Presentation Title: **Antioxidants decrease the apoptotic effect of 5-Fu in colon cancer by regulating Src-dependent caspase-7 phosphorylation**

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Abstract Body: Although the rate of development of drug resistance remains very high, 5-Fu is still the most common chemotherapeutic drug used for the treatment of colon cancer. A better understanding of the mechanism of why cancers develop resistance to 5-Fu could improve its therapeutic effect. Sometimes antioxidants are used simultaneously with 5-Fu treatment. However, a recent clinical trial showed no advantage or even a harmful effect of combining antioxidants with 5-Fu compared to administration of 5-Fu alone. The mechanism explaining this phenomenon is still poorly understood. In this study, we show that 5-Fu can induce reactive oxygen species-dependent Src activation in colon cancer cells. Mouse embryonic fibroblasts that are deficient in Src showed a clear resistance to 5-Fu, and knocking down Src protein expression in colon cancer cells also decreased 5-Fu-induced apoptosis. We found that Src could interact with and phosphorylate caspase-7 at multiple tyrosine sites. Functionally, the tyrosine phosphorylation of caspase-7 increases its activity, thereby enhancing cellular apoptosis. When using 5-Fu and antioxidants together, Src activation was blocked, resulting in decreased 5-Fu-induced apoptosis. Our results provide a novel explanation as to why 5-Fu is not effective in combination with some antioxidants in colon cancer patients, which is important for clinical chemotherapy.
Keywords: antioxidant; 5-Fu; Src; caspase-7; colon cancer; apoptosis

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Presentation Title: **NVP-BEZ235 decreases mutant p53 and downregulates miR-23a-24-27a to inhibit metastasis in breast cancer cells**

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Abstract Body: Nearly half of human cancers harbor TP53 mutations, which are predominantly through missense mutations that result in accumulation of mutant p53 proteins in cancer cells. More and more findings indicate

that the mutant p53 proteins acquire gain-of-function abilities to promote carcinogenesis, metastasis, tumor recurrence and chemoresistance. TP53 mutations are the most frequent genetic alterations in breast cancer, observed in 30% of breast carcinomas, especially the triple negative breast cancer (TNBC),which most easily metastasizes and confers chemoresistance. Therefore targeting TP53 mutation in breast cancer might be another promising approach of therapeutics. Our previous investigation showed that the AKT-mTOR dual inhibitor, NVP-BEZ235(called BEZ235 thereafter), could inhibit AKT-mTOR pathway activation, most interestingly mutant p53 was significantly reduced with BEZ235 treatment(unpublished data). In this study, three TNBC cell lines MDA-MB-231, MDA-MB-436, MDA-MB-468 as TP53 mutation group and MCF-7 as control were employed with BEZ235 treatment at different dosages and time points. We found that AKT-mTOR signaling pathway were highly activated in these TNBC cells but were inhibited obviously after BEZ235 treatment; most importantly, mutant p53 in these TNBC cells decreased significantly, but rare changes were detected in MCF-7 cells with BEZ235 treatment. Interestingly, we found miR-23a-24-27a cluster was about 2 fold upregulation in TNBC cells after BEZ235 treatment while exploring the decline of p53 by detecting microRNAs expression in those cell lines. To address whether BEZ235 could affect the metastasis ability of TNBC cells, cell invasion assay Transwell was used to measure the invasiveness of TNBC cells with BEZ235, and we observed that BEZ235 could inhibit the metastasis of TNBC cells, on the other hand miR-23a-24-27a cluster mimics could also induce the inhibition of metastasis in TNBC cells. Our results suggest that BEZ235 might target mutant p53 accumulation and downregulate miR-23a-24-27a cluster to inhibit the metastasis abilities of triple negative breast cancer cells, which might provide an important direction to further study the breast cancer harboring mutant p53, and also have promising therapeutic benefits for cancer patients carrying such p53 mutations.

