YW3-07 Inhibition of HIF is necessary for tumor suppression by the von Hippel-Lindau protein

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Inactivation of the von Hippel-Lindau tumor suppressor gene is linked to the development of hereditary (VHL Disease associated) and sporadic clear cell carcinoma of the kidney. The VHL gene product, pVHL, is a component of E3 ligase complex and targets the heterodimeric transcription factor HIF (Hypoxia inducible factor) for poly-ubiquitination in the presence of oxygen. Tumor-derived pVHL mutants are defective in this regard and restoration of pVHL function in VHL-/-renal carcinoma cells suppresses their ability to form tumors in nude mice. Nonetheless, it is unproven that inhibition of HIF is causally linked to tumor suppression by pVHL. Here we show that tumor suppression by pVHL can be overridden by a HIF variant that escapes pVHL control. Thus, inhibition of HIF is necessary for tumor suppression by pVHL. This study shows that activation of HIF-target genes can promote tumor growth and thus establishes that HIF can behave as an oncogene *in vivo*. This finding has broad implications, as most solid tumors contain hypoxic cells, which accumulate HIF.

YW3-08 Association of VEGF overexpression with VHL gene inactivation in sporadic renal cell carcinoma

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A high frequency of genetic alteration of the von Hippel-Lindau (VHL) gene and overexpression of the vascular endothelial growth factor (VEGF) gene have been observed independently in human sporadic renal cell carcinoma (RCCs). We examined in this study the association of the two in 27 primary sporadic RCCs. Mutations of the VHL gene were found in 14 of 27 RCC samples (52.9%). LOH occurred in 10 of 15 RCC samples (66.7%). Northern blot analysis showed that the VEGF mRNA was overexpressed in 17 of 27 RCC cases (63.0%). Out of the 17 cases, 15 were from 18 RCC samples estimated to have at least 1 hit of the VHL inactivation, and 2 were from 6 RCC samples with 0-1 hit. None overexpressed the VEGF mRNA in the 3 RCC samples in which the VHL gene was not inactivated. VEGF overexpression was found to be correlated with both monoallelic and biallelic inactivation. Alteration of the VHL gene is thought to cause angiogenesis in RCCs through the overexpression of VEGF.

YW3-09 Antitumoral Activity of Cationic Liposomes Containing Human Interferon- β Gene against Renal Cell Carcinoma

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Purpose: We examined the antitumor effect of cationic multilamellar liposome containing human Interferon- β (huIFN- β) gene (IAB-1) against RCC.

Experimental design: Concentrations of huIFN- β protein were measured by ELISA. Cytotoxicity of IAB-1 against RCC (NC65, ACHN and freshly isolated RCC cells), prostate and bladder cancer cell lines was examined by MTT assay. Apoptosis was assessed by the acridine orange staining. For *in vivo* study, we used NC 65 cells inoculated into SCID mouse.

Results: The RCC cells treated with IAB-1 secreted significant amounts of huIFN- β protein continuously. Drastic *in vitro* cytotoxic effect of IAB-1 against RCC was observed. In contrast, treatment with 1000 IU/ml of recombinant huIFN- β protein resulted in weak cytotoxicity. The cytotoxic effect against other cancer cell lines was less than that against RCC. Apoptosis was observed in the cells treated with IAB-1, but recombinant huIFN- β failed to induce apoptosis. The size of NC65 tumors transfected with IAB-1 in mice was significantly smaller than that receiving injection of empty liposome or recombinant huIFN- β protein.

Conclusions: These findings indicate that IAB-1 may have an antitumor activity against human RCC by inducing apoptosis, suggesting its potential clinical application for gene therapy against RCC.

YW3-10 Differential profiling analysis of proteins involved in anti-proliferative effect of interferon- α on RCC cell lines by protein biochip technology

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New biomarkers related with the susceptibility for IFN treatment on RCC are needed to improve the effect of IFN-treatment against RCC. The proteins expressed in the IFN-susceptible and-resistant RCC cell lysates were analyzed by surface enhanced laser desorption/ionization (SELDI) mass spectrometry using ProteinChip® technology, and unique proteins were detected by the comparison of the profiles between them.

We detected the following candidate markers: (a) in the IFN-susceptible cell lines, a marker was detected on a hydrophobic (H4) chip; (b) in the IFN-resistant cell lines, markers were detected on strong anion exchange (SAX2, pH 9, two types) H4, and weak cation exchange (WCX, pH 9) chips; (c) a marker was expressed in both type cell lines after the IFN treatment was detected on the H4 chip.

These data suggest that the ProteinChip® system is very useful in identifying proteins showing unique peaks in the RCC cell lines with different IFN susceptibility, and the comparison of proteins profiles detected may help to identify the IFN sensitivity. Furthermore, the discovery of a susceptibility and/or an inhibitory factor may eventually lead to the development of a novel drug targeting the respective factor for the improvement of anticancer chemotherapy.