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Letter to the Editor Open Access

A233 Peptide: A Growth Hormone Secretagogue that Promotes an Antiviral Signaling Pathway

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Letter to the Editor

Growth hormone secretagogues (GHS) such as ghrelin induce an array of effects in different types of organisms. The role of ghrelin in immune cell activation includes the release of pro- and anti-inflammatory cytokines via different signaling pathways [1,2]. For example, murine RAW 264.7 macrophages express both ghrelin and GHS receptors. Treatment of these cells with exogenous ghrelin decreases LPS-dependent NF-κB activation and subsequent IL-1β and TNF-α production (pro-inflammatory cytokines) while it increases IL10 (anti-inflammatory cytokine) levels through the p38MAPK pathway [3]. GHS also stimulates oxidized LDL uptake by macrophages and PPAR-γ signaling, both being functions associated with the pathogenesis of cardiovascular diseases such as atherosclerosis [4,5].

The A233 decapeptide is a novel synthetic GHS obtained from a massive docking experiment performed against a molecular model of the GHS receptor. Previous studies have demonstrated an impact of peptide treatment on growth, immune system function, and antioxidant defense in tilapia fish [1]. Additionally, this peptide stimulates *in vitro* and *in vivo* mammalian immune cells, promoting a signaling cascade that activates the innate immune response.

The treatment of J774.2 macrophage cell line with A233, impacts on glucose and fatty acid catabolism, the oxidation-reduction process, DNA repair, cysteine-type endopeptidase activity involved in apoptotic processes, and positive regulation of TNF production [6]. In fact, peptide treatment increases $O_2^{\bullet-}$ (superoxide anion) levels in this cell line. Activated M1 macrophages mediate their cytotoxic and pro-inflammatory effects by producing ROS and RNS such as $O_2^{\bullet-}$ and NO (nitric oxide), respectively [7]. ROS generation induces cell damage, for example, by reacting with NO to yield $ONOO^-$ (peroxynitrite anion) [8]. Sod1 and Prdx6 up-regulation might then suggest an increase in ROS production, while avoiding associated oxidative stress damage. Therefore, A233 treatment might potentiate macrophage activation and cytotoxic functions, as ROS production has been reported to have role in macrophage differentiation [9]. On the other hand, activated macrophages are protected against ROS induced oxidative damage by showing, for instance, an enhancement of DNA repair and resistance to apoptosis [10]. A233 up-regulates the DNA repair proteins in macrophages, which might promote survival under ROS high level conditions.

The activation of the transcription factors NF-κB, STAT1 and AP-1 by stimuli like LPS, and/or IFN-α/β/γ induces the expression of inflammatory and microbicidal M1 genes [11,12]. After A233 stimulation J774.2 macrophage cell line shows the up-regulation of Fabp4 protein, a target gene of the STAT1 transcription factor. The expression of Fabp4 in macrophages is induced by a pro-inflammatory environment that promotes NF-κB activation.

It has been shown that patients in every stage of the disease present strong correlations in the levels of DNA methylation at promoters of several NF-kB-related genes [13]. In fact, aberrant DNA methylation patterns have been found in a great number of human disorders, including inflammatory bowel disease, distal colonic neoplasia and even cancers [14-16]. As DNA methyltransferases (DNMTs) are required for the establishment and maintenance of DNA methylation in mammals [17] and some other histone methyltransferases such as G9a and GLP also play a role in the maintenance of DNA methylation [18], A233 might activate macrophage via affect the function of DNMTs or HMTs and alter their associated DNA methylation levels in the immune cells. Nevertheless, this hypothesis should be demonstrated in further experiments.

In vitro studies with A233 peptide have also provoked the secretion of IFN-γ in mouse spleen cells [6]. This cytokine play a crucial role in the antiviral activity against dengue viruses (DENV) [19]. Consequently, a therapeutic treatment of BALB/c mice with this peptide reduced the viral load in a mouse model of DENV intracranial infection. In fact, peptide treatment of J774.2 cells up-regulates the peroxisomal and mitochondrial protein MAVS required for innate immune defense against viruses. The mitochondrial isoform of MAVS activates an IFN-dependent signaling pathway that amplifies and stabilizes the antiviral response through the expression of type I IFNs and other antiviral cytokines [20]. The up-regulation of MAVS, the increase in IFN-γ levels, and the protection against DENV infection indicates an anti-viral effect of A233 peptide treatment, stimulating an innate immune response (Figure 1). Other mouse model studies examining mechanisms of antiviral innate immunity against DENV reveal an essential role for MAVS [21] and IRF-3/7 [22].

