ANTI-IVASION EFFECT OF BITTER MELON LEAF EXTRACT ON RAT PROSTATE CANCER CELL LINE

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Introduction: Cancer metastasis remains a major cause of death in cancer patients. Metastasis is a multi-step process. The invasive ability involving the first step of tumor metastasis is a major cause of treatment failure. Momordica charantia L. or bitter melon is widely consumed as vegetable and especially folk medicine in Asia. Extracts of bitter melon have been reported to possess anti-tumor activity such as inhibition of mouse spontaneous mammary tumourigenesis and benzo(a)pyrene-induced mice forestomach tumourigenesis, antioxidant activities, antiviral (HIV inhibitor), antidiabetic and immunomodulating properties. Our previous study revealed that bitter melon leaf extract (BMLE) inhibited P-glycoprotein activity and reverse multidrug-resistant in cervical carcinoma cell line (KB-V1), increased the intracellular accumulation of [^{3}H]-vinblastine, decreased the [^{3}H]-vinblastine efflux in KB-V1 cells and increased their sensitivity to vinblastine. However, there is no evidence whether BMLE has effects on prostate cancer cell invasion. **Purpose:** In this study we examined whether BMLE possessed inhibitory effects against invasive of rat prostate cancer cell line (PLS10). **Methods:** To determine cytotoxicity of BMLE on PLS10, WST-1 assay was used. The effect of BMLE on invasiveness and motility of PLS10 was determined by BD Biocoat^{TM} Matrigel^{TM} Invasion Chamber and BD Falcon inserts respectively. Gelatin zymography assay was performed to analyze matrix metalloproteinase (MMP)-2 and MMP-9 secretion from PLS10 cells. MMP-2, MMP-9 and tissue inhibitor of metalloproteinase (TIMP)-2 mRNA levels were analyzed by real time PCR. Collagenase type IV activity was assayed with the EnzChek Gelatinase/Collagenase Assay Kit. The results are presented as means ±SD (% of control) from three independent experiments. **Results:** WST-1 assay showed BMLE decreased cell viability on PLS10 cells (48 h; IC20<75 μg/ml, IC50: <150 μg/ml). 48 hr treatment of BMLE with non-toxic dose (25 and 50 μg/ml) significantly decreased PLS10 cell invasion (51±18; $p<0.05$ and 42±12; $p<0.005$ respectively) and gradually decreased cell migration at 24 hr (62±4 and 36±2; $p<0.005$ respectively). MMP-2 secreted in the culture media was significantly decreased when PLS10 cells were treated with 25 and 50 μg/ml for 24 hr (78±10 and 54± 18; $p<0.05$ respectively) and 50 μg/ml for 48 hr (67±12; $p<0.005$). After 24 hr 50 μg/ml BMLE treatment, MMP-9 secretion was significantly decreased (73±15; $p<0.05$). 25 and 50 μg/ml BMLE treatments significantly decreased MMP-9 secretions at 48 hour (83±10; $p<0.05$ and 71±8; $p<0.005$ respectively). We further analysis the effect of BMLE on MMP-2, MMP-9 and TIMP-2 gene expression using real time PCR. We found that BMLE (50 μg/ml) significantly decreased gene expression of MMP-2 (24 h; 25±6; $p<0.005$ and 48 h; 38±27 $p<0.005$) and MMP-9 (24 h; 39±27; $p<0.005$ and 48 h; 17±7 $p<0.005$ respectively) but markedly increase mRNA level of TIMP-2 (10, 25 and 50 μg/ml BMLE treatment; 24 h; 128±12; $p<0.05$, 153±20; $p<0.05$, 231±3; $p<0.005$ and 48 h; 124±8; $p<0.05$, 128±9; $p<0.005$, 222±67; $p<0.05$ respectively) which known to have inhibitory effect on the activity of on MMP-2. Detection of MMP activity was performed by an EnzChek Gelatinase/Collagenase Assay kit, the result showed that collagenase type IV activity was inhibit by BMLE (50 and 100 μg/ml BMLE treatment; 84±1; $p<0.005$ and 85±5 $p<0.05$ respectively). **Conclusion:** These results suggest that BMLE exerts anti-invasion effect on PLS10

cells through modulation of MMP-2 and MMP-9 secretion, down-regulated MMP-2 and MMP-9 gene expression, enhanced TIMP-2 gene expression and inhibits collagenase type IV activity. **Acknowledgement:** This work was supported by grants from the Royal Golden Jubilee Ph.D. Program of Thailand and the Society for Promotion of Pathology of Nagoya, Japan.