with a non-degradable IκB, restored tamoxifen sensitivity to our refractory Akt MCF-7 cells. These data suggest that activation of NF-κB via the PI3K/Akt signaling pathway is a significant mechanism for development of hormone refractory breast cancer, and that inhibition of NF-κB may be an effective treatment strategy for this relatively resistant disease.

#2038 Upregulation of Rab25 gene in ovarian cancer cells enhances cell proliferation by increasing AKT phosphorylation. Kwai Wa Cheng, John P. Lahad, and Gordon B. Mills. *UT-MD Anderson Cancer Center, Houston, TX.*

Rab proteins are small GTPases that form the largest branch of the Ras superfamily. Previously the family has been thought to function primarily in intracellular vesicle trafficking. Recently several Rab family members have been suggested to contribute to multiple signaling cascades including the PI3K and MAPK pathways. The Rab GTPase family member, Rab 25, is located at a site of DNA copy number increase at chromosome 1q23 in ovarian cancer. As assessed by RT-PCR and real-time QPCR, a 4 fold increased in Rab25 mRNA levels were found in ovarian tumor samples directly from the patient as compared to normal ovarian epithelial cells. Strikingly, expression of Rab25 gene was 2.7 fold higher in grade III than in grade I ovarian carcinoma suggesting the expression of Rab25 may contribute to the progression of ovarian cancer. Indeed, enforced expression of Rab25 induced increases in cell proliferation and colony forming cell ability of ovarian cancer cell lines including A2780, DOV13, OCC1 and HEY. In addition, over-expression of Rab25 improves cell survival ability under anoikis condition. A 48% decrease in apoptotic cells was observed in Rab25 transfected DOV13 cells cultured under stress condition when compared to the empty vector transfected cells. To understand the molecular mechanism of Rab25 induced increases in cell proliferation, several molecules involved in regulating cell growth were examined. Among them, an increase in phosphorylation of AKT at Ser 473 was observed in Rab25 over-expressed A2780 (3 fold), DOV13 (1.5 fold) and OCC1 (1.7 fold) cells after stimulation by 5% fetal bovine serum. Activation of AKT resulted in an increased phosphorylation of Bad at Ser 136 in A2780 (5.6 fold), DOV13 (2.4 fold) and OCC1 (3.2 fold). Furthermore, decreased Tyr15-phosphorylation of cdc-2 was observed in A2780 (48%) and OCC1 (37%), potentially releasing negative regulatory effects on cell cycle progression. Taken together, our data indicated that the novel Rab GTPase family member Rab-25 is over expressed in ovarian cancer cells in the patient and increases cell survival ability by activating the AKT pathway. KW Cheng was supported by Odyssey Fellowship Program at the MD Anderson Cancer Center.

#2039 Induction of *In vitro* chemotherapeutic drug resistance by activation of Raf/MEK/ERK or PI3K/PDK/AKT signal transduction pathways in MCF-7 breast cancer cells. Patrick M. Navolanic, Linda S. Steelman, Nick R. Leslie, Paul J. Coffer, Marina Konopleva, Wei Hu, Michael Andreeff, Mikhail V. Blagosklonny, Martin McMahon, and James A. McCubrey. *East Carolina University, Greenville, NC, University of Dundee, Dundee, UK, University Medical Center, Utrecht, Netherlands, M.D. Anderson Cancer Center, Houston, TX, New York Medical College, Hawthorne, NY, and UCSF Comprehensive Cancer Center, San Francisco, CA.*

