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# Transmembrane Signalling:

## I. Insulin Signal Transduction and Insulin Resistance

## II. Modulation of Cell Adhesion and Growth by Mammalian Lectins

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Our studies focus on two subjects. One involves insulin signal transduction, insulin receptor (IR) trafficking and the molecular basis of insulin resistance. The second relates to the biological role of galectin-8, a novel mammalian lectin that modulates receptor trafficking, cell adhesion, and cellular growth.

**A molecular basis for insulin Resistance**

Failure of target cells to respond to insulin, a state known as insulin-resistance, is a major attribute to the pathological manifestations associated with diabetes- an ever-increasing epidemic of the 21st century. In recent studies we found that agents that induce insulin resistance exploit phosphorylation-based negative feedback control mechanisms, otherwise utilized by insulin itself, to uncouple the insulin receptor from its downstream effectors, and thereby terminate insulin signal transduction. This involves aberrant Ser/Thr phosphorylation of insulin receptor substrates (IRS-1 and IRS-2) that impairs their interactions with the insulin receptor. Such impaired interactions abolish the ability of IRS proteins to undergo Tyr phosphorylation and further propagate insulin signaling. The objective of our present studies is to unravel the molecular basis for this uncoupling. Currently, we focus on two main questions: i. What is the nature of the Ser/Thr kinases that phosphorylate and uncouple IRS proteins from IR; ii. Which Ser/Thr residues are subjected to phosphorylation. We found that PKB (Akt) is a positive regulator of IRS functions. By contrast, phosphorylation of IRS proteins by insulin-stimulated PKCζ, dissociates the IR-IRS complexes, inhibits their ability to undergo insulin-stimulated Tyr phosphorylation and terminates insulin signaling. Furthermore, agent that induce insulin resistance such as TNF also trigger kinases such as PKCζ, implicating PKCζ and its downstream targets as potential IRS kinases. These kinases are activated either as part of a physiological negative feed back control mechanism, triggered by insulin itself, or by agents which induce insulin resistance (Fig. 1). We could further demonstrate that by mutation of several PKC phosphorylation sites we are able to generate IRS proteins that are resistant to the inhibitory effects of prolonged insulin treatment or to the action of inducers of insulin resistance. The mutated IRS proteins, not only couple better to IR, but they are resistant to degradation, induced by prolonged insulin treatment. These findings target us towards potential pharmacological interventions in disease states such as the prevalent form of obesity-induced insulin resistance and diabetes.

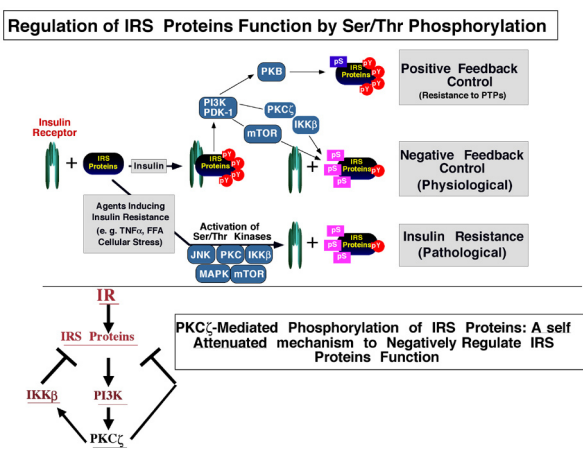


**Fig. 1** *Insulin-induced Ser/Thr phosphorylation of IRS-1 proteins serves either as a positive or a negative feed-back control mechanism under physiological conditions, as well as a mean to induce an insulin-resistant state under pathological conditions.*

**Insulin receptor (IR) trafficking**

The temporal and spatial communication of IR with downstream effectors is of major importance,

therefore we thought to identify novel elements involved in regulating IR trafficking. We found that IR endocytosis occurs independent of Tyr phosphorylation of IRS proteins, but it depends upon proper organization of actin filaments and the nature of the ECM proteins onto which the cells adhere. These findings implicate actin filaments and

