Role of Interleukin-6 (IL-6) in Cell Invasion of Cholangiocarcinoma Cell Lines

บทบาทของอินเตอร์ลิวคิน-6 ในการบุกรุกเซลล์เนื้อเยื่อของเซลล์มะเร็งท่อน้ำดี

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ABSTRACT

Interleukin-6 (IL-6) is a pleiotropic cytokine that has been implicated in the progression of cholangiocarcinoma (CCA), a bile duct cancer with high prevalence in the northeastern Thailand. In the present study, we aimed to investigate the role of IL-6 in cell invasion of human cholangiocarcinoma cell lines. The results showed that exogenous IL-6 induced cell invasion of KKU-M213 cells. To investigate the mechanisms underlying IL-6-induced cell invasion, the effect of IL-6 on secretion of MMP-2 and MMP-9 was measured by gelatin zymography. Zymograms from conditioned media of IL-6-stimulated KKU-M213 cells showed a clear band indicating MMP-9 activity. In conclusion, IL-6 promotes cell invasion of CCA by up-regulating the MMP-9 secretion and IL-6 signaling may be a potential anti-invasion target for the treatment of cholangiocarcinoma.

บทคัดย่อ

อินเตอร์ลิวคิน-6 เป็นไซโตไคน์ที่มีหน้าที่หลากหลายที่มีผลต่อการพัฒนาของมะเร็งท่อน้ำดีซึ่งเป็นมะเร็งที่พบมากในภาคตะวันออกเฉียงเหนือของไทย ในงานวิจัยนี้เราต้องการศึกษาบทบาทของอินเตอร์ลิวคิน-6 ในการบุกรุกเซลล์เนื้อเยื่อของเซลล์มะเร็งท่อน้ำดีของคน ผลการวิจัยแสดงให้เห็นว่าอินเตอร์ลิวคิน-6 ที่ได้รับจากภายนอกกระตุ้นให้เกิดการบุกรุกของเซลล์ในเซลล์ KKU-M213 เพื่อที่ต้องการศึกษากลไกการทำงานของอินเตอร์ลิวคิน-6 ที่กระตุ้นให้เกิดการบุกรุกของเซลล์ จึงทำการศึกษาผลของอินเตอร์ลิวคิน-6 ต่อการหลั่งของเอ็มเอ็มพี-2 และ เอ็มเอ็มพี-9 โดยการวัดเจลาตินไซโมกราฟี ผลไซโมแกรมส์ของอินเตอร์ลิวคิน-6 ที่กระตุ้นเซลล์ KKU-M213 ปรากฏให้เห็นแถบสว่างอย่างชัดเจนบ่งบอกถึงการทำงานของเอ็มเอ็มพี-9 โดยสรุป อินเตอร์ลิวคิน-6 ส่งเสริมการบุกรุกของเซลล์ของมะเร็งท่อน้ำดีโดยเพิ่มการหลั่งเอ็มเอ็มพี-9 และการยับยั้งสัญญาณของอินเตอร์ลิวคิน-6 อาจเป็นเป้าหมายที่เป็นไปได้ในการต้านการบุกรุกของเซลล์เพื่อการรักษามะเร็งท่อน้ำดี

Key Words: Interleukin-6, Cholangiocarcinoma, Cell invasion

คำสำคัญ: อินเตอร์ลิวคิน-6 มะเร็งท่อน้ำดี การบุกรุกเซลล์เนื้อเยื่อ

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Introduction

Cholangiocarcinoma (CCA) refers to all tumors arising from bile duct epithelium. Many risk factors that lead to CCA development have been reported, such as primary sclerosing cholangitis (PSC), specific carcinogens including nitrosamines, and liver fluke infestation (*Clonorchis sinensis* and *Opisthorchis viverrini*) (Bartlett et al., 2011). CCA was observed in a high percentage of liver cancers from northeast Thailand, where the prevalence of *O. viverrini* infection is higher than elsewhere in this country. Khon Kaen province in northeast Thailand has the highest incidence of this type of cancer in the world (Sripa et al., 2007).

