Signal Transduction by Insulin and Mammalian Lectins

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Our studies focus on two subjects. One involves insulin signal transduction, insulin resistance and diabetes. The second relates to the modulation of immune responses and mode of secretion of Galectin-8, a mammalian lectin.

A. Insulin signaling, insulin resistance and diabetes.

The homeostatic control of blood glucose is determined by two major factors the concentration of insulin in the circulation, which correlates to β cell function, and insulin sensitivity of target organs (e.g., muscle, adipose tissue and liver). Insulin resistance is defined as a failure of target organs to respond to insulin thus leading to the development of diabetes, an ever-increasing epidemic of the 21st century. Ongoing studies in our lab mainly focus on insulin action, insulin resistance, and growth and survival of the pancreatic insulin-producing β-cells.

I. We could show that inducers of insulin resistance exploit phosphorylation-based negative feedback control mechanisms, to uncouple the insulin receptor (IR) from its downstream effectors, the IRS proteins (IRS-1 and IRS-2) and thereby terminate insulin signal transduction. Ser/Thr phosphorylation of the IRS proteins was shown to be a pivotal player in the termination of insulin's action in target organs. Thus the identification of IRS kinases and their target Ser/Thr phosphorylation sites is of physiological importance, as they might serve as new potential targets for therapies. Indeed, we found that IRS proteins mutated at inhibitory Ser residues were resistant to the inhibitory effects of prolonged insulin treatment or to the action of inducers of insulin resistance. Accordingly, deletion of a specific Ser-rich-domain (we named DIDI) maintained the ability of IRS-1 to undergo ubiquitination while rendering it insensitive to insulin-induced proteasomal degradation. This deletion-mutant of IRS-1 better maintained insulin signaling and insulin action. These results identify DIDI as a novel domain, required for insulin-induced proteasomal degradation of IRS-1 at a post-ubiquitination stage.

The second approach focused on IRS-2 as a potential target, as this protein plays a pivotal role in β cell function (Fig. 1). We could show that a mutated form of IRS-2 in which five inhibitory Ser residues were replaced by Ala (IRS-2^{5A}) improves β cell function. Cytokine-treated isolated islets overexpressing IRS-2^{5A} secreted significantly more insulin in response to glucose, compared to islets overexpressing IRS-2^{WT}. Moreover, transplantation of a limited number of islets overexpressing IRS-2^{5A} into Streptozotocin-induced diabetic mice restored their ability to respond to glucose loads, significantly better than islets overexpressing IRS-2^{WT}. Taken together, these studies may open new pharmacological approaches to improve islets engraftment.

II. Other studies in the lab focus on the mode of action of inducers of insulin resistance. Selective serotonin reuptake inhibitors (SSRIs) which are common antidepressants act as inducers of insulin resistance. We could show that treatment of Min6, a β-cell line, with SSRIs activates IRS-kinases (e.g. c-Jun kinase) that inhibit insulin signaling. Insulin-stimulated Tyr-phosphorylation of IRS-2 was decreased concomitant with a reduction in PKB activity. We could further demonstrate that glucose-stimulated insulin secretion was inhibited upon SSRIs treatment. This was accompanied by pro apoptotic processes and increase caspase 3/7 activity. These results provide a molecular basis for the action of SSRIs as inducers of insulin resistance.

III. Islets transplantation is a potential therapeutic solution for diabetes. However, a substantial proportion of the transplanted islet mass fails to engraft due to death induced by apoptosis. Thus, improving islets engraftment is of a great concern. With this respect, the strategy that was applied was aimed at exploring novel molecular targets involved in the induction of β cell death. Screening of siRNA libraries consisting of ~5000 potential target genes, the inhibition of which could modulate cytokine-induced apoptosis of β cells, resulted in the identification of ~ 40 novel genes (Fig. 2). These genes might be potential key players in cytokine-induced β cell apoptosis and as such could be rational candidates for therapeutics. Validation of these findings is currently underway.

