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Involvement of Galectin-1 and Galectin-3 in Proliferation and Migration of Rat Hepatic Stellate Cells in Culture

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Introduction

Hepatic stellate cells (HSC), liver-specific pericytes, play a pivotal role in hepatic fibrogenesis. Galectin forms a group of animal lectins characterized by their specificity for beta-galactosides. At present, more than 10 galectins have been identified in mammals [1]. Galectin-1 forms a homodimer of 14 kDa subunits, and galectin-3 is a monomer having molecular weights of approximately 32 kDa. Galectin-1 and galectin-3 are localized not only in intracellular space such as the cytoplasm or the nucleus but also in extracellular space such as the cell surface or the extracellular matrix. Although the biological functions of galectin-1 and galectin-3 remain speculative in individual cells, tissues, or diseases, there is evidence that they play a role in cellular proliferation, differentiation, adhesion, neoplastic transformation, apoptosis, neoplastic and extracellular matrix interaction [2-4]. These functions are thought to act by cross-linking beta-galactoside containing glycoconjugates, resulting in modulation of cell signaling [5,6]. Here, we detail the expression pattern of galectin-1 and galectin-3 in activated HSC and in fibrotic liver tissues. We further show that both galectins are possible mitogens for HSC activating MAP kinase pathways presumably by cross-linking extracellular beta-galactoside.

Methods

Sinusoidal cells and hepatocytes were isolated from rat liver as described previously and cultured in DMEM containing 10% FCS. Expression of galectin-1 and galectin-3 in these cells and fibrotic livers was evaluated in protein and mRNA level. Effect of recombinant galectin-1 and galectin-3 on DNA synthesis of HSC and hepatocytes was measured by [^{3}H]thymidine incorporation. Activation of MEK1/2-ERK1/2 and Akt signal pathways was analyzed by western blot using phospho-specific antibodies. HSC migration was evaluated by using a cell culture insert. Collagen alpha 2(I) gene expression in stimulated HSC was assessed.

Results

In hepatic constituent cells isolated from intact liver, only Kupffer cells exhibited the expression of galectin-3. Expression of mRNAs and proteins for galectin-1 and galectin-3 increased time-dependently in HSC during the primary culture. In TAA-induced fibrotic livers, galectin-1 was expressed in HSC and galectin-3 in HSC and Kupffer cells. Both galectin-1 and galectin-3 dose-dependently stimulated DNA synthesis of HSC, but not hepatocytes. Galectin-1 was found to be a stronger mitogen for HSC

than galectin-3. Among ERK1/2, p38 MAPK, SAPK/JNK and Akt, both galectin-1 and galectin-3 induced phosphorylation of ERK1/2 at 120 min after addition. Phosphorylation of MEK1/2, upstream signal of ERK1/2, also happened. Thiodigalactoside, a beta-galactoside-binding inhibitor, significantly suppressed the galectin-dependent both ERK1/2 activation and DNA synthesis. These galectin-dependent DNA synthesis and ERK1/2 phosphorylation were hampered dose-dependently by PD98059 and U0126, but neither by SB203580 nor LY294002. Although galectin-1-stimulated ERK1/2 phospholyration was affected neither by GF109203X, a PKC inhibitor, nor H-89, a PKA inhibitor, galectin-3-stimulated ERK1/2 phospholyration was attenuated dose-dependently by these agents. Galectin-1, but not galectin-3, significantly promoted HSC migration. Neither galectin-1 nor galectin-3 affected collagen alpha 2(I) mRNA expression in activated HSC.

Discussion

Our present study revealed that expression of galectin-1 and galectin-3 was induced in activated HSC and fibrotic livers, and that galectin-1 was found to be more potent mitogen for HSC than galectin-3. There have been little reports on the intracellular signal pathways through which galectins stimulate cell proliferation. We clarified that HSC proliferation in response to galectin-1 and galectin-3 could be mediated by a MEK1/2-ERK1/2 pathway. However, PKC and PKA were involved in galectin-3-, but not galectin-1-, dependent activation of ERK1/2 in HSC. These results indicated that signal stream toward ERK1/2 stimulation, thereby DNA synthesis of HSC, could be differently regulated between galectin-1 and galectin-3. We speculate that counter receptor of galectin-1 in HSC surface may be different from that of galectin-3, or that the conformations of cell surface glycoproteins are differently modified by either galectin-1 or galectin-3.

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