Breast cancer chemotherapy often fails to achieve long lasting remission due to emergence of drug resistant cells. Mechanisms of chemotherapeutic drug resistance include increased activity of anti-apoptotic proteins, such as Bcl-2, as well as drug efflux pumps, such as P-gp. These proteins may be regulated by signal transduction cascades linked to breast cancer development, including Raf/MEK/ERK and PI3K/PDK/Akt pathways. For example, some breast cancers are associated with loss of PTEN phosphatase activity that would otherwise inhibit the PI3K/PDK/Akt pathway. The hypothesis that Raf/MEK/ERK and PI3K/PDK/Akt cascades modulate *in vitro* breast cancer cell sensitivity to P-gp substrates doxorubicin and paclitaxel was tested in MCF-7 cells. Doxorubicin resistance was increased by overexpression of a mutant form of PTEN, PTEN(C124S), that lacks both lipid and protein phosphatase activity. Doxorubicin resistance was also increased by overexpression of another mutant form of PTEN, PTEN(G129E), which lacks lipid phosphatase activity yet retains protein phosphatase activity. These results suggest overexpression of either of these two mutant PTEN proteins lacking lipid phosphatase activity has a dominant negative effect that inhibits the PI3K/PDK/Akt pathway. In contrast, overexpression of wild type PTEN, PTEN(WT), or PTEN truncated at its carboxyl terminus, PTEN(399stop), did not significantly affect doxorubicin sensitivity. Doxorubicin resistance was also increased by overexpression of v-Ha-Ras or ΔRaf-1, which are constitutively active forms of Ras and Raf-1, respectively. Doxorubicin resistance induced by overexpression of ΔRaf-1 was accompanied by increased Bcl-2 and P-gp mRNA levels as well as elevated P-gp pump activity. Additionally, treatment of MCF-7/ΔRaf-1:AR cells with testosterone, which activates Raf-1, increased resistance to both doxorubicin and paclitaxel. Overexpression of Bcl-2 induced doxorubicin resistance that was observed both for short term exposure lasting 5 days or less as well as for long term exposure lasting at least 8 weeks. Bcl-2 overexpression also enhanced doxorubicin resistance induced by testosterone treatment of MCF/ΔRaf-1:AR cells. Finally, the hypothesis that Bcl-2 induces doxorubicin resistance by preventing increased expression of proteins associated with DNA damage was tested. Doxorubicin treatment increased levels of $p21^{Cip1}$ and its transcriptional activator, p53, in both the presence and absence of Bcl-2 overexpression, which implies Bcl-2 induces doxorubicin resistance by mechanisms other than abrogation of DNA damage sensing. These results indicate Raf/MEK/ERK and PI3K/PDK/Akt pathways induce chemotherapeutic drug resistance in MCF-7 cells and suggest roles for P-gp and Bcl-2 as downstream mediators of this effect.

#2040 Signaling pathways involved in MCL1 regulation are cell type and inducer specific. Julie A. Vrana and Ruth W. Craig. *Dartmouth Medical School, Hanover, NH.*

MCL1, a viability promoting member of the BCL2 family, was originally identified as a gene upregulated early in the differentiation of ML-1 myeloid leukemia cells. Since its discovery, MCL1 expression has been reported to be upregulated in a variety of cell lines by numerous signaling pathways including PKC/ERK, PKB/Akt, and p38 MAP kinases. In this study we have undertaken a thorough examination of the role of various pathways in the control of MCL1 expression in leukemic cell lines, with the primary concentration on ML-1 cells. These studies focus on MCL1 regulation in response to both the differentiation-inducing agent phorbol 12'-myristate 13'- acetate (PMA) and cytotoxic microtubule disrupting agents such as vinblastine (VBL). PMA induced MCL1 expression in all four leukemic cell lines tested ML-1, THP-1, U937, and HL-60, however microtubule disrupting agents only induced MCL1 in two of the four lines (ML-1 and THP-1). We found that induction of MCL1 mRNA by PMA exposure was blocked by pretreatment with the MEK inhibitors, PD98059 or U0126 while induction by microtubule disrupting agents were only partially responsive to inhibition of this pathway. In contrast, induction of MCL1 mRNA by microtubule disrupting agents was partially blocked by pretreatment with the JNK inhibitor SP600125 while induction by PMA was unaffected. Blocking p38 MAP kinase signaling with SB201290, did not inhibit MCL1 induction by either PMA or microtubule disrupting agents suggesting that this pathway did not influence MCL1 expression. MCL1 upregulation by either type of agent was not dependent on PI3-kinase signaling since it was not inhibited by LY294002. Consistent with this result, PKB/Akt activation via TNF-α treatment did not induce MCL1 expression. In sum, the PMA-induced increase in MCL1 expression involves ERK but not stress-activated branches of the MAP kinase network while microtubule disrupting agents appear to use both the ERK and JNK signaling pathways. Neither type of agent appears to use the PI3K/PKB-Akt pathway. Since this pathway is involved in other systems, different cell types appear to utilize different signaling pathways to induce expression of MCL1. Furthermore, since the effects of microtubule disrupting agents differ from those of PMA both between leukemic cell lines and within a leukemic cell line, different inducers may also act through varying pathways within a particular cell type. Overall, expression of antiapoptotic BCL2 family members such as MCL1 appear to be controlled through a variety of pathways in a cell type- and inducer-specific manner.

