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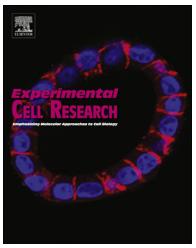

Research Article

Soluble Lutheran/basal cell adhesion molecule is detectable in plasma of hepatocellular carcinoma patients and modulates cellular interaction with laminin-511 in vitro

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ABSTRACT

Lutheran (Lu), an immunoglobulin superfamily transmembrane receptor, is also known as basal cell adhesion molecule (B-CAM). Lu/B-CAM is a specific receptor for laminin α5, a subunit of laminin-511 (LM-511) that is a major component of basement membranes in various tissues. Our previous study showed that Lu/B-CAM was cleaved by MT1-MMP and released from cell surfaces. In this study we examined the soluble Lu/B-CAM in culture media and in plasma of mice bearing HuH-7 hepatocellular carcinoma (HCC) cells and patients with HCC. Two HCC cell lines, HepG2 and HuH-7, released Lu/B-CAM into the culture media. Although Lu/B-CAM was cleaved by MT1-MMP in HuH-7 cells, HepG2 cells released Lu/B-CAM in a MMP-independent manner. The concentration of Lu/B-CAM released into mouse plasma correlated with tumor size. Moreover the soluble Lu/B-CAM in plasma of HCC patients was significantly decreased after resection of the tumor. Immunohistochemical studies showed that although the expression of Lu/B-CAM was observed in most HCCs, MT1-MMP was not always expressed in tumor tissues, suggesting that a part of Lu/B-CAM in plasma of HCC patients was also released in a MMP-independent manner. In vitro studies showed that the soluble Lu/B-CAM released from HCC cells bound to LM-511. Moreover the soluble Lu/B-CAM influenced cell migration on LM-511. These results suggest that soluble Lu/B-CAM serves as not only a novel marker for HCC but also a modulator in tumor progression.

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Introduction

Progression of HCC results from a multi-step process of carcinogenesis that involves hepatitis virus infection and sequential genetic alterations [1]. In addition to imaging observations, pathological and serological diagnoses of HCC are important for selecting the most appropriate treatments. So far α-fetoprotein (AFP) has been routinely used as a serum markers for diagnosis of HCC. However, because it is often undetectable in serum of HCC patients, novel markers are required for more precise diagnosis of HCC.

Tumor invasion and metastasis have critical impacts on survival of patients suffering from HCC [2]. They are characterized by cells that lose their cell–cell contacts, destroy extracellular matrix (ECM), invade the stroma, spread through blood vessels, and form metastatic regions in other organs. In multiple steps of invasion and metastasis, tumor cells must adhere to and migrate through the surrounding ECM, with local degradation of the matrix. Our previous reports showed that HCC cells were surrounded by ectopic laminins [3]. Laminins are a diverse group of α/β/γ heterotrimers formed from five α, three β and three γ chains and are major components of all basal laminae. Of laminins, α5 chain-containing laminins are ectopically deposited in well- and poorly- differentiated HCCs. The ectopic expression of laminin α5 leads to increased levels of its receptors in HCCs [3]. Of the receptors for laminin α5, Lu/B-CAM is widely expressed in well and poorly differentiated HCCs. Lu is a transmembrane protein in which the extracellular domain contains one variable (V), one constant-1 (C1) and three intermediate (I) immunoglobulin-like domains, V-C1-I-I-I [4–6]. A splice variant of Lu known as B-CAM has the same extracellular and transmembrane domains as Lu, but it lacks the cytoplasmic tail [7]. So far Lu has been studied mainly as the antigen of the Lutheran blood group system and in the context of sickle cell disease. On the other hand, B-CAM was identified as an up-regulated antigen in ovarian carcinoma, suggesting its involvement in tumor progression [7]. However, although the interaction between laminin α5 and Lu/B-CAM is expected to be involved in tumor invasion and metastasis, it is still unproven.

Matrix metalloproteinases (MMPs) are endopeptidases responsible for extracellular matrix degradation. The expression of MMPs in tumor cells enhances behavior consistent with increased malignancy, such as rapid tumor growth, invasion, and metastasis [8]. Of the MMPs, membrane-type 1 matrix metalloproteinase (MT1-MMP) cleaves not only ECM (collagens, fibronectin, vitronectin, laminins, and others) but also cell adhesion molecules (CD44, syndecan-1, and αv integrin) [9,10]. The cleavage of cell adhesion molecules with MT1-MMP is involved in tumor cell migration [11]. Recently, we reported that MT1-MMP associated with Lu/B-CAM and cleaved it in human epidermoid carcinoma A431 cells [12]. Lu/B-CAM was cleaved at the juxtamembrane region of the extracellular domain and released into the culture media. Therefore, it is likely that released Lu/B-CAM is detectable in the plasma of tumor patients.

In this study we found soluble Lu/B-CAM in the culture media of human HCC cell lines and characterized its release from the cell surface. In addition, we could detect soluble Lu/B-CAM in the plasma of mice bearing the human HCC cells. Moreover, we measured the concentration of soluble Lu/B-CAM in the plasma of HCC patients using sandwich ELISA. In vitro studies revealed the possibility that Lu/B-CAM is released from cell surfaces as a modulator in the progression of HCC.

Materials and methods

Antibodies and reagents

Monoclonal antibody against MT1-MMP (128527) was purchased from R&D systems (Minneapolis, MN). Polyclonal antibody against the hinge region of MT1-MMP was purchased from Millipore (Temecula, CA). Monoclonal antibodies against Lu/B-CAM (87202 and BRIC221) were purchased from R&D systems and Serotec (Oxford, UK), respectively. Polyclonal antibody against the extracellular domain of Lu/B-CAM has been described [3]. IgG purified from antiserum was labeled with a biotinylation kit (GE Healthcare, Little Chalfont, UK). Alexa488- and 594-conjugated secondary antibodies were purchased from Life technologies (Carlsbad, CA). Recombinant proteins containing the Lu/B-CAM extracellular domain fused with a 6xHis-Tag (Sol-Lu) or Fc-Tag (Lu-Fc) were produced and characterized as described previously [13]. Metalloproteinase inhibitor (BB-94) was purchased from Tocris Bioscience (Ellisville, MO).

Patients and tissues

HCCs and surrounding non-cancerous tissues were obtained from nine patients (6 males and 3 females, Tables 1 and 2) who underwent surgical resection at the Sapporo Medical University Hospital, Japan, as described in our previous study [3]. The patients' ages ranged from 43 to 71 years old. Hepatitis B surface

Table 1 – Expression of Lu/B-CAM and MT1-MMP in HCC tissues.

Patient no.	Sex	Differentiation	Lu/B-CAM	MT1-MMP	Merge
1	Male	Well	+++	–	–
2	Male	Well	++	++	+
3	Male	Well	+++	+++	++
4	Female	Moderate	+++	+	–
5	Male	Moderate	+	–	–
6	Male	Poor	+++	+++	++
7	Female	Poor	++	–	–
8	Male	Poor	++	++	+
9	Female	Poor	++	–	–
			(9/9)	(5/9)	(4/9)

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