Presentation Title: **Kaempferol suppresses solar ultraviolet radiation-induced skin cancers by targeting RSK2 and MSK1**

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Abstract Body: Ultraviolet (UV) irradiation is the leading factor in the development of skin cancer, which is the most common form of cancer in the United States. Discovering novel chemopreventive agents against this disease is extremely important. Kaempferol, a natural flavonol isolated from tea, broccoli, grapes, apples and other plant sources, is known to have anticancer activity, but its molecular mechanisms and direct target(s) in cancer chemoprevention are still unclear. In this study, our pull-down assay results showed that RSK2 and MSK1 directly interact with kaempferol in both *ex vivo* and *in vitro* systems. ATP competition and *in vitro* kinase assay data revealed that kaempferol interacts with RSK2 and MSK1 at the ATP-binding pocket and inhibits their respective kinase activities. Mechanistic investigations determined that kaempferol acts as an inhibitor of RSK2 and MSK1 kinase activities to attenuate solar UV-induced phosphorylation in mitogen-activated protein kinase signaling cascades in JB6 P+ mouse skin epidermal cells. In a mouse skin tumorigenesis study, kaempferol significantly suppressed solar UV-induced skin carcinogenesis. Further analysis showed that the kaempferol-treated group had a substantial reduction in solar UV-induced phosphorylation of CREB and c-Fos in mouse skin. Taken together, our results identify kaempferol as a safe and novel chemopreventive agent against solar UV-induced skin carcinogenesis that acts by targeting RSK2 and MSK1.

Presentation Title: **6-c-(e-phenylethenyl)-naringenin suppresses colorectal cancer growth by inhibiting cyclooxygenase-1**

Author Block: Haitao Li[1], Feng Zhu[1], Hanyong Chen[1], Ka Wing Cheng[2], Tatyana Zykova[1], Naomi Oi[1], Ronald A. Lubet[3], Ann M. Bode[1], Mingfu Wang[2], Zigang Dong[1]. [1]The Hormel Institute, Austin, MN; [2]Univeristy of Hong Kong, Hong Kong, China; [3]National Institutes of Health, National Cancer Institute, Bethesda, MD

Abstract Body: Recent clinical trials raised concerns regarding the cardiovascular toxicity of selective cyclooxygenase-2 (COX-2) inhibitors therefore COX-1 is now being reconsidered as a target for chemoprevention. Our aim was to examine whether selective COX-1 inhibition could delay or prevent cancer development and clarify the underlying mechanisms. Data clearly showed that COX-1 was required for maintenance of malignant characteristics of colon cancer cells or tumor promoter(s)-induced transformation of pre-neoplastic cells. We also successfully applied a ligand docking computational method to identify a novel selective COX-1 inhibitor, 6-*C*-(*E*-phenylethenyl)-naringenin (designated herein as 6CEPN). 6CEPN could bind to COX-1 and specifically inhibit its activity both *in vitro* and *ex vivo.* In colorectal cancer cells, it suppressed anchorage-independent growth by inhibiting COX-1 activity. 6CEPN also effectively suppressed tumor growth in a 28-day colon cancer xenograft model without any obvious systemic toxicity. Taken together, COX-1 plays a critical role in human colorectal carcinogenesis, and the specific COX-1 inhibitor merits further investigation as a potential preventive agent against colorectal cancer.

Keywords: colon cancer; natural product; cyclooxygenases; signal transduction

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Presentation Title: **Direct down-regulation of eEF1A2 by Tumor suppressor p16^{INK4a} inhibits cancer cell growth.**