#2040A VEGF expression is inhibited by cisplatin at the transcriptional level through HIF-1α expression. Xiaosong Zhong, Heath Skinner, Min Ding, Lesly Anne Lopez, Eddie Reed, and Bing-Hua Jiang. *West Virginia University, Morgantown, WV, National Institute for Occupational Safety and Health, Morgantown, WV, and MBR Cancer Center, Morgantown, WV.*

Cisplatin (CDDP) is an effective anti-neoplastic agent against a variety of human solid tumors, particularly those of the ovary. However, the molecular mechanism of its action is poorly understood. Vascular endothelial growth factor (VEGF) is a potent inducer of angiogenesis, and is upregulated in many human cancers. The expression of VEGF is regulated by hypoxia-inducible factor-1 (HIF-1). HIF-1 is a heterodimeric basic helix-loop-helix transcription factor, composed of HIF-1α and HIF-1β subunits. HIF-1 activity is regulated by the level of HIF-1α expression in the cells. In this study, we demonstrated that CDDP inhibited VEGF expression in human ovarian cancer cells. To determine whether CDDP inhibited VEGF expression at the transcriptional level, we analyzed the effects of CDDP on a reporter containing human VEGF 5'-flanking sequences inserted into pGL2-basic luciferase vector, and found that CDDP inhibited the reporter activity in a dose dependent manner in ovarian cancer cells, indicating that CDDP inhibited transcriptional activation of VEGF. To determine whether CDDP inhibited transcriptional activation of VEGF through the activation of HIF-1, the HIF-1 binding site at the VEGF 5'-flanking sequences was mutated. We found that: 1) the luciferase activity mediated by VEGF reporter containing the mutation of HIF-1 binding site was much lower than the reporter containing the wild-type HIF-1 binding site in the ovarian cancer cells. This result confirms that HIF-1 is a major transcriptional regulator for VEGF expression. 2) CDDP greatly inhibited the VEGF reporter activity containing the wild-type HIF-1 binding site, but increased the reporter activity containing the mutation at the HIF-1 binding site. This result indicates that CDDP inhibited VEGF transcriptional activation specifically by decreasing HIF-1 activity. To further confirm the role of HIF-1 in CDDP-mediated VEGF expression, A2780-CP70 cells were co-transfected with VEGF reporter and a dominant negative construct of HIF-1. Co-transfection of a dominant negative construct of HIF-1 inhibited VEGF reporter activity in a dose dependent manner in ovarian cancer cells. CDDP inhibited HIF-1 activity through the expression of HIF-1α, while level of HIF-1β did not change by the treatment. HIF-1α expression in OVCAR-3 cells was inhibited by CDDP at much lower dose than in A2780-CP70 cells which are more resistant to CDDP, indicating that HIF-1α expression is also correlated with the sensitization of cells to CDDP. Taken together, these results suggest a novel mechanism of CDDP's anti-tumor activity in ovarian cancer cells through the transcriptional regulation of VEGF expression and HIF-1 activation in the cells.

CELLULAR, MOLECULAR, AND TUMOR BIOLOGY 63: Angiogenesis and Lymphangiogenesis

#2041 G-CSF stimulates neovascularization and promotes tumor growth: Potential contribution of bone marrow-derived endothelial progenitor cells. Takeshi Natori, Masataka Sata, Kenji Miki, and Masatoshi Makuuchi. *Department of Surgery, Graduate School of Medicine, University of Tokyo, Tokyo, Japan and Department of Cardiovascular Medicine, Graduate School of Medicine, University of Tokyo, Tokyo, Japan.*

Solid tumors require neovascularization for their growth. Recent evidence indicates that bone marrow-derived endothelial progenitor cells (EPCs) contribute to tumor angiogenesis. We show here that granulocyte colony-stimulating factor (G-CSF) markedly promotes growth of the colon cancer inoculated into the subcutaneous space of mice, whereas G-CSF had no effect on cancer cell proliferation in vitro. The accelerated tumor growth was associated with enhancement of neovascularization in the tumor. We found that bone marrow-derived cells participated in new blood vessel formation in tumor. Our findings suggest that G-CSF may have potential to promote tumor growth, at least in part, by stimulating neovascularization in which bone marrow-derived EPCs play a role.

