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Application of the MALDI-MS and MALDI-IMS in the identification of the GPR146 receptor in tissue preparations

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Introduction

G protein-coupled receptor 146 (GPR146, PGR8) belongs to membrane protein with molecular mass around 36.6 kDa. Its activity is regulated by seven typical transmembrane domains (Fig. 1). GPR146 is highly expressed in the livers (especially in hepatocytes) and adipose tissues of humans and mice. The activation of a G-protein coupled receptor can trigger a number of intracellular signalling cascades and responses. Which pathway is activated varies depending on the class of the coupled protein. Regardless of class, the G proteins initiate interactions and relocation of cellular components.

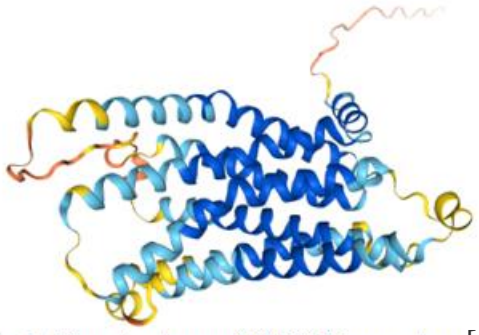

Fig.1. The structure of GPR146 receptor. [5]

Despite the fact that signalling mechanisms have been well studied and established, it has been increasingly clear that GPR146 signalling biology is remarkably more complex than originally thought. Moreover, the number of potential ligands, compatible to GPR146 receptor could be also more, which makes it possible to expand the use of this protein in the diagnosis and treatment of many diseases. Based on human genetic findings and on mouse studies, the G protein-coupled receptor emerges as a promising target against hypercholesterolemia and atherosclerosis [1,2]. It is also known their potential in diabetes treatment – current GPR-targeting therapies include glucagon-like peptide 1 (GLP-1) based agonists, the related DPP4 antagonists, bromocriptine, and serotonin agonists [3]. In turn, proinsulin C-peptide has gained recognition as a potential therapeutic target for the treatment of metabolic disorders and there are some reports indicate on their correlation with GPR146 receptor activity [1,2,4]. Therefore, in order to develop more effective methods of treating type I and II diabetes, it is important to know the correlation between receptor expression and the stage of disease advancement.

Results and Discussion

The attempts were made to detect the transmembrane receptor GPR146 in biological material (fresh tissue and tissue homogenates) collected from various organs of patients with known insulin resistance using matrix-assisted laser desorption/ionization spectrometry (MALDI-MS) and imaging mass spectrometry (MALDI-IMS) equipped in time of flight analyzer (TOF). Sinapic acid (SA) was used as a matrix in both techniques, and a mixture of water and acetonitrile with addition of 1% trifluoroacetic acid as the solvent (in a volume ratio of 1:1).

In the first stage, MALDI-MS measurements were performed for tissue homogenates, collected from patients with known insulin resistance, from: the saphenous vein (Z), right atrium of the heart (U), para-aortic fat tissue (TKO), epicardial fat tissue (TKN) and postthymic fat tissue

(TKP). Furthermore, MS spectra for the standard sample of GPR146 were also recorded and compared with homogenates results (Fig.2).

