## 21P Urethane-induced lung carcinogenesis in genetically edited C57Bl/6 mice with CHEK2 and GPRC5A heterozygous inactivating mutations

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**Background:** Some lung cancer (LC) patients carry germline truncating mutations in DNA repair or other cancer-related genes, however it is unclear whether these allelic variants play a causative role in LC predisposition, or, alternatively, are occasionally present in these subjects due to a chance. Case-control comparison is often not efficient due to rarity of gene-inactivating variants in population, therefore alternative approaches are required. We evaluated whether germ-line inactivation of GPRC5A and CHEK2 genes facilitates LC in mice.

**Methods:** C57BL/6 mice with heterozygous inactivating mutations in the GPRC5A or CHEK2 genes were created using the CRISPR/Cas9 system. Donor eggs were obtained from the first generation of crossing mice of C57BL/6 (males) and CBA (females) inbred strains. Offspring mice F1 bearing inactivating mutations in GPRC5A and CHEK2 were used in further breeding with C57BL/6 wild-type strain for the study purposes. Altogether, 33 females and 21 males without mutations, 11 females and 19 males with mutations in CHEK2, and 13 females and 14 males with mutations in GPRC5A were analyzed. At the age of 3 months, mice were intraperitoneally injected with urethane (1 g/kg). The experiment was stopped after 45 weeks, all animals were autopsied and the lung tumor nodes were counted.

**Results:** In the wild-type (control) mice, the number of affected animals was 16/33 (48%) in females and 12/21 (57%) in males (pooled males/females: 28/54 (52%)). The rate of tumor development was not increased in CHEK2-heterozygous mice (females: 6/11 (55%); males: 7/19 (37%); pooled males/females: 13/30 (43%)). However, animals carrying GPRC5A heterozygous inactivating mutations showed a trend towards elevated occurrence of carcinogen-induced lung neoplasms (females: 9/13 (69%); males: 11/14 (79%); pooled males/females: 20/27 (74%); $p = 0.06$ when compared to controls).

**Conclusions:** These data support evidences for the involvement of GPRC5A gene in pathogenesis of lung cancer.

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## 22P Salicylidene Acylhydrazides attenuate SH-SY5Y neuroblastoma cell survival through mitotic regulator Speedy/RINGO and ERK/ MAPK - PI3K/ AKT pathways

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**Background:** Iron is an essential element for cell proliferation, growth and cellular activities. Iron has been shown to be important for tumorigenesis and metastasis. Therefore, targeting the metabolic pathways of iron can provide new tools for cancer treatment. In this study, effects of iron chelating ME0053, ME0055 and ME0192 salicylidene acylhydrazides were investigated in SH-SY5Y cells. By targeting iron in the cell, iron chelators are known to act on cyclins and CDKs, as well as on AKT and MAPK signaling which function in tumorigenesis. Therefore, in this study the effect of used iron chelators on cell viability was investigated both on MAPK and AKT signaling and on the mitotic Speedy/RINGO protein, which potentially regulates the communication of these two signaling paths. In addition, the apoptotic states of the cells were examined by active caspase-3 analysis.

**Methods:** Appropriate administration dose of the ME053, ME055 and ME0192 compounds was determined by MTT analysis and SH-SY5Y cells were treated with these compounds. Then, the effect of iron chelators on Speedy/RINGO expression, AKT and MAPK signaling and also on the apoptotic state of cells were determined by western blotting.

**Results:** These compounds have been shown to reduce the phosphorylation level of AKT, one of the signaling molecules associated with survival in SH-SY5Y cells. A relatively less but significant decrease in the activity of the MAPK signaling was observed. Besides, it has been demonstrated for the first time that Speedy/RINGO protein expression was significantly reduced by these compounds with an yet unknown mechanism. Finally, the active caspase-3 analysis in SH-SY5Y cells showed that the compounds ME0053, ME0055 and ME0192 increased the amount of active caspase-3 by 218%, 90% and 175%, respectively.

**Conclusions:** It was shown for the first time that ME0053, ME0055 and ME0192 compounds showed a suppressive effect on the MAPK and AKT pathways, and also on the anti-apoptotic protein Speedy/RINGO, thereby causing apoptotic death of SH-SY5Y cells.

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## 23P DAB2IP inhibits metastasis in NSCLC by governing cell-matrix and cell-cell adhesions

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**Background:** Metastasis is a critical factor for the high mortality in Non-small-cell lung carcinoma (NSCLC), but the mechanism is still not completely understood. DAB2IP, as a Ras GTPase-activating protein and novel scaffold protein, is involved in the progression of various cancers. Previously, it was reported that DAB2IP methylation status in circulating DNA could predict response to erlotinib in EGFR-mutated NSCLC patients. However, the role of DAB2IP in NSCLC has rarely been investigated.

**Methods:** We collected 148 paraffin embedding tissues from primary NSCLC without distant metastases, and analyzed the relationship between DAB2IP and clinical pathology. We also used a lentivirus system to build the stable DAB2IP knockdown cell line A549-KD and compared the metastatic ability *in vitro* with A549 cells transfected with control shRNA (shcon). Cell-cell and cell-matrix adhesion was also investigated *in vitro*. The clinical tissue analysis was approved by the Medical Ethics Committee in the First Affiliated Hospital of Chongqing Medical University.

**Results:** In 148 patients, regional metastases were found in 96 patients. In 96 patients with metastases, no or low-expression of DAB2IP was observed in a high number of patients (79 no or low/96 patients; 17 high/96 patients), while high expression was frequently detected in patients without metastasis (8 no or low/52 patients; 44 high/52 patients). This suggests that loss of DAB2IP could indicate the occurrence of metastases in lung cancer patients($p<0.0001$). The patients with high expression of DAB2IP also had longer disease-free survival after surgery ($p=0.013$). *In vitro*, A549-KD cells migrated faster in wound healing assays. Further, knocking down DAB2IP expression reduced the adhesion of A549 on Laminin 111 and fibronectin, which destroyed cell-matrix adhesion. Meanwhile, the expression of some cell-cell adhesion proteins (E-cadherin, Epcam and MelCAM) also decreased in A549-KD by western blot experiment.

**Conclusions:** In summary, our study identified that DAB2IP has a role in predicting metastasis and poor survival in NSCLC. DAB2IP could regulate metastasis by governing cell-matrix and cell-cell adhesion.

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## 24P Identification of Zfp161 as a regulator of the c-Myc oncogene in human cells

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**Background:** Deregulated expression of the c-Myc oncogene is an important molecular hallmark of cancer. Previous studies have shown that the murine zinc finger protein ZF5 is a putative transcriptional regulator of c-Myc and can affect cell growth in mouse cell lines. The human zinc finger protein ZFP161 is 98% homologous to murine ZF5. The human gene Zfp161 is localized to 18p11.21 and cDNA of the gene has an open reading frame of 1347bp. We tested the regulatory effects of ZFP161 on c-Myc in human cells.

**Methods:** Correlation of Zfp161 and Myc in human colon cancer cells was derived from the R2 database. A Zfp161 knockout Hct116 cell line was prepared. Hct116 cells were transfected with PLK01, ZFP161 60shRNA and ZFP161 50shRNA to make Zfp161 knockdown cells. Western blot analysis was performed to determine ZFP161 and c-MYC protein levels in wild type Hct116 cells, Zfp161 knockout cells, control cells, 60sh-Zfp161 cells and in 50sh-Zfp161 cells. To test Zfp161 expression in the same cells, quantitative PCR (qPCR) analysis was performed. Clonogenic assays of 5 different cell types *in vitro* were performed to monitor the growth regulatory activity of Zfp161 in human cells.