#2042 Tumor cell dissemination in blood and bone marrow of colon cancer patients undergoing surgery of hepatic metastases. Katja Schoppmeyer, Nils Fruehauf, Karl Oldhafer, Siegfried Seeber, and Sabine Kasimir-Bauer. *Department of Molecular Biology (Cancer Research), Essen, Germany, Center of General and Transplantation Surgery, University of Essen, Essen, Germany, Clinic for General and Visceral Surgery, General Hospital Celle, Celle, Germany, and Department of Internal Medicine (Cancer Research), West German Cancer Center, Essen, Germany.*

Purpose of the study: Patients undergoing resection of hepatic metastases of colorectal cancer have a high risk for extrahepatic recurrence, probably caused by early tumor cell dissemination or the manipulation of liver tumors during surgical resection. Experimental Design: Using immunocytochemistry, we studied 47 patients for cytokeratin (CK)-positive (+) cells in blood and bone marrow (BM) samples before surgery and in blood samples taken from the hepatic vein during surgery of liver metastases during time points when the liver is mostly mobilized. Normal and malignant tissue of the liver were studied for the expression of the urokinase plasminogen activator (uPA), the oncoprotein c-erbB2 and the Epithelial Growth Factor-receptor (EGF-r), using sandwich enzyme immunoassays. Results: CK+ cells were detected in the BM of 26/47 patients (55%), in blood samples before surgery in 14/47 patients (30%) and during surgery in 11/47 patients (23%). In 9/11 patients of the latter group, CK+ cells were also found in the BM. No CK-positive cells were found in 15/47 patients (32%) in any sample studied. Since the few detected CK-positive cells were distributed on different slides which makes double labelling for characterization of the cells difficult, we analyzed normal and tumor tissue of the liver for the expression of EGF-r, uPA, and c-erbB2. Tumor tissue could be obtained from 32/47 patients and normal liver tissue from 24/32 patients. EGF-r was found to be significantly higher in normal tissue (mean 647 ng/mL, range 275-1400 ng/mL) as compared to tumor tissue (mean 210ng/mL, range 5-1136 ng/mL, $p < 0,001$). uPA was markedly enhanced in 22/35 patients (mean 283 pg/mL, range 88-765 pg/mL, $p < 0,001$) as compared to 2561 pg/mL (range 204-5864 pg/ml). Although the mean values for c-erbB2 in normal tissue (0,609 ng/μg protein, range 0,306- 0,903 ng/μg) were comparable to those in tumor tissue (mean: 0,595 ng/μg, range 0,156-1,512 ng/μg), significant differences in expression were found in 15/32 (47%, $p < 0.001$) patients. The comparison of normal and malignant tissue in these 15 patients resulted in markedly higher c-erbB2 values in normal tissue in 9/15 patients and markedly enhanced values in tumor tissue were found in 6/15 patients, respectively. Conclusion: Although CK+ cells were present before surgery in many patients, the spread of CK+ cells during surgery of hepatic metastases occurs in 23% of all cases. Whether these cells are able to form new metastases has to be clarified in further studies. Up to now, follow-up time for our patients is too short to determine whether CK+ cells present in the BM or spread during surgery as well as overexpression of uPA in the liver metastases are of prognostic significance for disease-free or overall survival. Nevertheless, these results may help to think about modifying resection techniques to avoid or reduce tumor cell spread during surgery.

#2043 Dendritic cells activate blood endothelial cell proliferation in vivo through basic FGF. Christine F. Odoux, Simon C. Watkins, and Michael K.K. Wong. *University of Pittsburgh, Pittsburgh, PA.*

Dendritic cells (DCs) play a central role in immunity by initiating primary immune responses. Promising anti-tumor immuno-therapies using dendritic cells are being intensively studied for their broadly potential application in cancer treatments. DCs originate in the bone marrow and traverse multiple body compartments before maturing in lymphoid organs. They capture and process exogenous antigens in peripheral tissues, presenting them to naive T-cells, thus triggering immunoreactivity. Their ability to traffic through tissues using endothelial vessels is essential to their immune function but little is known on the processes of DC migration other than DC interactions initiated through lymphatic endothelial cell mechanisms. Previously, we showed that human blood-derived DCs produce angiogenic cytokines: Vascular Endothelial Growth Factor (VEGF), basic Fibroblast Growth Factor (bFGF), Endothelin-1 (ET-1), and Oncostatin-M (OSM). The purpose of this work is to determine the biologic activity of the DC angiogenic factor production and to establish the mechanism of DC angiogenesis. We generated green fluorescent protein (GFP)-DCs from GFP-trans-