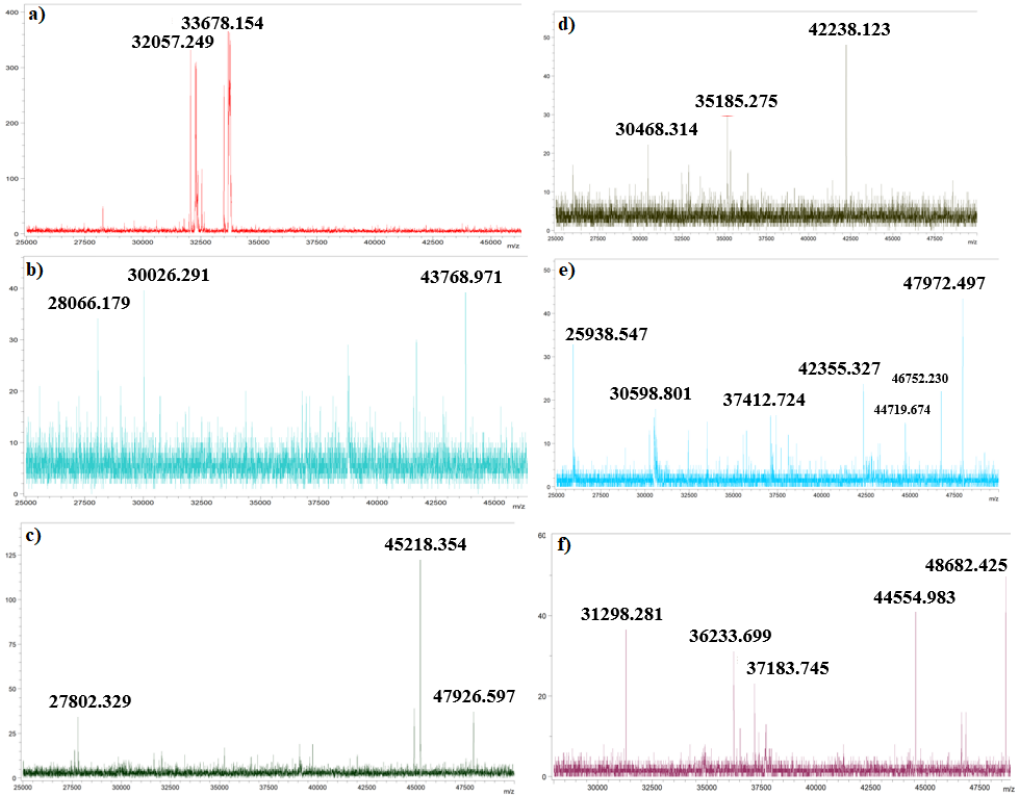

Fig.2. The MALDI-MS spectra registered for the following homogenates: a) TKN, b) TKO, c) Z; d) TKP; e) U and for f) GPR146 standard sample. SA concentration 20 mg/ml.

The presence of GPR146 depends on the type of homogenate – for that receptor the most characteristic and repetitive peaks in MALDI-MS analyses were detected in postthymic fat tissue (TKP) and right atrium of the heart (U). On the other hand the results registered for the saphenous vein (Z) didn't confirm expression of the receptor within tissue homogenate. Nextly, MALDI-IMS imaging was performed for tissue specimens taken from a healthy patient (reference sample) and from a patient with diagnosed metabolic disorders (Fig.3). It was compared occurrence and intensity of peaks characteristic for GPR146 within both of them. Firstly, the ionization efficiency was higher in the case of healthy tissue – probably, changes occurring in the diabetic's body definitely

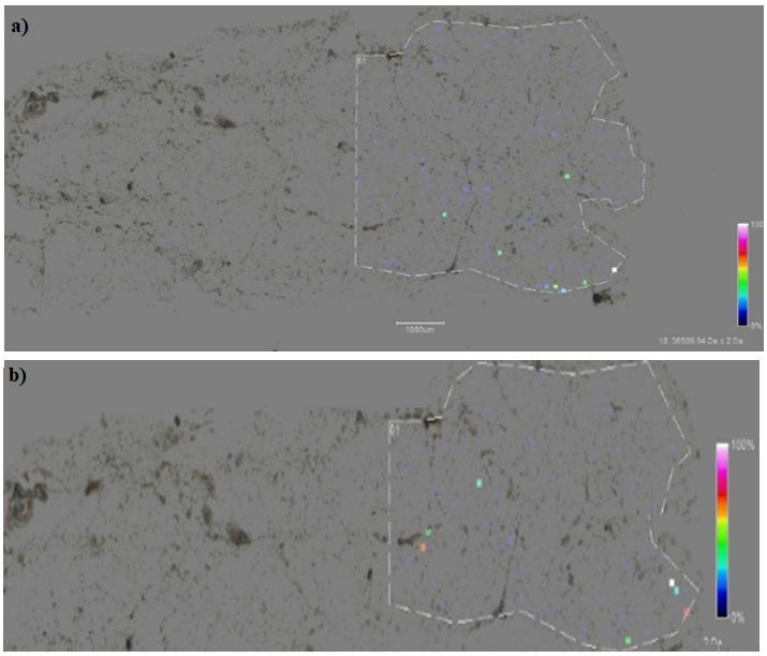

Fig.3. GPR146 distribution within: a) diabetic tissue, b) healthy tissue.

modify its structure. In addition, the occurrence of GPR146 was more intensive and visible in the pancreas section taken from a patient with diagnosed insulin resistance.

Conclusions

Based on the presented results we concluded that GPR146 expression within human tissues may be higher during metabolic disorders. Moreover the same tendency we observed for peptide C in previous studies, thus both of them should be take into account during designing innovative and more effective methods for treatment of diabetes mellitus. In samples collected from circulatory system the largest number of peaks corresponding to transmembrane protein were identified, which confirms the relationship between diabetes and heart diseases. Moreover, increased expression of the receptor in tissue taken from a patient with known insulin resistance may also indicate possible correlations between the amount of C-peptide and the GPR146 receptor.

Literature

[1] Lindfors, L., Sundström, L., Fröderberg Roth, L., Meuller, J., Andersson, S., Kihlberg, J. (2020). Is GPR146 really the receptor for proinsulin C-peptide? Bioorganic and Medicinal Chemistry Letters, 30, 127208. doi: 10.1016/j.bmcl.2020.127208

[2] Kolar, G.R., Grote, S.M., Yosten, G.L.C. (2017). Targeting orphan G protein coupled receptors for the treatment of diabetes and its complications: C-peptide and GPR146. J Intern Med. 281(1), 25–40. doi:10.1111/joim.12528.

[3] Ahren, B. (2009). Islet G protein-coupled receptors as potential targets for treatment of type 2 diabetes. Nat Rev Drug Discov. 8(5), 369–385.

[4] Yosten, G.L.C., Kolar, G.R. (2015). The Physiology of Proinsulin C-Peptide: Unanswered Questions and a Proposed Model. PHYSIOLOGY, 30, 327–332. doi:10.1152/physiol.00008.2015

[5] https://www.proteinatlas.org/ENSG00000164849-GPR146/structure (accsess date: 02.07.2024)