Coexpression of RasG12V and shp53 in the liver cancer model

**Methods:** Transgenic liver cancer mouse models expressing different Ras isoforms were developed using a hydrodynamic injection method and the Sleeping Beauty Transposon System. Transposon vectors, each encoding an oncogene (HrasG12V, KrasG12V, NrasG12V) or downregulating a tumor suppressor gene (shp53), were constructed. To induce liver cancer, 40 µg of the three plasmids, encoding the sleeping beauty transposase and two transposons, was diluted in 2.5 mL of 0.9% saline and injected into the lateral tail veins of 6-week-old C57BL/6 mice. Mice were observed at 23 days post-hydrodynamic injection or near to death.

**Results:** Coexpression of H-, K-, N-RasG12V and shp53 resulted in a massive abdomen enlargement within 4 weeks after injection. Several nodular lesions emerged from the liver parenchyma, finally occupying most of the liver surface in 23 days after injection. The ratio of liver/body weight in KrasG12V group increased significantly compared to the HrasG12V group (P=0.0005) or NrasG12V group (P=0.0181) individually. Although the ratio of NrasG12V group showed a mild increase compared to the HrasG12V group, but statistically it was not significant (P=0.3819). Survival curve of these groups corresponded to the ratio of liver/body weight. All mice became moribund by 36 days.

**Conclusion:** Coexpression of RasG12V and shp53 in the mouse liver promotes rapid hepatocarcinogenesis. In particular, we found that Kras was the most oncogenic in the liver among the Ras isoforms when co-expressed with shp53.

**Keywords:** Ras isoform, Liver cancer

**Kahweol Induces Cell Death through Caspase-Dependent Pathway and Autophagy Dysfunction in Hepatocellular Carcinoma Cell Line**

**Background:** Coffee is one of the most widely consumed pharmacologically active beverages in the world. Several studies showed that a daily intake of coffee has the protective effect of coronary heart disease, diabetes mellitus, nonalcoholic steatohepatitis and cancer. Recent research suggests that kahweol, a diterpene molecule founded in the coffee bean, has anti-carcinogenic, anti-tumor and anti-inflammatory properties. However, little is known about the anti-tumor mechanisms of kahweol. Here, we examined the anti-tumor effect of kahweol in HepG2 human hepatocellular carcinoma cells.

**Methods:** The effect of kahweol on apoptosis is determined by hoechst and propidium iodide (PI) staining analysis on cultured human hepatocellular carcinoma HepG2 cells. The expression of cleaved caspase-3, cleaved PARP, pAkt, pERK, LC3 and p62 were determined by western blot analysis. To investigate whether kahweol induced apoptosis is related with autophagy, autophagy inhibitor baflinomycin or autophagy related gene 7 siRNA (SiATG7) were treated.

**Results:** Kahweol increased nuclear condensation and PI staining positive cells and induced cleaved caspase-3 and cleaved PARP expression. In addition, kahweol inhibits insulin induced phosphorylation of Akt, ERK, STAT3. Kahweol also increased expression of LC3-II and p62, indicating inhibition of autophagy. When kahweol was treated with baflinomycin or siATG7, apoptosis was significantly enhanced.

**Conclusions:** This study shows that kahweol increases HepG2 cell apoptosis through caspase 3-dependent pathway and inhibition of autophagy. The present study suggest that kahweol has anti-tumor effect in hepatocellular carcinoma.

**Keywords:** Kahweol, Hepatocellular carcinoma, Caspase, Autophagy, Apoptosis

**Knockdown of 14-3-3ζ Enhance the Radio-sensitivity and Radiation Induced Apoptosis in CD133+ Liver Cancer Stem-like Cells**

**Background:** Elevated expression of 14-3-3ζ in cancer cells has been reported to enhance radio-sensitivity and radiation induced apoptosis. We previously showed that 14-3-3ζ expression is elevated in CD133+ liver cancer stem-like cells.

**Materials and Methods:** CD133+ liver cancer stem-like cells were isolated from human liver cancer specimens. Knockdown of 14-3-3ζ was achieved by siRNA transfection. In vitro radio-sensitivity was assessed by colony formation assays and apoptosis was analyzed by Annexin V-FITC/PI double staining.

**Results:** Knockdown of 14-3-3ζ significantly enhanced the radio-sensitivity of CD133+ liver cancer stem-like cells. Additionally, knockdown of 14-3-3ζ increased radiation induced apoptosis in CD133+ liver cancer stem-like cells.

**Conclusion:** Knockdown of 14-3-3ζ enhances the radio-sensitivity and radiation induced apoptosis in CD133+ liver cancer stem-like cells.

**Keywords:** 14-3-3ζ, Radio-sensitivity, Apoptosis, Radiation, CD133+ liver cancer stem-like cells