WS1-1 : ワークショップ 1-1 「Renal Cell Cancer」 WS1-1-1

Nitrogen-Containing Bisphosphonates Inhibit the Growth of Renal Cell Carcinoma Cells

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【Objectives】 Originally, BPs were developed as a drug for bone diseases. However, recent studies demonstrated that the 3rd generation nitrogen-containing bisphosphonates (BPs) induce apoptosis in not only osteoclasts, but also several types of cancer cells and have anti-cancer activities. In the present study, we investigated the effects of NBPs on the growth of renal cancer cells. **【Materials and Methods】** 1) BPs: incadronate (IC), zoledronic acid (Zol). 2) RCC cells: ACHN, LUS-M cells (established from clear cell, grade 2 RCC) 3) Cell growth assay: Cells were incubated with or without BP for 5 days and the viable cell number was determined. 4) Detection of apoptosis: Cells were cultured with or without BPs for 48h and apoptotic index was determined. 5) VEGF measurement: Cells were treated with or without BPs for 24 h. Concentration of VEGF in the conditioned media was measured by ELISA. 6) Effects of isoprenoids on the growth inhibitory effects of BPs: Cells were cultured with isoprenoids (FOH or GGOH) in the presence or absence of BPs. **【Results】** 1) IC and Zol inhibited the growth of renal cancer cells in a dose-dependent manner. 2) IC and Zol induced apoptosis in renal cancer cells. Zoledronic acid was stronger than incadronate in those activities. 3) IC and Zol significantly inhibited the secretion of VEGF protein by renal cancer cells. 4) Inhibitory effect of BPs was overcome by replenishing cells with isoprenoid substrates. **【Conclusions】** Those results suggested that nitrogen-containing BPs inhibit the growth of renal cancer cells and that the inhibition of geranylgeranylation is associated with this antineoplastic action of BPs. Exploring the anti-cancer mechanisms of BPs may provide a novel clue for the development of renal cancer treatments.

WS1-1-2

Wnt-antagonist Family Genes as Biomarkers for Diagnosis, Staging and Prognosis of Renal Cell Carcinoma Using Tumor and Serum DNA

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Objective: Aberrant promoter hypermethylation of Wnt-antagonist genes contributes to the pathogenesis of several cancers. We hypothesized that combined methylation analysis of Wnt-antagonist genes could serve as a panel of biomarkers in renal cell carcinoma (RCC). **Methods:** Samples (n=62) of RCC and corresponding normal kidney tissue (NRT) were analyzed using methylation-specific PCR (MSP) for methylation of six Wnt-antagonist genes (sFRP-1, 2, 4 and 5, Wif-1, and Dkk-3) after validation of our semi-quantitative conventional MSP results by quantitative real-time MSP. The methylation score (M-score) for multi-gene methylation analysis was developed. ROC curve analysis was used to determine the optimal sensitivity/specificity of the M-score. In addition, the M-score was compared with the clinico-pathological outcome. Thirty-three serum DNA samples were also used to investigate the methylation status of Wnt-antagonist genes. **Results:** Correlation analysis revealed a significant positive association between relative methylation level using conventional semi-quantitative MSP and using quantitative real-time MSP. The methylation levels of all Wnt-antagonists were significantly higher in RCC than in NRT. In multivariate regression analysis, the methylation level of sFRP-1 was a significant independent predictor of RCC, while for sFRP-2 and 4 there was a trend towards significance as independent predictors. The M-score of Wnt-antagonist genes was significantly higher in RCC than in NRT. Overall, the M-score had a sensitivity of 79.0% and a specificity of 75.8% (AUC=0.808) as a diagnostic biomarker. In addition, the M-score could significantly distinguish grade, pT category, M category and overall survival of RCC patients. The M-score was independent of age and gender in predicting overall survival by the Cox proportional hazards model. In RCC patients, 72.7% of the MSP results had identical methylation in samples of tumor and serum DNA. No serum DNA in normal controls showed aberrant methylation of the Wnt-antagonist genes. In addition, the methylation status of Wnt-antagonist genes in serum DNA was significantly correlated with tumor grade and stage. **Conclusions:** This is the first report demonstrating that M-score analysis of Wnt-antagonist genes can serve as an excellent epigenetic biomarker panel for detection, staging and prognosis of RCC using serum DNA.