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Tumor necrosis factor α promotes invasiveness of cholangiocarcinoma cells via its receptor, TNFR2

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Abstract

We studied the effect of TNF-α stimulation on a cholangiocarcinoma cell line, CCKS1. CCKS1 expressed only one type TNF receptor, TNFR2. Treatment of CCKS1 with TNF-α substantially activated NFκB, MAPK and Akt signalings which in turn activated matrix metalloproteinase-9 (MMP-9) secretion and in vitro invasiveness of CCKS1. Pretreatment of cells with anti-TNFR2 neutralizing antibody inhibited the TNF-α-dependent signaling and MMP-9 secretion and subsequently blocked invasion in vitro. Moreover, an inhibitor for matrix metalloproteinase, Galardin, suppressed the invasion in a dose-dependent manner. Similarly, pharmacological inhibition of signaling clearly suppressed the TNF-α dependent MMP-9 secretion. These results strongly suggest that TNF-α-TNFR2 signaling plays an important role to convert the cholangiocarcinoma cells to be more aggressive one.

Keywords: Cholangiocarcinoma; Matrix metalloproteinase; Tumor necrosis factor-α; Invasion

Abbreviations: TNF, tumor necrosis factor; MMP, matrix metalloproteinase; TNFR, tumor necrosis factor receptor; RT-PCR, reverse-transcription-PCR.

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1. Introduction

Cholangiocarcinoma is a malignant tumor derived from the bile duct epithelium. While cholangiocarcinoma is a relatively rare tumor in temperate countries, it is one the most common liver tumors in some areas and the incidence of it is higher in south-east Asia [1,2]. Although the progress of the modern combined therapy greatly improved the treatment of cholangiocarcinoma [3], its prognosis at advanced stage is extremely miserable. Epidemiological and experimental studies suggest the significant correlation of cholangiocarcinoma with hepatolithiasis which is frequently associated with recurrent inflammation [1,2,4–6]. A large number of recent studies rendered the concept that inflammation is a critical component of tumor progression [7,8]. Tumor microenvironment orchestrated by inflammatory cells is an indispensable participant in the tumor progression, invasion and metastasis [7–9].

To study overall change in gene expression associated with cholangiocarcinoma, we compared the gene expression between cholangiocarcinoma tissues associated with hepatolithiasis and paired normal tissues by the cDNA array method. As a result of the assay, we observed frequent overexpression of tumor necrosis factor receptors (TNFR) genes in cholangiocarcinoma associated with hepatolithiasis. Tumor necrosis factor (TNF)-α, a member of proinflammatory cytokines, is a mediator of inflammation with actions directed towards both tissue destruction and recovery. Accumulated evidence suggests that TNF-α may act as an endogenous tumor promoter in addition to its role in immune responses (reviewed by Balkwill) [7]. TNF-α can induce of matrix metalloproteinase (MMP)-9 that plays a critical role in tumor invasion and metastasis. MMP-9 is a member of MMPs, a family of zinc-required matrix-degrading enzymes. MMP-9 specifically targets type-4 collagen, a major component of basement membrane, and appears to play a crucial role in tumor invasion across the basement membrane [10]. Indeed, expression of MMP-9 in intrahepatic cholangiocarcinoma is a prognostic factor related to lymph node metastasis [11]. However, regulation of MMP-9 expression in cholangiocarcinoma remains largely unclear.

TNF-α elicits responses through its binding to the receptors, 55 kDa TNF receptor 1 (TNFR1) and 75 kDa TNF receptor (TNFR2). Both TNFR1 and TNFR2 contain similar extracellular domain but have clear difference in their cytoplasmic domains. TNFR1 has a so-called death domain (DD) region, whereas TNFR2 does not. The DD domain plays a critical role in signaling for cell death. Compared with TNFR1, function of TNFR2 is less understood. Activation of TNFR2 in some cells appears to be proliferative [12], whereas other reports suggest its involvement in death signaling, although it lacks the DD domain. So-called 'ligand-passing' mechanism from TNFR2 to TNFR1 is proposed for the induction of cell death by TNFR2 [13,14].

To obtain more clues, we examined the role of TNF-α in tumor invasion of CCKS1 cells, a cell line established from cholangiocarcinoma with intraperitoneal dissemination [15]. In this report, we show that TNFR2 in the absence of TNFR1 plays a critical role in MMP-9 secretion and subsequent activation of invasion in CCKS1.

2. Materials and methods

2.1. Cell culture

Cell lines derived from human cholangiocarcinoma, HuH-28, CCKS1, TFK-1, HuCCT1, cell line from human T-cell lymphoma, Jurkat, and Rat fibroblast 3Y1 cells transformed with v-Src were cultured as described previously [16]. HuH-28, TFK-1 and HuCCT1 were provided by Cell Resource Center for Biomedical Research Institute of Development, Aging and Cancer, Tohoku University.

2.2. Reverse-transcription-polymerase chain reaction (RT-PCR)

RT-PCR was performed as described previously [17]. Briefly, total RNA was purified from cells with TRIzol Reagent (Life Technologies. Inc.). First strand cDNA was generated using random hexamer primers (TOYOBO, ReverTra Ace). RT-PCR was performed with TNFR1, TNFR2 and Gluceralaldehyde-3-phosphate dehydrogenase