Omentin-1 increases cell proliferation and decreases apoptosis in cultured porcine anterior pituitary cells: a description of the potential signaling pathways involved

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Omentin-1 (OMNT-1) is an adipokine involved in the regulation of energy metabolism and ovarian functions. Our previous study showed OMNT-1 expression in the porcine anterior pituitary and its influences on *in vitro* endocrine functions including gonadotropins secretion. However, the specific role of OMNT-1 on anterior pituitary cell proliferation and apoptosis is not thoroughly established. Hence, the research goal is to understand the influence of OMNT-1 on cell proliferation and programmed cell death, while also clarifying the related molecular pathways. Pituitary glands were isolated from sexually mature female pigs (Large White breed) in the mid-luteal phase. Next, cultured anterior pituitary cells were treated with OMNT-1 at increasing doses of 10, 50, and 100 ng/ml alone and with the combination of GnRH (100 ng/ml) for 24, 48, and 72 h. Next, we assessed cell viability (alamarBlue), cell cycle (flow cytometry), and executive caspase activity (Caspase-Glo 3/7 assay). Additionally, we examined the gene and protein expressions of cyclins A, B, D, and E, proliferating cell nuclear antigen PCNA, and key components involved in programmed cell death pathways (caspase-3, -8, -9, Bax, Bcl2, p53, Fas, Fadd, Xiap) using quantitative reverse transcription polymerase chain reaction and Western Blot. Also, we studied the influence of OMNT-1 (50 ng/ml) on extracellular signal-regulated kinases, protein kinase B, 5'AMP-activated protein kinase alpha, and signal transducer and activator of transcription 3 phosphorylation at various time points (5, 15, 30, 60 min). Pharmacological blockers (PD098059 (25 μM), LY294002 (15 μM), AG490 (3 μM), and Compound C (1 μM), respectively) for these kinases were used, and OMNT-1 impact on cell viability and caspase activity was assessed. Statistical analyses included Student's t-test, one-way analysis of variance, and Tukey's post-hoc test ($p<0.05$) with six repetitions for each group. Our results affirmed the mitogenic properties of OMNT-1, promoting increased cell growth in the G2/M phase and upregulating cyclins A, B, D, E, and PCNA expression, particularly at a concentration of 50 ng/ml, also in combination with GnRH after 24 h of incubation. OMNT-1 stimulated time-dependent phosphorylation of selected kinases, while inhibiting levels of executive caspases (-3, -7) at doses 10 and 50 ng/ml, suggesting an anti-apoptotic effect in pituitary cells. Additionally, we observed a modulatory effect of OMNT-1 on proteins involved in the process of programmed cell death and confirmed the mitogenic and anti-apoptotic effects of OMNT-1 using pharmacological inhibitors targeting kinases. In summary, our studies demonstrated for the first time a stimulatory effect of OMNT-1

on proliferation and an inhibitory effect on apoptosis in porcine anterior pituitary cells by the regulation of different signaling pathways. Due to the intricate regulation of apoptosis and the crucial balance between cell proliferation and death that maintains tissue homeostasis, OMNT-1 may apply a significant physiological influence. Thus, OMNT-1 might be a new regulator of the balance proliferation/apoptosis of the porcine anterior pituitary cells.

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