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Original Research

Effect of PPARδ Agonist on Stearoyl-CoA Desaturase 1 in Human Pancreatic Cancer Cells: Role of MEK/ERK1/2 Pathway

Shima Byagowi MSc [a], Taghi Naserpour Farivar PhD [a], Reza Najafipour PhD [a], Mehdi Sahmani PhD [a], Masoud Darabi PhD [b], Shabnam Fayezi DVM [c], Shahab Mirshahvaladi PhD [d], Maryam Darabi PhD [a,*]

[a] *Cellular and Molecular Research Center, Faculty of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran*
[b] *Liver and Gastrointestinal Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
[c] *Students Research Committee, Faculty of Medicine, Department of Anatomy and Cell Biology, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[d] *Department of Biotechnology, Cellular and Molecular and Burns Research Centers, Iran University of Medical Sciences, Tehran, Iran*

A R T I C L E I N F O

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A B S T R A C T

Objective: The stearoyl-CoA desaturase 1 (SCD1), also known as Δ9-desaturase, is a regulatory enzyme in the cellular lipid modification process that has been linked to pancreatic cancer and diabetes. The aim of the present study was to investigate the effect of peroxisome proliferative-activated receptor δ (PPARδ) agonist and ERK1/2- and EGF receptor (EGFR)-dependent pathways on the expression of SCD1 in human pancreatic carcinoma cell line PANC-1.
Methods: PANC-1 cells cultured in RPMI-1640 were exposed to the commonly used MEK inhibitor PD98059, EGFR-selective inhibitor AG1478, and PPARδ agonist GW0742. Changes in mRNA, protein expression and activity index of SCD1 were then determined using real-time reverse transcription polymerase chain reaction, Western blot and gas liquid chromatography, respectively.
Results: The activity index and expression of SCD1 ($p<0.01$) decreased following treatment with PPARδ agonist at both mRNA and protein levels, whereas significant increases were observed after treatment with MEK or EGFR inhibitor. It was also found that the activity index of SCD1 were lower ($p<0.01$) in the combined treatment compared to the incubation with either inhibitor alone.
Conclusions: PPARδ and MEK/ERK1/2- and EGFR-dependent pathways affect the expression and activity of SCD1 in pancreatic cancer cells. Furthermore, the aforementioned kinase signalling pathways were involved in an inhibitory effect on the expression and activity of SCD1 in these cells, possibly via PPARδ activation.

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Mots Clés :
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R É S U M É

Objectif : Le stéaryle-CoA désaturase 1 (SCD1), également connu sous le nom de Δ9-désaturase, est une enzyme régulatrice dans le processus de modification cellulaire des lipides qui a été liée au cancer du pancréas et au diabète. Le but de la présente étude était d'examiner l'effet de l'agoniste du récepteur δ activé de la prolifération des peroxysomes (PPARδ) et les voies dépendantes de ERK1/2 et du récepteur EGF (R-EGF) sur l'expression du SCD1 dans la lignée cellulaire PANC-1 de l'adénocarcinome du pancréas humain.
Méthodes : Les cellules PANC-1 mises en culture en RPMI-1640 ont été exposées aux inhibiteurs les plus fréquemment utilisés : PD98059, l'inhibiteur de MEK, AG1478, un inhibiteur spécifique du R-EGF, et GW0742, un agoniste du PPARδ. Les modifications de l'ARNm, de l'expression de la protéine et de l'indice d'activité du SCD1 ont été déterminées de manière respective par la transcription inverse de la réaction en chaîne par polymérase en temps réel, le buvardage de Western et la chromatographie gaz-liquide.
Résultats : L'indice d'activité et l'expression du SCD1 ($p < 0{,}01$) ont diminué au niveau de l'ARNm et au niveau de la protéine à la suite du traitement par l'agoniste du PPARδ, tandis que des augmentations significatives ont été observées après le traitement par MEK ou par l'inhibiteur R-EGF. Il a également été

* Address for correspondence: Maryam Darabi, PhD, Cellular and Molecular Research Center, Faculty of Medicine, Qazvin University of Medical Sciences, Qazvin 3411975981 Iran.
E-mail address: darabim@tbzmed.ac.ir

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observé que l'indice d'activité du SCD1 était plus faible ($p < 0,01$) lors du traitement combiné que lors de l'incubation avec l'un ou l'autre des inhibiteurs.
Conclusions : Le PPARδ et les voies dépendantes du MEK/ERK1/2 et du R-EGF affectent l'expression et l'activité du SCD1 dans les cellules cancéreuses du pancréas. De plus, les voies de signalisation des kinases ci-dessus mentionnées ont été impliquées dans l'effet inhibiteur de l'expression et l'activité du SCD1 dans ces cellules, possiblement par l'activation du PPARδ.
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Introduction

Fatty acid desaturases are important lipid-modifying enzymes that play critical roles in multiple biologic functions, such as cell membrane fluidity, signal transduction, differentiation and inflammatory responses (1). The stearoyl-CoA desaturase 1 (SCD1), also known as Δ9-desaturase, is a key regulatory enzyme in desaturating the products of *de novo* fatty acid synthesis. This enzyme provides monounsaturated fatty acids (MUFA) used by other lipogenic enzymes like acyl-CoA cholesterol acyltransferase and diacylglycerol acyltransferase (2). Increased levels of SCD1 activity have been reported in various human cancers (3,4). Recent studies in patients with pancreatic cancer have shown elevated SCD1 activity, which was reflected by an increased proportion of serum MUFA (3,5).

