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Remnant lipoproteins stimulate proliferation and activate MAPK and Akt signaling pathways via G protein-coupled receptor in PC-3 prostate cancer cells

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Abstract

Background: Hypertriglyceridemia was recently shown to be a risk factor for prostate cancer; however, there are only a few reports about the relationship between prostate cancer and TG (triglycerides) rich lipoproteins. Remnant lipoproteins (RLP) are TG-rich lipoproteins, which are produced by the hydrolysis of very low density lipoproteins and chylomicrons. We examined the direct effect of RLP on the proliferation and signal transduction of prostate cancer cells.
Methods: RLP were isolated from human serum with an immunoaffinity mixed gel containing anti-apoA-1 and anti-apoB-100. We evaluated RLP-induced cell proliferation by using MTS assay. Moreover we examined the direct effect of RLP on the MAPK and Akt signal transductions which are reported to be correlated with prostate cancer by using Western blotting.
Results: Incubation in the presence of RLP for 48 h induced the proliferation of prostate cancer PC-3 cells more significantly than prostate cancer LNCaP cells and human prostate stromal cells. In PC-3 cells, RLP also induced the phosphorylation of MEK/ERK via a G protein-coupled receptor-protein kinase C dependent pathway. Moreover, activation of Akt pathway was observed after RLP treatment of PC-3.
Conclusion: These findings suggested that hypertriglyceridemia, especially remnant hyperlipoproteinemia, might be one of the progressive factors for prostate cancer.

Keywords: Remnant lipoproteins; Prostate cancer; Signal transduction

1. Introduction

The incidence of prostate cancer (PC) is significantly higher in Western countries than in Asian countries, being shown to be very similar with the incidence of cardiovascular diseases [1]. But interestingly, the incidence of latent prostate cancer is well known to be very similar between Western and Asian countries [2]. One of the explanations for this discrepancy is based on a dietary factor: a high intake of dietary fat has been implicated as a risk factor for the development of PC. The epidemiological studies have shown that men who consumed a high-fat diet had more risk of developing PC [3,4].

Blood lipid levels are elevated by an intake of high-fat diet. In blood, they exists as lipoproteins, such as chylomicrons (CM), very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL). Recently, hypertriglyceridemia was shown to be a possible risk factor for PC [5]. Serum triglycerides (TG) are mainly contained in VLDL and CM in the circulation. Remnant lipoproteins (RLP) are TG-rich lipoproteins, which are produced by the hydrolysis of VLDL and CM in plasma [6]. Recently, clinical studies have revealed

Abbreviations: PC; Prostate cancer; CM; chylomicrons; RLPs; remnant lipoproteins; MAPK; mitogen-activated protein kinase; EGFR; epidermal growth factor receptor; GPCR; G protein-coupled receptor; PKC; protein kinase C; PI3K; phosphoinositide 3-kinase; FBS; fetal bovine serum; PrSC; prostate stromal cells; PTX; pertussis toxin; CMe; culture medium.

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that RLP may be a major risk factor for coronary atherosclerosis [7–9]. Furthermore, RLP have some biological activities in vitro, e.g. lipid accumulation by mouse peritoneal macrophages, stimulation of platelet aggregation, impairment of the endothelium, and stimulation of arterial smooth muscle cell proliferation [10–13]. Concerning PC and hyperlipidemia, we have shown previously that RLP-TG increased after estrogen treatment for PC patients [14].

It was reported that RLP induce the proliferation of smooth muscle cells via the activation of mitogen-activated protein kinase (MAPK) and epidermal growth factor receptor (EFGR) transactivation, along with the involvement G protein-coupled receptor (GPCR)-dependent protein kinase C (PKC) [15]. The activation of MAPK mediates important roles in the progression of PC [16], and some growth factors, such as EGF, induce PC cell proliferation by activating MAPK [17]. In addition, activated GPCR allows the activation of phosphoinositide 3-kinase (PI3K) [18]. PI3K effects the activation of Akt, which modulates a number of downstream targets that affect apoptosis and cell cycling [19], and usually promotes tumor progression [20].

As the patients suffering from both metabolic syndrome and malignancies have been increasing greatly, it may be very important to find out the mutual causes of these diseases. Hypertriglyceridemia, especially remnant hyperlipoproteinemia, may be one of the causes common between these diseases, but there have been no reports about the relationship between PC and TG-rich remnant lipoproteins. This is the first report described about the relationship between RLP and malignant cell proliferation in vitro. We investigated the direct effect of RLP on the proliferation of PC and clarified the effect of RLP at the MAPK and Akt pathways in PC cells using RLP isolated from human plasma [21].

