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ORIGINAL ARTICLE

Effect of sitagliptin on down-regulation of KAT7 and SIRT1 gene expression in breast cancer cell line MCF7

Efecto de la sitaglipina en la regulación a la baja de la expresión de los genes KAT7 y SIRT1 en la línea celular de cáncer de mama MCF7

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Abstract

Background. *To date, the main clinical interest in DPP4 is focused on its inhibition in diabetic patients to prolong the half-life of incretins. Epigenetic alterations resulting from DPP4 inhibition have been poorly explored.* **Objective.** *The objective of this study was to determine, whether sitagliptin, a DPP4 inhibitor, has effects on the expression of KAT7 and SIRT1 (genes encoding a histone acetyltransferase and a histone deacetylase, respectively) in MCF7 breast cancer cells, which play an essential role in modulating the epigenetic landscape of chromatin.* **Material and methods.** *MCF7 cells were incubated for 20 h with sitagliptin at concentrations of 0.5, 1.0 and 2.0 μM. Total RNA was isolated and the relative mRNA expression of KAT7 and SIRT1 was determined by RT-qPCR.* **Results.** *There was downregulation in the relative expression of both genes; for KAT7, downregulation reached up to 0.49 ($p = 0.027$) and for SIRT1, it reached up to 0.55 ($p = 0.037$).* **Conclusions.** *These results suggest that sitagliptin has effects on the histone epigenetic landscape. This topic deserves further study due to the current sample use of DPP4 inhibitors in diabetic patients.*

Keywords: *Acetylation. DPP4. KAT7. MCF7. SIRT1.*

Resumen

Antecedentes. *Hasta la fecha, el principal interés clínico de la DPP4 se centra en su inhibición en pacientes diabéticos para prolongar la vida media de las incretinas. Las alteraciones epigenéticas resultantes de la inhibición de DPP4 han sido poco exploradas.* **Objetivo.** *Determinar si la sitagliptina, un inhibidor de DPP4, tiene efectos sobre la expresión de KAT7 y SIRT1 (genes que codifican una histona acetiltransferasa y una histona desacetilasa, respectivamente) en células de cáncer de mama MCF7, que desempeñan un papel esencial en la modulación del paisaje epigenético de la cromatina.* **Método.** *Las células MCF7 se incubaron durante 20 h con sitagliptina a concentraciones de 0.5, 1.0 y 2.0 μM. Se aisló el ARN total y se determinó la expresión relativa de ARNm de KAT7 y SIRT1 mediante RT-qPCR.* **Resultados.** *Hubo una regulación a la baja en la expresión relativa de ambos genes; para KAT7, la regulación negativa alcanzó hasta 0.49 ($p = 0.027$) y para SIRT1 alcanzó hasta 0.55 ($p = 0.037$).* **Conclusiones.** *Estos resultados sugieren que la sitagliptina tiene efectos sobre el paisaje epigenético de las histonas. Este tema merece más estudios debido al uso actual de inhibidores de DPP4 en pacientes diabéticos.*

***Palabras clave*:** *Acetilación. DPP4. KAT7. MCF7. SIRT1.*

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Introduction

Dipeptidyl peptidase 4 (DPP4) is an integral membrane protein with aminopeptidase activity since it hydrolyzes the N-terminal or basic end of peptides when aside to the last residue is found proline, hydroxyproline, dehydroxyproline, or alanine. It is encoded by the *DPP4* gene, which is located on the long arm of chromosome 2 and is ubiquitously expressed, having small intestine, prostate, kidney cortex, adipose, and mammary tissues the highest transcriptional levels, according to the database of the GTEx project[1].

In the human being, DPP4 participates through modulation of peptidic factors in many cell processes, which include proliferation, adhesion, and signal transduction. In cancer, the role of DPP4 is controversial; on the one hand, it has been associated with metastatic processes in cancer[2]; on the other hand, it has been described as a tumor suppressor[3].