Interleukin-6 (IL-6) is now known as a multi-functional cytokine that can be produced by various cell types, including tumor cells. IL-6 plays important roles with a wide range of biological activities in immune regulation, hematopoiesis, and oncogenesis. Most importantly, IL-6 is involved in the proliferation and differentiation of various malignant tumor cells (Guo et al., 2012). IL-6 signaling generally induces phosphorylation of STAT3 which allows for dimerization of STAT3. The dimer form of STAT3 translocates into the nucleus and binds to DNA at specific binding sites, thereby directing transcription of target genes (Huang et al., 2005). Recently, supporting report suggested that a single plasma IL-6 measurement has excellent positive predictive value for the detection of bile duct cancer in regions with high *O. viverrini* transmission (Sripa et al., 2012).

Metastasis is the process whereby cancer cells spread throughout the body, establishing new colonies in organs at a distance from the one where the primary tumor originated (Bacac, Stamenkovic, 2008). In order to break away from the primary tumor and initiate the metastatic process, individual or small groups of cancer cells must acquire the ability to migrate and invade. These traits enable cells to degrade and move through the extracellular matrix of the surrounding tissue toward blood and lymphatic vessels, which in turn provide highways for their passage to distant secondary sites. Understanding the mechanisms of this process is likely to be important for preventing metastasis in patients who are diagnosed with early cancer lesions (Chaffer, Weinberg, 2011). However, mechanisms of cell invasion that are triggered by IL-6 in CCA still unknown.

Objective of the study

The objective of this study was to investigate whether IL-6 plays a role in cell invasion of human CCA cell lines.

Methodology

Cell culture

Two human cholangiocarcinoma cell lines, KKU-M213 and KKU-100, were obtained from Thai patients and cultured in Ham's F12 culture medium (Invitrogen, New Zealand) supplemented with 10% fetal bovine serum (FBS), 100 U/ml antibiotic-antimycotic, 100 µg/ml (Gibco BRL, Paisley, Scotland) and 15 mM HEPES (Merck, Darmstadt, Germany). Cells were incubated at 37°C in a humidified atmosphere with 5% CO_2.

In-vitro Transwell invasion assay

Invasion assay was performed by Matrigel Transwell in vitro invasion assay. In brief, the upper chamber of a Transwell unit (6.5 mm diameter polycarbonate membrane with 8-µm-pore size) (Corning, United States) was coated with 30 µg of

Matrigel (BD Biosciences, United States). Cells (1 × 10^5 cells) in 200 µl of serum-free medium with 20 ng/ml recombinant human IL-6 were seeded to the upper chamber. The lower chamber was filled with 600 µl of media containing 1% FBS. After 12 hrs of incubation at 37°C under CO_2 atmosphere, non-invading cells in the upper chamber were removed and cells that invaded the Matrigel and had attached to the lower surface of the Transwell membrane were fixed with 25% methanol for 15 min and stained with 0.25% crystal violet. Invaded cells were counted in 5 random fields under light microscope at 10x magnification.

Gelatin zymography

To examine the expression and activation of matrix metalloproteinases (MMPs), cells were seeded on 60×15 mm tissue culture dishes to reach 80% confluence for 24 hrs. Then, cells were treated with a recombinant human IL-6 (20 ng/ml) for 24 hrs at 37 °C and conditioned media was collected by centrifugation. Samples were mixed with sample buffer in the absence of reducing agent and separated by 7.5% acrylamide gel containing 1% gelatin at 4°C. Then, the gel was washed in 2.5% Triton X-100 solution at room temperature with gentle agitation and soaked in incubation buffer (500 mmol/L Tris-HCl pH 8, 100 mmol/L $CaCl_2$, 1 mmol/L $ZnCl_2$ and 1% TritonX-100) at 37°C overnight. Afterwards, gel was stained with 0.25 % Coomassie blue for 2 hrs at room temperature, destained for 10 min and dried with cellophane paper. MMPs were detected as clear band against a blue background.