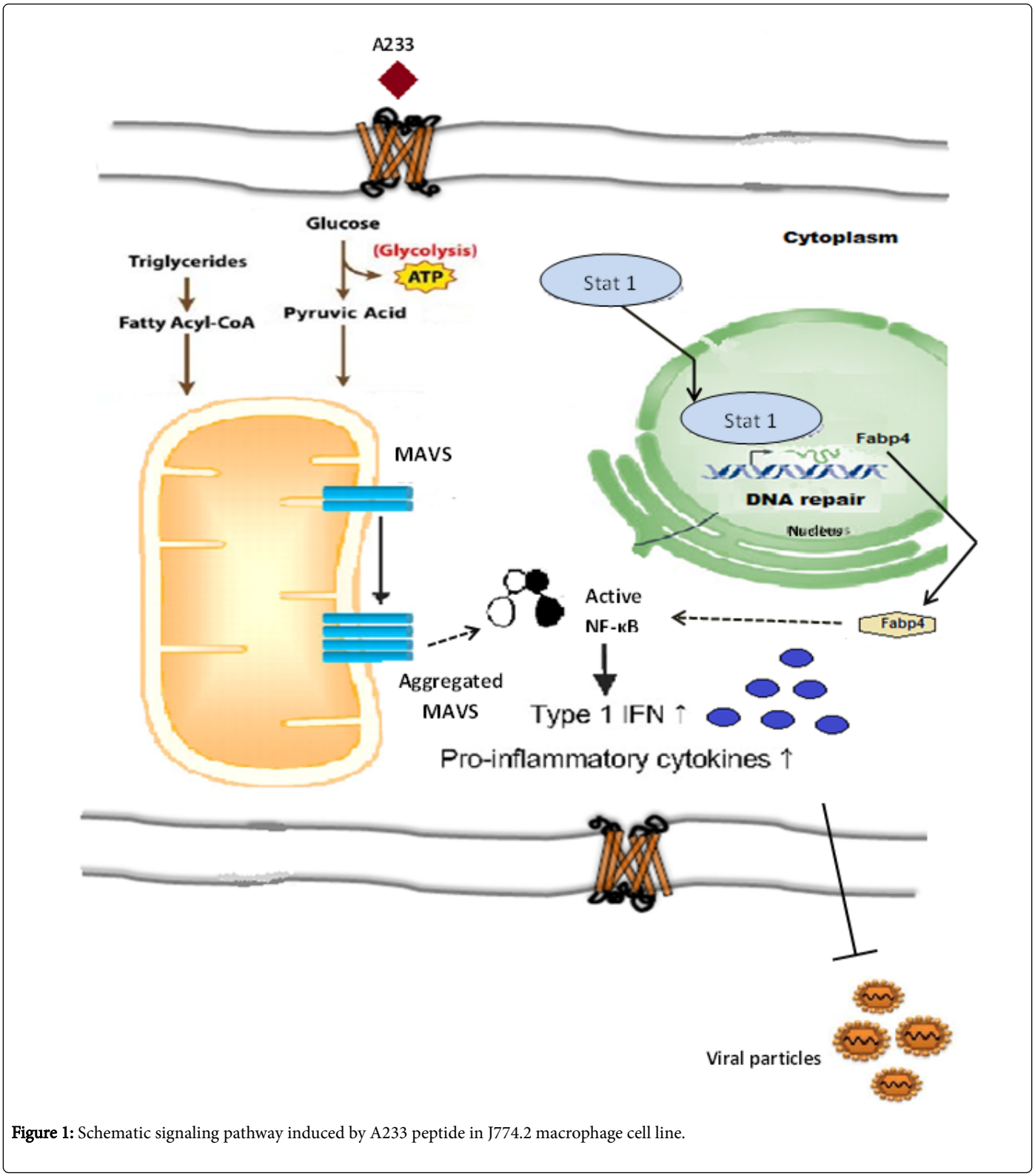

Figure 1: Schematic signaling pathway induced by A233 peptide in J774.2 macrophage cell line.

Further studies should be conducted to evaluate the use of A233 in biomedical applications. Other growth hormone releasing peptides, that recognized the same cellular receptor (GHSR-1a), have shown to be cardio, neuro and broadly cytoprotective candidates. These molecules have been capable of control cardiac electric potentials and ion pumps and promote cardiomyocytes survival, the attenuation of inflammatory soluble messengers and distal cellular effectors [23]. Additionally they have impacted in the control of peripheral vascular tone, necrosis and apoptosis prevention in a variety of epithelial and non-epithelial structures, the control of sarcopenia by stimulating

skeletal muscle trophism, cachexia and catabolism lessening [23]. That is why A233 could have important effects as cyto-protective agent over different tissues. Other important applications, for example in the treatment of autoimmune diseases and in the modulation of immune conditions, should be demonstrated in the appropriated animal models. Also, safety and toxicological studies will be necessary to introduce A233 in the biomedical practices.

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