To date, the main interest in DPP4 is focused on its effect on incretins such as GLP1. Incretins are delivered to the blood by the small intestine in response to food ingest, inducing in the pancreas the insulin secretion; however, the duration of the effect is brief because DPP4 rapidly degrades incretins[4]. As such, DPP4 is a therapeutic target for intervention in diabetes mellitus and with this aim, several DPP4 inhibitors, generically named gliptins, have been developed in the past years to prolong the incretin stimulated insulin secretion. Currently, DPP4 inhibitors are among the most widely used as second-line therapy in the treatment of diabetes mellitus[5].

However, considering the wide distribution of DPP4 expression and its participation in diverse cell processes, much is ignored about the effects of DPP4 inhibitors in other sites than pancreas[6]. Gliptins have shown cardioprotective effects[7], but information on different issues is lacking. Sitagliptin has also been shown to be vasculoprotective, acting through reversion of epigenetic alterations such as increased H3K27Me3, linked to vascular endothelial dysfunction in patients with metabolic syndrome[8].

In cancer, DPP4 promotes epithelial cell transformation and tumorigenesis. In breast cancer, recent data drawn from human specimens and the MCF7 cancer cell line by Choi et al.[9] suggest that DPP4 is an essential mediator of tumorigenesis acting through *PIN1* expression. Furthermore, these authors also showed that the DPP4 inhibitor sitagliptin suppresses the DPP4-induced events in MCF7 cells.

To the best of our knowledge, there are no data related to DPP4 inhibitors in breast cancer and the epigenetic landscape. Hence, as a first approach, this work aimed to determine whether gliptins have effects on the expression of genes related to the epigenetic landscape. We selected the *KAT7* and *SIRT1* genes because encode enzymes related to modifications of the epigenetic landscape at histone level.

Lysine acetyl transferases 7 (KATs7), also known as HBO1, pertains to the MYST family of KATs, which have regulating roles on DNA replication, cell proliferation, and development; it acts in concert with scaffolding proteins of the JADE family to acetylate histones, mainly H3 and H4, unfolding the chromatin and activating gene transcription; additionally, KAT7 acetylates non-histone proteins[10]. On the contrary, *SIRT1* encodes a histone deacetylase of the sirtuin family; its deacetylating activity on histones promotes gene repression; besides, it is involved in deacetylation of non-histone proteins like P53, although its precise role to date is not known. In breast cancer, SIRT1 is supposed to contribute to the chromatin remodeling involved in tumor progression. *SIRT1* overexpression has been found in estrogen receptor-positive breast cancer cases; likewise, estradiol stimulation promotes *SIRT1* expression mediated by estrogen-receptor alpha[11]. Hence, the modulation of the epigenetic landscape by DPP4 inhibitors is an issue that merits research focused on the more rational use of this kind of drugs beyond their current use in diabetes.

Materials and methods

Experimental model

Discovery study in the estrogen-receptor positive breast cancer cell line MCF7 (ATCC® HTB-22™) acquired from American Type Culture Collection (Manassas, VA). The cells were cultured in Eagle's Minimum Essential Medium supplemented with 10% fetal bovine serum, amphotericin, and streptomycin. At the time of the experiments, the cell line maintained its phenotype of estrogen-receptor alpha gene expression evaluated by RTqPCR. High-density triplicate cell cultures in 35 mm polystyrene dishes were incubated by 20 h with the DPP4 inhibitor sitagliptin at concentrations of 0.5, 1.0, and 2.0 μM; these drug concentrations were chosen because they are within the range reached in plasma *in vivo* with therapeutic schemes of sitagliptin in humans. Controls without the drug were included in the study. The cultures were lysed with TRIzol®, and total RNA and proteins were obtained following the manufacturer's recommendations.

Relative expression of KAT7 and SIRT1 mRNA

The relative expression of *KAT7* and *SIRT1* mRNA was determined by real-time PCR using the One-step RT-qPCR kit and an Eco™ illumina® (San Diego, CA) thermal cycler equipped with the Eco Software V.2.0. *PPIA* gene was used as normalizer. The thermal profile was 50°C/30', 95°C/15', 42 cycles of 95°C/15', 55°C/30', and 72°C/30'. The specificity of the products was analyzed by DNA HRM analysis melting curves and the amplicon size verified by agarose gel electrophoresis. The data were analyzed with the REST software v.2.0.13 (Qiagen) using 2000 randomizations.

