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Transmembrane signaling:

I. Molecular basis of insulin resistance
II. Regulation of cell adhesion and cell cycle progression by mammalian lectins

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Our studies focus on insulin signal transduction, insulin receptor (IR) trafficking and the molecular basis of insulin resistance. A major thrust is also given to elucidate 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, the IRS proteins, and thereby terminate insulin signal transduction. We demonstrated that Ser phosphorylation of IRS proteins plays either a positive or a negative role in insulin signaling. Phosphorylation of IRS proteins by insulin-stimulated protein kinase-B enables them to maintain their Tyr-phosphorylated active conformation, thus implicating PKB as a positive regulator of IRS functions. By contrast, phosphorylation of IRS proteins, mediated by insulin-stimulated PKCζ, dissociates the IR-IRS complexes, inhibits their ability to undergo insulin-stimulated Tyr phosphorylation and terminates insulin signaling. Agents that induce insulin resistance such as TNF also induce Ser phosphorylation and inhibit insulin-stimulated Tyr phosphorylation of IRS proteins. TNF's effects are mimicked by ceramide analogs, suggesting that TNF triggers a ceramide-activated kinase such as PKCζ. These findings implicate PKCζ and its downstream targets IKKβ 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 to uncouple IRS proteins from the insulin signaling pathway (Fig. 1). These findings target us towards potential pharmacological interventions in disease states where this mechanism can be the underlying cause of insulin resistance, such as the prevalent form of obesity-induced diabetes.

Insulin Receptor (IR) Trafficking

The temporal and spatial communication of IR with downstream effectors, such as IRS proteins 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. Accordingly, annexin II, an actin-binding protein, undergoes insulin-induced Tyr phosphorylation only when receptor endocytosis takes place, and overexpression of annexin II potentiates IR internalization. These findings implicate actin, ECM molecules, and annexin-II as a candidate players in regulating insulin receptor trafficking.

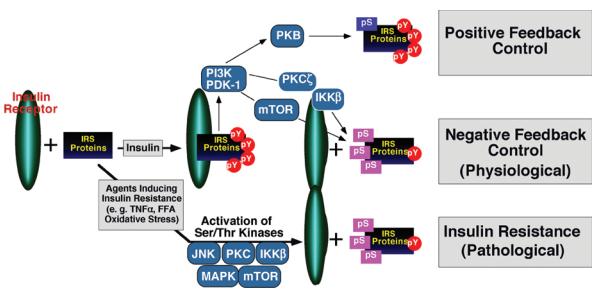

Fig. 1 *Insulin-induced Ser/Thr phosphorylation of IRS-1 proteins serves either as a positive or a negative feed-back control mechanism under physiological conditions, as well as a mean to induce an insulin-resistant state under pathological conditions.*

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, and we wish to determine its mode of action and physiological role. Galectin-8 is a secreted, surface-expressed protein having two-carbohydrate recognition domains. Immobilized galectin-8 is equipotent to fibronectin (Fn) in promoting cell adhesion, spreading, and migration, by forming protein-sugar complexes with cell surface integrins. Adhesion to galectin-8 triggers integrin-mediated signaling including Tyr phosphorylation of FAK, paxillin and

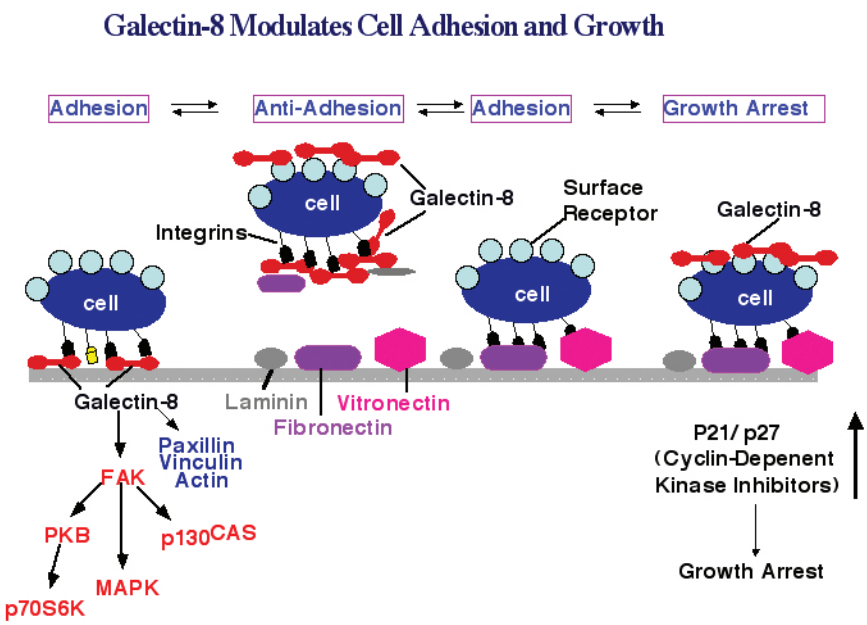

Fig. 2 *Soluble galectin-8 interacts with integrins to inhibit cell adhesion and induce growth arrest, while immobilized galectin-8 serves as an ECM protein. These results implicate galectin-8 as novel modulator of cell adhesion and cellular growth.*

P130cas; and activation of a Rho-family GTPases, MAPK and PI3K cascades. In contrast, soluble galectin-8 forms complexes with integrins and Fn that negatively regulate cell adhesion. Such a mechanism allows local signals emitted by secreted galectin-8 to specify territories available for cell adhesion. Due to its dual effects on the adhesive properties of the cells and its association with fibronectin, galectin-8 might be considered as a novel matricellular protein. Attempts to overexpress galectin-8 revealed that the secreted lectin negatively regulates cellular growth and colony formation. This effect can be attributed to elevated expression of cyclin-dependent kinase inhibitors p21 and p27, induced by soluble galectin-8. Studies in progress are aimed at identifying the mechanisms which mediate the growth-inhibitory effects of soluble galectin-8.

Selected Publications

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