Enzyme-digested fucoidan extracts derived from seaweed Mozuku of Cladosiphon novae-caledoniae kylin inhibit invasion and angiogenesis of tumor cells

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Abstract

Fucoidan is a uniquely-structured sulfated polysaccharide found in the cell walls of several types of brown seaweed that has recently, especially as enzyme-digested fucoidan extract, attracted a lot attention due to its anti-tumor potential. In this study, we evaluated the effects of enzyme-digested fucoidan extracts prepared from seaweed Mozuku of Cladosiphon novae-caledoniae kylin on in vitro invasion and angiogenesis abilities of human tumor cells. First, we evaluated the effect of the fucoidan extracts on oxidative stress of tumor cells, and demonstrated that intracellular H₂O₂ level and released H₂O₂ from tumor cells were both greatly repressed upon the treatment with the fucoidan extracts, suggesting that fucoidan extracts ameliorate oxidative stress of tumor cells. Next, we tested for the effects of fucoidan extracts on invasion ability of human fibrosarcoma HT1080 cells, showing that fucoidan extracts significantly inhibit their invasion, possibly via suppressing matrix metalloproteinases (MMPs) MMP-2/9 activities. Further, we investigated the effects of the fucoidan extracts on angiogenesis of human uterine carcinoma HeLa cells, and found that fucoidan extracts suppressed expression and secretion of an angiogenesis factor vascular endothelial growth factor (VEGF), resulting in suppressed vascular tubules formation of tumor cells. The results taken together clarified that enzyme-digested fucoidan extracts from Cladosiphon novae-caledoniae kylin possess inhibitory effects on invasion and angiogenesis of tumor cells. These effects might, at least partially, be elicited by the antioxidative potential of enzyme digested fucoidan extracts.

Introduction

Metastasis is a major problem in cancer treatment/therapy; there being several sequential steps in cancer cell metastasis. Cancer cells migrate from the primary cancerous site to other parts of the body via the bloodstream or lymph system, absorb to extracellular matrix (ECM) and degrade its surrounding proteins. Matrix metalloproteinases (MMPs) are key enzymes involved in tumor
invasion, where MMPs degrade ECM proteins such as collagen, proteoglycan, elastin, laminin, and fibronectin (Johnson et al. 1998). In human, MMP-2 (gelatinase A/M, 72,000 type IV collagenase) and MMP-9 (gelatinase B/M, 92,000 type IV collagenase) are thought to be key enzymes for degrading type IV collagen, which is a major component of the basement membrane (Westermarck and Kahari 1999). Both MMP-2 and MMP-9 are abundantly expressed in various malignant tumors (Johnsen et al. 1998) and contribute to invasion and metastasis (Liabakk et al. 1996).

Tumor angiogenesis, the formation of new blood capillaries by vascular endothelial cells from existing vessels, is an important mechanism for supplying nutrients to tumor cells that are distant from existing blood vessels. Tumor angiogenesis is thought to be controlled by angiogenic factors including fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF). VEGF is a highly conserved dimeric heparin-binding glycoprotein and has a pivotal role in the regulation of normal and abnormal angiogenesis (Ferrara 1993). At present, reactive oxygen species (ROS) are thought to be involved in the regulation of angiogenesis, suggesting that ROS also regulate the expression of VEGF, and that ROS-scavengers is ideal anti-angiogenesis agents.

Fucoidan is a uniquely-structured sulfated polysaccharide found in the cell walls of several types of brown seaweed. Recently, fucoidan has attracted a lot of clinical attention due to its strong anti-tumor potential, which has been intensively investigated. Fucoidan suppresses the growth of tumor cells in vivo and activates the immune system against tumors (Usui et al. 1980; Yamamoto et al. 1984; Noda et al. 1990; Itoh et al. 1993; Zhuang et al. 1995; Maruyama et al. 2003). Sulphation of fucoidan enhances its antitumor activity (Yamamoto et al. 1984). Koyanagi et al. (2003) reported that fucoidan inhibited tube formation following migration of human umbilical vein endothelial cells (HUVEC) and its chemical over-sulfation enhances the inhibitory potency. They suggested that fucoidan inhibited the binding of VEGF to the VEGF receptor. Recently it has been reported that a low molecular weight fucoidan (MW, ca. 4 kDa) prepared by radical degradation promotes basic fibroblast growth factor-induced tube formation of endothelial cells (Chabut et al. 2003, 2004). Here we present the first evidence that enzyme-digested fucoidan extract derived from Mozuku of Cladosiphon novae-caledoniae kylin, which originates in the Kingdom of Tonga, scavenges intracellular ROS and suppresses the invasion and angiogenesis abilities of tumor cells.

Materials and methods

Preparation of fucoidan and reagents

The abalone glycosidase-digested fucoidan extract prepared from seaweed Mozuku of Cladosiphon novae-caledoniae kylin from the Kingdom of Tonga, commercially available as a product named ‘Power fucoidan’, was donated for the study by the Daiichi Sangyo Corporation (Osaka, Japan). An undiluted solution (pH 3.7) was neutralized to pH 7.0 with NaOH. The precipitants were removed by centrifugation at 2200×g for 15 min. The supernatants were then sterilized with a 0.2 µm pore filter (Millipore, MA, USA), and stored as ‘fucoidan extract (43.5 mg/ml)’ at 4 °C. 2’,7’-Dichlorodihydro-fluorescin diacetate (H₂DCFDA) was purchased from Molecular probes (Eugene, OR, USA). 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt (WST-1) was purchased from Wako (Osaka, Japan). Matrigel and type I collagen were obtained from Funakoshi (Tokyo, Japan). The human VEGF immunoassay kit was obtained from R&D system (MN, USA). Angiogenesis tubule staining kit (for staining CD31) was purchased from TCS Cellworks (Buchingham, UK).

Cell culture and treatment

The human fibrosarcoma cell line HT1080, human uterine carcinoma cell line HeLa and human normal fibroblast TIG-1 were cultured in Minimum Eagle’s medium (MEM: Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS) (Biowest, France), 2 mM L-glutamine and 10 mM HEPES (10% FBS/MEM). HUVEC were cultured in EBM-2 medium (Cambrex, MD, USA). The fucoidan extract (final conc.: 10–20%) was mixed with 10×MEM, and diluted with