Author Block: Mee-Hyun Lee1, Bu Young Choi2, Yong-Yeon Cho1, Myoung Ok Kim3, Sung-Hyun Kim3, Cheol-Jung Lee1, Ji Hong Song1, Ann M. Bode4, Zigang Dong4, Yong-Joon Surh5. 1College of Pharmacy, The Catholic University of Korea, Bucheon, Korea, Republic of; 2Pharmaceutical Science and Engineering, School of Convergence Bioscience and Technology, University of SeoWon, Chungju, Chungbuk, Korea, Republic of; 3Kyungpook National University, School of Animal BT Science, Center for Laboratory Animal Resources, Daegu, Korea, Republic of; 4The Hormel Institute, University of Minnesota, Austin, MN; 5Tumor Microenvironment Global Core Research Center, College of Pharmacy, Seoul National University, Seoul, Korea, Republic of

Abstract Body:

The tumor suppressor protein p16INK4a is a member of the INK4 family of cyclin-dependent kinase (Cdk) inhibitors, which are involved in the regulation of the eukaryotic cell cycle. However, the mechanisms underlying the anti-proliferative effects of p16INK4a have not been fully elucidated. Using yeast two-hybrid screening, we identified the eukaryotic elongation factor (eEF)1A2 as a novel

interacting partner of p16INK4a. eEF1A2 is thought to function as an oncogene in cancers. The p16INK4a protein interacted with all but the D2 (250-327 aa) domain of eEF1A2. Computational docking study predicted that D24/D131 residues of p16INK4a interacted with eEF1A2 and it was confirmed by pull-down assay with mutant p16INK4a (D24A/D131E). Ectopic expression of p16INK4a decreased the expression of eEF1A2 and inhibited cancer cell growth. Furthermore, suppression of protein synthesis by expression of p16INK4a ex vivo was verified by luciferase reporter activity. Microinjection of p16INK4a mRNA into the cytoplasm of Xenopus embryos suppressed the luciferase mRNA translation, whereas the combination of p16INK4a and morpholino-eEF1A2 resulted in a further reduction in translational activity. We conclude that the interaction of p16INK4a with eEF1A2, and subsequent downregulation of the expression and function of eEF1A2 is a novel mechanism explaining the anti-proliferative effects of p16INK4a.

Presentation Title: **Rack1 protects N-terminal phosphorylated c-Jun from Fbw7-mediated degradation**

Author Block: Jinhui Zhang, Feng Zhu, Xiang Li, Yanming Xu, Cong Peng, Shengqing Li, Yong-Yeon Cho, Ke Yao, Tatyana A. Zykova, Ann M. Bode, Zigang Dong. University of Minnesota, The Hormel Institute, Austin, MN

Abstract Body: The c-Jun transcription factor is a highly unstable oncoprotein. Several ubiquitin ligases mediate c-Jun degradation. However, c-Jun can be stabilized once it is phosphorylated at the N-terminus by c-Jun N-terminal kinases (JNKs) or other protein kinases. This phosphorylation decreases c-Jun ubiquitination and degradation. The underlying mechanism for this phenomenon is still unknown. We show that receptor for activated C-kinase 1 (Rack1) can bind with c-Jun and ubiquitin ligase Fbw7 to form a complex. When c-Jun is phosphorylated at the N-terminus, c-Jun is released from the complex and cannot be ubiquitinated by Fbw7, which leads to increased stabilization and accumulation of c-Jun. These results reveal that Rack1 plays a very important role in tumorigenesis by maintaining the stability of c-Jun that has been phosphorylated at its N- terminus by JNKs or other kinases.

Presentation Title: **TRAF4 is a key molecule for lung cancer through regulating AKT ubiquitination and activation**

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Abstract Body:

TRAF4 is a member of TRAF family. Although TRAF4 was reported to be overexpressed in cancer tissues, the role of TRAF4 in tumorigenesis remains unclear. Here, we found TRAF4 is over expressed in lung cancer cell lines as well as lung cancer tissues. Knockdown TRAF4 dramatically attenuated lung cancer malignant phenotype including cell proliferation, anchorage independent growth and tumor development in a xenograft mouse model . To our surprised, it is that TRAF4, but not skp2 is required for Akt activation through ubiquitination in lung cancer. Overall, our study suggests that TRAF4 might be a potential molecular target for lung cancer prevention and therapy.