B. Secretion and Action of Galectin-8, a mammalian lectin

Galectins, a family of mammalian lectins, are implicated in cell adhesion, cancer and immunity. We focus on galectin-8, cloned by us. Galectin-8 is a secreted, integrin-binding protein

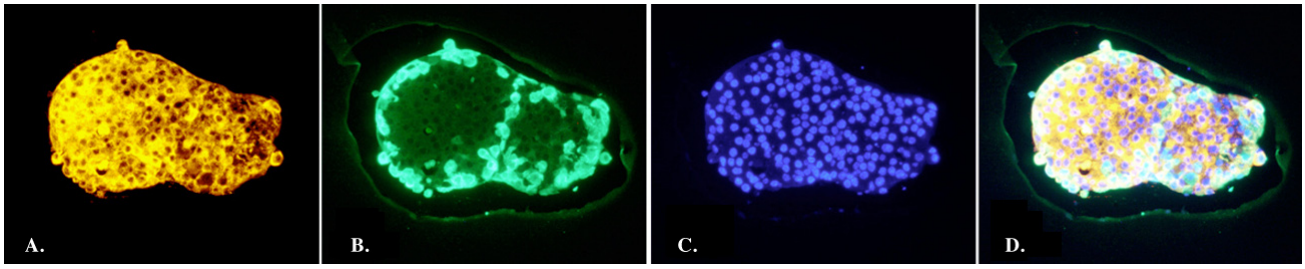

Fig. 1 *Sections of mouse islets were stained with insulin (Yellow), GFP/IRS-2 (Green) and nuclei (Blue). Merged picture is presented in D.*

that modulates integrin interactions with the extracellular matrix. However, galectin-8, like other galectins, lacks a signal peptide suitable for ER/ Golgi mediated secretion. Therefore, galectin-8 is assumed to be secreted by an atypical unknown mechanism. We have previously shown (Fig. 1) that internalized galectin-8 co-localizes with early endosomes (Fig. 3). Following internalization a portion of galectin-8 is recycled and secreted by the endocytic pathway. Further studies conducted in the lab provide evidence that secretion of endogenous galectin-8, like its exogenous counterpart, is mediated by the endocytic cycle, suggesting that internalization and secretion of galectin-8 are tightly coupled. We could also demonstrate that secretagogues known to enhance histamine and neurotransmitters secretion, caused an increase in galectin-8 secretion. These findings suggest that some of the molecular machinery involved in galectin-8 secretion is shared with that of triggered exocytosis.

Another aspect of our work deals with the autocrine or paracrine effects of galectin-8 upon its secretion by tumor cells. We found that ligation of integrins by secreted galectin-8 triggers transcription of a unique set of genes, some of which are associated with bone remodeling and prostate cancer progression. Modulation of gene expression by galectin-8 may represent a novel attribute associated with cancer development and bone metastasis. Our findings so far suggest that galectin-8 secreted by tumor cells at the bone

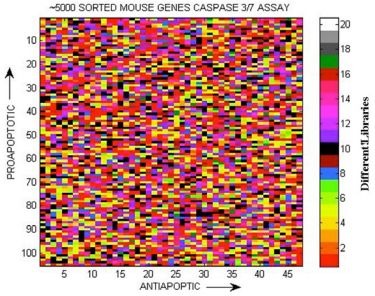

Fig. 2 *Min6 β cells were screened with siRNAs libraries representing 18 different gene families. Genes were sorted according to their apoptotic index. Colors represent the different libraries.*

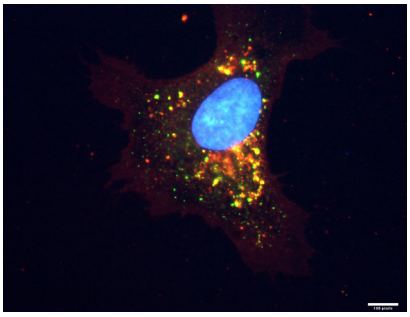

Fig. 3 *Co-localization of internalized Galectin-8 (Red) with early endosome antigen-1. (Green) is presented as Yellow.*

microenvironment is involved in the tumor-bone interplay by promoting the osteoblastsic and osteolytic metastasis process.

Ongoing studies in the lab attempt to resolve the mode of galectin-8 secretion; to elucidate the signaling pathway utilized by this protein to promote tumor development and bone remodeling, and to evaluate the inflammatory effect of galectin-8 in-vitro and in-vivo using mice over-expressing galectin-8 generated at our lab. These studies might help clarify the mode of action of galectin-8 and its cellular functions.

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Acknowledgements

Y. Z. holds the Marte R. Gomez Professorial Chair. This study was supported by funds from: The Juvenile Diabetes Research Foundation; The Israel Science Foundation; The MINERVA Foundation; and D-Cure Diabetes Care in Israel

INTERNAL support

Women Health Research Center ; the Center for scientific Excellence and the Joseph Cohen Minerva Foundation.