2. Materials and methods

2.1. Cells and chemicals

The human prostate cancer cell lines PC-3 and LNCaP were from Dainippon Pharmaceutical (Tokyo, Japan) and cultured in RPMI (Sigma, St Louis, MO) supplemented with 10% fetal bovine serum (FBS) (Moregate, Bulimba, Australia). Human prostate stromal cells (PrSC) were purchased from Sanko Junyaku (Tokyo, Japan) and cultured in MEM (Invitrogen, Carlsbad, CA) supplemented with 10% FBS. PrSC was isolated from a 19-year-old Caucasian male prostate, which exhibited prostate stromal cell characteristics (vimentin positive, cytokeratin negative). The chemical inhibitors used in this study were as follows: PD98059; a MEK inhibitor, AG1478; an EGFR inhibitor, pertussis toxin (PTX); a Gi protein inhibitor, GF109203X; a PKC inhibitor, and LY294002; a PI3K inhibitor (Calbiochem, LA Jolla, CA). Antibodies (rabbit anti-ERK1/2 polyclonal antibody, rabbit anti-phospho-ERK1/2 (Thr202/Tyr204) polyclonal antibody, rabbit anti-MEK1/2 polyclonal antibody, rabbit anti-phospho-MEK1/2 (Ser217/221) polyclonal antibody, rabbit anti-EGF receptor polyclonal antibody, rabbit anti-phospho-EGF receptor (Tyr 845) polyclonal antibody, rabbit anti-Akt polyclonal antibody, and rabbit anti-phospho-Akt (Ser473) polyclonal antibody) were from Cell Signaling (Beverly, MA).

2.2. Isolation of lipoproteins

EDTA plasma was obtained from 16 male patients with hypertriglyceridemia who did not suffer from prostate cancer after informed consent. They were younger than 50 years old or their prostate specific antigen levels were <4.0 ng/ml. Total VLDL (composed of VLDL, CM, VLDL remnants and CM remnants) (d<1.006) was isolated from the plasma by ultracentrifugation for 1.5 h at 120,000 rpm and 4 °C in a Himac CS120FX (Hitachi, Tokyo, Japan) with a S120AT2 rotor (Hitachi, Tokyo, Japan). RLP were isolated from plasma samples using immunoaffinity mixed gels containing 2 clones of monoclonal antibodies (JIMRO, Takasaki, Japan), as described previously [21]. RLP and total VLDL were dialyzed overnight against 5 1×2 of PBS (pH 7.4) and then sterilized using a 0.22 μm filter unit (Millipore, Billerica, MA). In this study, we decided the dose of RLP and total VLDL by measuring TG concentrations, because RLP and total VLDL were TG-rich lipoproteins. The TG concentration of lipoproteins was measured by biochemical autoanalyzer TBA-20R (Toshiba, Tokyo, Japan). This study was approved by the Ethical Committee of Gunma University.

2.3. Cell Proliferation assay of human prostate cancer cells and PrSC

Approximately 2×10^3 PC-3 cells per well, 3×10^3 LNCaP cells per well or 2×10^3 PrSC cells per well were seeded into a 96-well microtiter plate in 100 μl of culture medium (CMe) with 1.0% FBS for 48 h at 37 °C in a 5% CO_2 atmosphere. Thereafter, the CMe was aspirated and the cells were incubated with CMe containing various concentrations of RLP. For chemical inhibitor experiments, 20 μmol/l PD98059, 1 μmol/l AG1478, 100 ng/ml PTX, 1 μmol/l GF109203X, or 2.5 μmol/l LY294002 was added prior to RLP stimulation. After incubation at 37 °C in 5% CO_2 for 48 h, the number of living cells was measured using an MTS assay (Celltiter 96 Aqueous one solution cell proliferation assay, Promega, Madison, WI) according to the manufacturer's instructions. Briefly, MTS solution was added to each well and the cells were incubated for a final 3 h. The absorbance at 490 nm was measured using a multiwell plate reader (Model 680, Bio-Rad, Richmond, CA), with wells containing medium but no cells serving as blank controls.

2.4. Western blotting assays

Phospho-ERK1/2, phospho-MEK1/2, phosphor-EGFR and phosphor-Akt were detected using Cell Signaling kits with modification of the manufacturer's instructions. PC-3 cells, in CM with 1.0% FBS, were pretreated with the indicated concentrations of inhibitors prior to stimulation with RLP, as described in the figure legends. Cell lysates were prepared in a buffer containing 62.5 mmol/l Tris-HCl (pH 6.8 at 25 °C), 2% w/v SDS, 10% glycerol, 1 mmol/l sodium orthovanadate (Sigma) and protease inhibitors (Complete TM-without EDTA, Roche Diagnostics, Penzberg, Germany). Cell lysates were sheared through a 26-gauge needle and clarified by centrifugation. Protein concentrations were determined by DC protein assay (Bio-Rad) according to the manufacturer's instructions. After determination of the protein content, β-mercaptoethanol (6%) and bromophenol blue (0.001%) were added to each sample. Samples were boiled for 5 min and equal amounts of proteins (~30 μg per lane) were electrophoresed on a 5–20% SDS-polyacrylamide gel and transferred onto a nitrocellulose membrane. Each membrane was incubated with the primary antibodies described above. Blots were developed with a 1:2000 dilution of horseradish peroxidase-conjugated secondary antibody (cell signaling). Proteins were visualized using Immobilon Western HRP Reagent (Millipore).

2.5. Statistical analysis

Data are expressed as the mean±SD. Differences between the values were evaluated with one-way analysis of variance (one-way ANOVA) by Tukey's post-hoc analysis. In all analyses, a $P<0.05$ were considered statistically significant.

3. Results

3.1. Effects of RLP on the proliferation of PC-3, LNCaP and PrSC

First, we performed a cell proliferation assay using MTS to examine the effect of RLPs on PC-3, LNCaP and PrSC. The viable cell number of PC-3 was increased after RLP treatment in a dose-dependent manner as shown in Fig. 1. The proliferations of PC-3 by RLP (50 mgTG/dl) were more remarkable than by