059, 25μM), prior to hypoxic conditions for 0,1, 6 and 24 hours. Inhibition of MEK did not have any effect on HIF-1 transactivation and mRNA gene expression as determined by luciferase assays and RT-PCR, respectively. The combined inhibition of the MAPK and PI3K pathways mediated by PD098059 treatment and AKT dominant negative transfection of Hep3B cells unexpectedly led to similarly reduced HIF-1 transactivation following hypoxia as that observed with the AKT dominant negative alone. Our results show that only PI3K signaling pathways mediate HIF-1 activation following hypoxic stress to Hep3B cells whereas MAPK signaling pathways play no role.

#2019 Activation of the phosphatidylinositol 3-Kinase AKT pathway in acute myeloid leukemia and mTOR-inhibition by rapamycin. Christian H. Brandts, Christoph Biermann, Beate Lindtner, Horst Burger, Carsten Muller-Tidow, Wolfgang E. Berdel, and Hubert Serve. *University of Muenster, Muenster, Germany.*

The most common genetic aberrations seen in acute myeloid leukemia (AML) are oncogenic K-RAS and N-RAS-mutations as well as activating mutations of the receptor tyrosine kinase FLT3, characterized by in-frame internal tandem duplications in the juxtamembrane region (designated FLT3-ITD). Both oncogenic RAS and FLT3-ITD activate the phosphatidylinositol 3-Kinase (PI3K) pathway, a signalling cascade implicated in cancer development. In AML, additional mechanisms of activation of the pathway may also occur. Critical to this pathway is the activation of the serine/threonine-kinase AKT, which regulates proliferation and apoptosis in cancer cells. Recent evidence suggests that the mammalian target of rapamycin (mTOR) is a downstream target of AKT. We have screened a large panel of myeloid leukemia cell lines for expression and activation of both AKT and mTOR in the presence and absence of growth factor stimulation. Using phosphospecific antibodies against phospho-Ser473-AKT and phospho-p70S6 kinase (a phosphorylation site of mTOR on p70S6 kinase) we showed that a subset of cell lines show evidence of constitutive AKT and mTOR activation in the absence of growth factors. Furthermore the AKT activation status correlated with mTOR activity. Of particular interest, we demonstrated that the stable expression of the AML-specific mutation FLT3-ITD in the murine myeloid cell line 32Dcl3 led to con-