Results

Effect of exogenous IL-6 on cell invasion in CCA was determined in two CCA cell lines, KKU-M213 and KKU-100. As shown in Figure 1, KKU-M213 cell line displayed higher basal level of invasiveness than KKU-100 cell line. Moreover, IL-6 treatment (20 ng/ml) could increase cell invasion of KKU-M213 cells but not of KKU-100 cells.

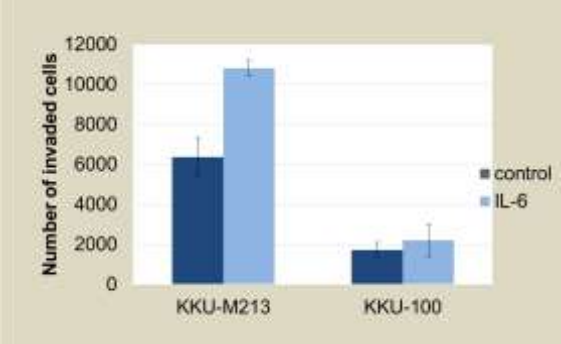

Figure 1 Matrigel-Transwell assay was used to determine cell invasion after KKU-M213 and KKU-100 cell lines were incubated without or with IL-6 (20 ng/ml) for 12 hrs.

The proteolysis of basement membrane by MMPs is one of metastasis process that successfully promotes cell invasion. Thus, to investigate the mechanisms underlying IL-6-induced cell invasion, the effect of IL-6 on secretion of MMP-2 and MMP-9 in KKU-M213 cells was measured by gelatin zymography.

The results showed that IL-6 could significantly increase MMP-9 secretion but not MMP-2 in KKU-M213 cells (Figure 2). A clear band of MMP-9 indicated that MMP-9 (gelatinase enzyme) could digest gelatin substrate in gel.

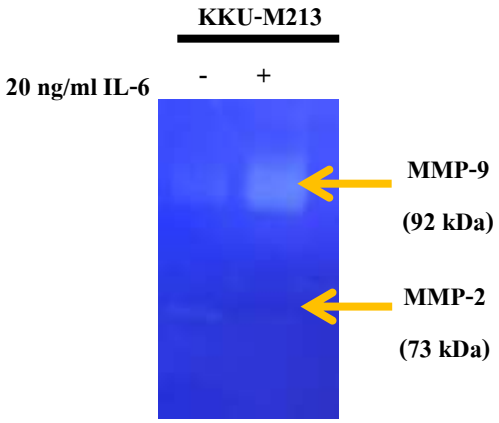

Figure 2 MMP-2 (73 kDa) and MMP-9 (92 kDa) secretion were determined by gelatin zymography after KKU-M213 cells were treated without or with IL-6 (20 ng/ml).

Discussion and Conclusions

IL-6 is a multifunctional cytokine which plays an important role in a wide range of biological activities in different types of cells including tumor cells. IL-6 is involved in the host immune defense mechanism as well as the modulation of growth and differentiation in various malignancies. Clinical studies have revealed that increased serum IL-6 concentrations in patients are associated with advanced tumor stages of various cancers and short survival in patients. Therefore, blocking IL-6 signaling is a potential therapeutic strategy for cancer. Several investigators have reported an aberrant IL-6 pathway activation in a variety of human cancer cell lines and solid tumors and IL-6 has been found to play an important role in various tumor behaviors including cell invasion. In this study, we used two different CCA cell lines, KKU-M213 and KKU-100, as models to study *in vitro* cell invasion of CCA. The results showed that IL-6 could increase cell invasion through MMP-9 secretion in KKU-M213 cells. Our results are consistent with the previous studies showing the effect of IL-6 in human malignant non-Hodgkin's lymphomas (Kossakowska et al., 1999) and human cerebral endothelial cell (Yao et al., 2006). However, IL-6 had no effect on cell invasion in KKU-100 cells. The lack of IL-6 effect may be due to lack of IL-6 receptors expression in this cell line.

In conclusion, IL-6 can promote cell invasion by up-regulating MMP-9 secretion in CCA and IL-6 signaling may be a potential target for the treatment of cholangiocarcinoma.

Acknowledgements

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