Other measurements

Morphology and cell proliferation were evaluated by Phase contrast microscopy. The peptidic profile of the total TRIzol® extract of MCF7 cells was assessed by SDS-PAGE, followed by Coomassie-G staining.

Materials

Sitagliptin (Januvia, Merck Sharp and Dhome Limited, Cramlington, Northumberland, UK) was obtained dissolving 25 mg tablets in water; the absorption spectrum was analyzed in the UV-VIS region, and the concentration was determined by spectrophotometry at λ 266 using a molar absorptivity (L. mole-1 cm-1) of 3258.512[12]. Eagle's minimum essential medium, fetal bovine serum, and other cell culture reagents were supplied by Gibco® ThermoFisher™ (Waltham, MA). TRIzol® was acquired from ThermoFisher. RT-qPCR kit was from Qiagen (Germantown, MD). Primers were custom made by IDT Technologies (Coralville, IO); their sequences were designed using the on-line PrimerQuest® Tool software (IDT) (Table S1).

Results

Effect of sitagliptin on the relative expression of KAT7 and SIRT1 Mrna

The amplification products of RT-qPCR were specific according to melting profiles and electrophoretic analysis (Fig. 1 and 2). The relative mRNA expression of *KAT7* and *SIRT1* were downregulated in a statistically significant way at the three sitagliptin

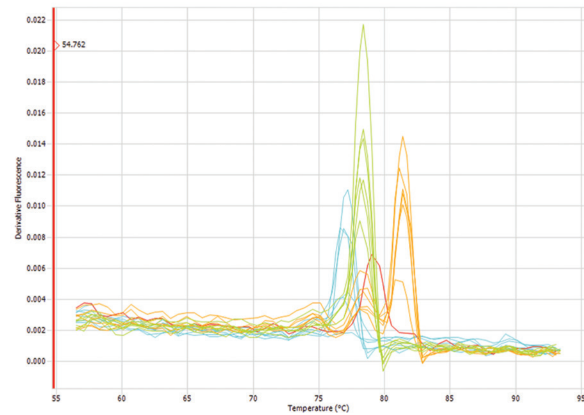

Figure 1. *Representation of the specificity of amplification products through RT-qPCR. The blue color corresponds to the PPIA gene, the orange color to the KAT7 gene, and the green color to the SIRT1 gene.*

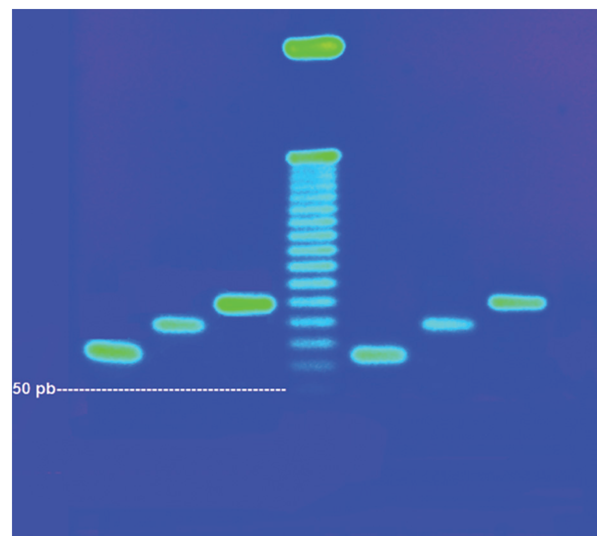

Figure 2. *Specificity of the RT-PCR products in agarose gel for the PPIA, KAT7 and SIRT1 gene, from left to right. The marker has a molecular weight of 50 base pairs.*

Table 1. Effect of sitagliptin on the mRNA expression of *SIRT1* and *KAT7* in the *MCF7* breast cancer cell line

