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IGF-I overexpression stimulates CCN5/Wisp2 expression in pancreatic β-cells, which promotes cell proliferation and survival against streptozotocin

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Abstract

Insulin-like growth factor I (IGF-I) is normally produced from hepatocytes and various other sources including the pancreas. Acting through its receptor, IGF-IR, IGF-I promotes embryonic development, postnatal growth, and maturation of major organ systems. In order to explore novel targets of IGF-I action within the pancreatic islets, we have recently performed a whole-genome DNA microarray analysis on total RNA prepared from IGF-I overexpressing islets and found ~100 genes specifically up- or down-regulated. Prominently among them, CCN5 mRNA level was found increased 2.7 and 3.4 fold respectively in the islets of MT-IGF mice using microarray and real-time PCR; the protein level was increased 2-fold in isolated islets from MT-IGF mice; using dual-labeled immunohistochemistry, CCN5 was normally expressed at low level in the β-cells of wild-type islets, but exhibited significant induction upon IGF-I overexpression. Our results demonstrated for the first time that CCN5 is expressed in the β-cells and its expression is stimulated by IGF-I overexpression. The IGF-I effect seemed to be directly on the islet cells as 6-24 h treatment increased CCN5 mRNA level significantly in parimary islets.

To define the role of CCN5 in islet function, we stably overexpressed its cDNA in insulinoma MIN6 cells using pcDNA3.1 vector. Using Western blots, we confirmed that normally CCN5 expression was very low in those cells, and the level was increased 30-fold in stably transfected cell lines (MIN6-CCN5). To explore possible effect of CCN5 on cell proliferation, we detected 2-fold increases in the cell numbers of three independent lines of MIN6-CCN5 cells vs. parental cells after 3-d culture, using MTT cell viability assay. In order to investigate intracellular mechanism, in CCN5-expressing cells, we detected 3-fold increase in the level of cyclin D1 which paralleled a 2-fold increase in the rate of Akt phosphorylation at Ser-473 and 2.5-fold elevation in Erk1/2 phosphorylation. It is established that β-cell replication is associated with increased cyclin D1 and CDK4 levels (1); deficiency in CDK4 or cyclin D2 results in reduced β-cell mass and type 1 diabetes. Our results suggest that CCN5 stimulates β-cell replication, by activating Akt and Erk1/2 kinases and increasing the levels of cyclin D1. Finally, CCN5 overexpressing cells were resistant to streptozotocin-induced cell death by decreasing the % apoptotic cells and the level of caspase-3 cleavage.

CCN proteins are involved in cell adhesion and extracellular matrix remodelling, skeletal development and chondrogenesis, angiogenesis and wound repair, proliferation and tumorigenesis (2). Among them, CCN5 is a secreted protein of 29 kDa, known to play positive or negative roles in cell proliferation, carcinogen and other events (3). As a putative growth factor, its expression in breast cancer cells can be induced by steroid hormones, serum, EGF, phorbol esters and IGF-I (4). In normal human pancreas, the mRNA and protein expression was detected in both ducts and acini; however, its expression in endocrine islet cells has not been explored. We have shown that CCN5 is normally expressed in islet β-cells, IGF-I directly stimulates its expression, and IGF-I overexpression causes increased level in the islets. We further demonstrate CCN5 overexpression accelerates the proliferation of MIN6 cells, activates Akt and/or Erk1/2 kinase, and is anti-apoptotic against streptozotocin. Thus, increased CCN5 expression may mediate IGF-I stimulated islet cell growth and/or survival.

Biography

Dr. Jun-Li Liu studied Biology/Physiology at Peking University, China, where he obtained his BSc (1982) and MSc (1985) degrees. He came to Canada in 1989 for postgraduate study in molecular endocrinology under the late Dr Yogesh C. Patel and received his PhD degree in Experimental Medicine at McGill University in 1995. Dr Liu subsequently worked for five years as a postdoctoral fellow with Dr Derek LeRoith (Diabetes Branch, NIDDK, NIH, Bethesda, MD). There he initiated the work on Cre/loxP-mediated conditional targeting of the mouse IGF-I gene and studied the effect of liver-specific IGF-I gene deficiency in somatic growth and glucose metabolism. In 2000 he was recruited back by McGill University as Assistant Professor of Medicine. The focus of his current research is the role of growth factors in pancreatic islet growth and insulin production, using various knockout and transgenic mice.