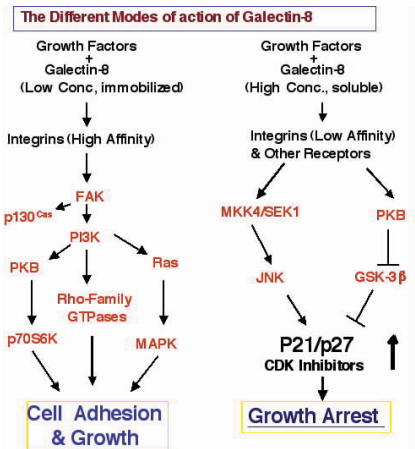


**Fig. 2** *Soluble galectin-8 interacts with integrins to inhibit cell adhesion and induce growth arrest, while immobilized galectin-8 serves as an ECM protein. These results implicate galectin-8 as novel modulator of cell adhesion and cellular growth.*

ECM molecules as candidate players in regulating insulin receptor trafficking.

**B. Galectin-8 modulates Cell Adhesion and Cell Growth.**

A different aspect of our work involves studies of galectins, mammalian lectins implicated as mediators of cell adhesion and apoptosis. We focus on galectin-8, cloned by us, whose expression is markedly enhanced in certain tumor cells including prostate cancer. Galectin-8 is a secreted, integrin-binding protein that modulates integrin interactions with the extracellular matrix. When present at high concentrations as a soluble ligand, or when it is overexpressed in cells, galectin-8 acts as an inhibitor of cell growth. Soluble galectin-8 triggers the activation of JNK and PKB/Akt which promote the accumulation of the cyclin-dependent kinase inhibitor, p21. These results implicate galectin-8 as a modulator of cell growth through up-regulation of proteins involved in cell cycle progression. In contrast, cell adhesion to immobilized galectin-8 triggers a unique integrin-mediated signaling cascade (Fig 2). This results in massive remodeling of the cellular cytoskeleton, and leads to the formation of extensive actin containing microspikes. As a result, galectin-8 potentiates the adhesive functions of integrins and promotes cell migration. Of no less importance, ligation of integrins by galectin-8 triggers transcription of a unique set of genes, some of which are directly associated with bone remodeling and prostate cancer progression. Hence, modulation of gene expression by galectin-8 may represent a novel attribute associated with cancer development and bone metastasis. Collectively, these findings suggest that galectin-8 can act in different modes, depending ton its cellular context and the extracellular environment. Ongoing studies in the lab, attempting to generate mice that overexpress galectin-8, or mice that are galectin-8-null, might help clarify these questions.

***Selected Publications***


Hadari, et. al. (2000) Galectin-8 Binding to Integrins Inhibits Cell Adhesion and Induces Apoptosis. J. Cell Sci. 113, 2385-2397

Liu, et. al. (2001) Insulin Stimulates PKCζ-Mediated Phosphorylation of Insulin Receptor Substrate-1 (IRS-1): A Self-Attenuated Mechanism Negatively Regulates IRS-1 Function. J. Biol. Chem., 276, 14459-14465

Levy, et. al. (2001) Galectin-8 Functions as a Matricellular Modulator of Cell Adhesion. J. Biol. Chem., 276, 31285-31295

Zick, Y. (2001) Insulin Resistance: a Phosphorylation-based uncoupling of Insulin Signal Transduction. Trends. Cell Biol. ,11, 437-441.

Hemi et. al. (2002) Transactivation of ErbB2 and ErbB3 by tumor necrosis factor-alpha and anisomycin leads to impaired insulin signaling through serine/threonine phosphorylation of IRS proteins *J. Biol. Chem*., 277, 8961–8969

Levy et. al. (2003) Sustained Induction of ERK, PKB and p70S6K Regulate Cell Spreading and Formation of F-actin Microspikes Upon Ligation of Integrins by Galectin-8, a Mammalian Lectin. *J. Biol. Chem* , 278, 14533-14542,

Boura-Halfon et. al. (2003) Extracellular Matrix Proteins Modulate Endocytosis of the Insulin Receptor *J. Biol. Chem* , 278, 16397-16404,

Zick, Y. et. al. (2004) *Glycoconjugate J*. 19, 517-526



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