Gene	Sitagliptin	Relative Expression	95% C.I.	p-value
KAT7				
	0.5 μM	0.49	0.36-0.73	0.027
	1.0 μM	0.54	0.37-0.91	0.044
	2.0 μM	0.57	0.43-0.81	0.041
SIRT1				
	0.5 μM	0.55	0.43-0.69	0.037
	1.0 μM	0.64	0.50-0.84	0.013
	2.0 μM	0.65	0.42-0.94	0.031

The effect was calculated using linear regression

concentrations assayed; the observed effect was not concentration dependent (Table 1).

Effect of sitagliptin on cell morphology and the peptidic pattern of cell extracts

Sitagliptin at all the three assayed concentrations did not have effects on cell morphology observed by phase-contrast microscopy, nor on the peptidic profile of protein extracts resolved by SDS-PAGE.

Discussion

This work aimed to determine in the breast cancer cell line MCF7 the effect of the DPP4 inhibitor sitagliptin on the mRNA expression of two genes, *KAT7* and *SIRT1*, involved in acetylation and deacetylation of histones, respectively. It was found that sitagliptin downregulated the relative mRNA expression of both genes in a statistically significant manner. The size of the effect was similar at all the three assayed concentrations; this fact can be explained because they were in the same order of magnitude, selected to be within ranges of plasma levels reached *in vivo* when sitagliptin is used with the therapeutic aim in the treatment of DT2.

To date, there is no information regarding the use of sitagliptin and its association with expression of enzymes related to the histone epigenetic landscape. We speculate that the decrease in *KAT7* and *SIRT1* mRNA expression could be reflected in a reduction of the encoded enzymes, in which through their impact on histone modifications could model the epigenetic landscape in MCF7 cells, without discard deacetylating effects of SIRT1 in non-histone proteins.

In clinical practice, some evidence has been generated that supports our hypothesis, such is the case of the study carried out by Tseng CH, who undertook the task of reconstructing a cohort of women with "*de novo*" diagnosis of type 2 diabetes and breast cancer. Tseng concluded that sitagliptin may reduce breast cancer risk in female patients with type 2 diabetes mellitus, especially 1 year after its use[13]. For their part, Shah et al. carried out a study in patients diagnosed with prostate, pancreatic, and breast cancer, assuming that the use of sitagliptin mediated by DPP4 must have a crucial role in the biology of the tumor. On subgroup analyses of pancreas cancer, the survival favored the group taking DPP4i, irrespective of stage, use of chemotherapy, androgen-deprivation therapy, and prostatectomy or radiation therapy. No association was found in pancreatic or breast cancer. However, given the limited information and contradictory data, the researchers suggest conducting prospective studies and at other levels of research to clarify the mechanisms involved between the use of sitaglipin and these tumors[14].

As DPP4 has ubiquitous pleiotropic effects related to cell proliferation, the obtained data suggest that inhibition of DPP4 by inhibitors currently used in T2D could potentially have distinct therapeutic aims in other diseases, through epigenetic effects. The subjacent mechanisms are beyond the objective of this study, but the results help to support the idea that DPP4 inhibitors could have some beneficial preventive effects for breast cancer in diabetic women under sitagliptin regimens[9]. Likewise, sitagliptin could help as a coadjuvant in breast and another kind of cancer types; therefore, further studies are warranted to shed light in this matter. It is also proposed for future research to include similar experiments on other cell lines.

In summary, here we depicted by the 1st time the effects of DPP4 inhibitors on the mRNA expression of genes encoding enzymes that cause chemical modifications in histones, which contributes to paving the way for more in-depth research on DPP4 inhibitors and their effects beyond those already known regarding the half-life of incretins.

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Conflicts of interest

The authors declare no conflicts of interest.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Supplementary data

Supplementary data are available at online DOI: 10.24875/CIRU.22000189. These data are provided

by the corresponding author and published online for the benefit of the reader. The contents of supplementary data are the sole responsibility of the authors.

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