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Molecular mechanism of green microalgae, *Dunaliella salina*, involved in attenuating balloon injury-induced neointimal formation

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The pathological mechanism of restenosis is primarily attributed to excessive proliferation of vascular smooth muscle cells (VSMC). The preventive effects of ethanol extract of *Dunaliella salina* (EDS) on balloon injury-induced neointimal formation were investigated. To explore its molecular mechanism in regulating cell proliferation, we first showed that EDS markedly reduced the human aortic smooth muscle cell proliferation via the inhibition of 5′-bromo-2′-deoxyuridine (BrdU) incorporation at 40 and 80 μg/ml. This was further supported by the G_0/G_1-phase arrest using a flow cytometric analysis. In an *in vivo* study, EDS at 40 and 80 μg/ml was previously administered to the Sprague–Dawley rats and found that the thickness of neointima, and the ratio of neointima:media were also reduced. EDS inhibited VSMC proliferation in a dose-dependent manner following stimulation of VSMC cultures with 15 % fetal bovine serum (FBS). Suppressed by EDS were 15 % FBS-stimulated intracellular Raf, phosphorylated extracellular signal-regulated kinases (p-Erk) involved in cell-cycle arrest and proliferating cell nuclear antigen. Phosphorylated focal adhesion kinase (p-FAK) was also suppressed by EDS. Also active caspase-9, caspase-3 and cleaved poly(ADP-ribose) polymerase (PARP) protein expression levels were increased by administration with EDS; the apoptotic pathway may play an important role in the regulatory effects of EDS on cell growth. These observations provide a mechanism of EDS in attenuating cell proliferation, thus as a potential intervention for restenosis.

Human aortic smooth muscle cells: Angioplasty: Neointima formation: Restenosis: *Dunaliella salina*

Dunaliella salina, Teod. (Chlorophyceae), the unicellular halophilic green microalga, is known as a major source of β-carotene. Administration of *D. salina* decreased the levels of cholesterol and lactate dehydrogenase as well as increasing the activities of catalase, superoxide dismutase, serum aspartate aminotransaminase and serum alanine aminotransferase[1]. Aside from being a precursor for vitamin A, *D. salina* has also been known to possess a potent antioxidant activity, as shown in an *in vivo* study[2]. Analysing the constituents of an ethanol extract of *D. salina* (EDS) in our previous study demonstrated 6 % of β-carotene, 0·12 % of α-carotene, 0·2 % of xanthophyll, 0·3 % of zeaxanthin, and scarse amounts of lycopene and chlorophyll[3]. It has been shown that 9-*cis* β-carotene-rich powder of the alga *D. bardawil* increases plasma HDL-cholesterol in fibrate-treated patients[4]. Levy *et al.* found a significant increase in the lag time of oxidising LDL-cholesterol following a 3-week β-carotene supplementation (60 mg/d), suggesting the antioxidant effects of β-carotene[5].

Percutaneous transluminal coronary angioplasty (PTCA) has been used in patients with angina and acute myocardial infarction[6]. However, restenosis in about 30 % of patients within 6 months following the angioplasty procedure has been a major disadvantage of this therapy[7]. Stents were then developed to decrease restenosis rate; however, 20 to 30 % of the patients are still affected by restenosis after coronary stenting[8]. The regulation of this pathological process remains elusive. One of the major causes leading to arterial reocclusion after PTCA has been linked to the outgrowth of vascular smooth muscle cells (VSMC)[9,10]. During this time, growth and prothrombotic factors released from platelets and leucocytes trigger the VSMC cell cycle from the G_1 to S phase[11]. Preventing the cell cycle of VSMC from the G_1 to S phase may be beneficial in reducing cell proliferation or migration[12]. For this reason, drugs associated with cell-cycle blocking are considered as potential candidates to reduce the incidence of restenosis[13]. Restenosis emerges

Abbreviations: BrdU5, 5′-bromo-2′-deoxyuridine; EDS, extract of *Dunaliella salina*; Erk, extracellular signal-regulated kinase; FBS, fetal bovine serum; HASMC, human aortic smooth muscle cells; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PARP, poly(ADP-ribose) polymerase; PCNA, proliferating cell nuclear antigen; p-FAK, phosphorylated focal adhesion kinase; PI, propidium iodide; PTCA, percutaneous transluminal coronary angioplasty; VSMC, vascular smooth muscle cells.

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