The delta isoform of the peroxisome proliferator-activated receptor (PPAR) δ is a family of nuclear receptors that is involved in various cellular processes, especially in glucose and lipid metabolism (6). Agonist-induced activation of PPARδ increases fatty acid catabolism (7), improves insulin sensitivity (8) and induces differentiation (9). PPARδ expression levels were found to be significantly increased in human pancreatic cancer and were correlated with increased risk for tumour recurrence and metastasis (10). However, conflicting data concerning the antioncogenic (11) or tumour promoting effects of PPARδ have been reported (12), and the underlying mechanisms of its action have not yet been fully understood.

PPARδ is a phosphoprotein, and its state of phosphorylation can be modulated by signalling pathways that are related to lipid metabolism and tumorigenesis, such as protein kinase cascades (13). Recent findings link ERK1/2 kinases with endogenous fatty acid desaturation. Inhibition of ERK1/2 could increase the expression level of SCD1 in hepatocellular carcinoma cell line HepG2 (14,15) and Δ6-desaturase in pancreatic carcinoma cell line PANC-1 (16). Thus, it is possible that ERK1/2 signalling affects SCD1 expression through the PPARδ pathway.

Based on the importance of PPARδ activity and ERK1/2 signalling in the regulation of lipogenesis and cancer progression, we tested the effects of PPARδ agonist and ERK1/2 inhibition on SCD1 expression and activity in human pancreatic carcinoma cell line PANC-1. To further elucidate the upstream signalling involved in this pathway, the effects of a selective inhibitor of EGF receptor (EGFR) was also examined in this study.

Methods

PANC-1 cells were grown in RPMI 1640 (Sigma-Aldrich, St. Louis, Missouri, USA) containing 10% FBS, L-glutamine (2 mM), penicillin (100 units/mL), and streptomycin (100 μg/mL) at 37°C, 5% CO_2/95% humidity. Following 48 hours of incubation with the specific inhibitor of the MEK PD 98059 or the selective inhibitor of EGFR AG1478 simultaneously, with or without PPARδ agonist GW0742, the culture medium was removed; the cell monolayer was washed and collected for fatty acids and mRNA and protein expression studies. Details of the experiments and measurements have been reported earlier (15,16) and are briefly summarized below.

Real-time reverse transcription polymerase chain reaction analysis

SCD1 (NM_005063) primers (17) for reverse transcription polymerase chain reaction (RT-PCR) were designed to amplify a segment in the cDNA sequence. The TaqMan (Life Technologies, Grand Island, New York, USA) probes were labelled with a reporter dye (FAM) and a quencher dye (TAMRA). The quantity of SCD1 mRNA was divided by GAPDH mRNA content, and all quantities are expressed as an ×-fold difference relative to a calibrator.

Western blot analysis

Western blot analysis was performed according to the standard procedures (Bio-Rad, Richmond, California, USA). Briefly, 30 μg of whole cell extract were separated by SDS-PAGE (Life Technologies). After electrotransfer to Immobilon-P membrane (Millipore, Bedford, Massachusetts, USA), the blots were blocked and subjected to Western blot analysis with either polyclonal anti-SCD1 or anti-β-actin (Abcam, Cambridge, Massachusetts, USA). For quantification, the developed films were scanned and the pixel intensity of SCD1 signal was normalized against β-actin for each sample.

Fatty acid analysis

Fatty acid methyl esters were extracted and analyzed for fatty acid composition, as described previously by us (15). Briefly, fatty acid methyl ester derivatives formed from isolated cellular lipids were separated on a 60 × 0.25 mm Teknokroma TR-CN100 column (Barcelona, Spain) using a Buck Scientific model 610 gas chromatograph (Norwalk, Connecticut, USA) equipped with a split injector and a flame ionization detector. Helium was used as the carrier gas. The oven temperature program was 170° to 210°C, 1°C per minute, and then isothermal for 45 minutes. Tridecanoic acid (13:0) was used as the internal standard. The ratio of oleic acid (18:1n-9)/stearic acid (18:0) was calculated as a metabolic index of SCD1 activity.

Statistics

Data presented are the mean ± SE of 3 separate experiments. Calculation of significance among groups was done according to ANOVA. A Tukey multiple-comparison post hoc test was performed to compare mean values among groups. A p value of <0.05 was considered statistically significant.

Results

To define whether there is a connection between PPARδ and ERK1/2 signalling pathway on the expression of SCD1 enzyme, PANC-1 cells were treated with a specific PPARδ agonist (GW0742), a selective inhibitor of MEK kinase (PD98059) or an EGFR-selective tyrosine kinase inhibitor (AG1478).

To optimize the assay, cultured PANC-1 cells were incubated with differing concentrations of GW0742 (0-20 μM); PD98059 (0-40 μM) or AG1478 (0-10 μM) for 48 hours at 37°C (Figure 1). At 1 μM concentration, GW0742 induced no apparent effect on SCD1 mRNA expression. At 10 to 20 μM of GW0742, SCD1 expression was

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