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STRUCTURAL AND FUNCTIONAL CHARACTERISTICS OF INSULIN RECEPTORS

Ivanova F.

Laboratory of Clinical Immunology, MU - Varna

Reviewed by: Assoc. Prof. Sv. Balev, MD, PhD

ABSTRACT

The insulin receptor delivers the signal from the hormone insulin to the target cells. Structurally and functionally it belongs to the superfamily of the receptors with tyrosin kinase activity. Insulin receptor is known for more than 30 years and during this time a lot of assays for detecting it have been developed. Analyzing the expression and functional characteristics of this receptor is helpful for better understanding the pathogenesis of different diseases.

INTRODUCTION

The isulin is long known to the medical science. This hormone was found in 1921 by the Canadian scientists Frederick Banting and Chars Best. The first practical approach of the gene engineering was the synthesizing of human insulin. The presence of a specific insulin receptor was first proposed by Roth and coworkers in 1971(2). The mechanism of the biologic effect of this receptor is due to its tyrosin kinase activity.

The ligands and receptors of the family of the Insulin/Insulin-like growth factor (IGF) take an important role in the regulation of different processes in the organism - growth, metabolism, reproduction (5). Besides the two non-allelic insulin genes another nine ones are discovered, which code insulin-like peptides. There are at least three different receptors interacting with these ligands - insulin receptor (IR), receptors for IGF-1 and IGF-2. IR belongs to the family of ligand activated receptor kinases. Other members of this family are Phospho-c-Abl, EGF Receptor, SAPK/JNK, p70 S6 Kinase e.c.

Biochemical structure of the IR

The IR is a transmembrane glycoprotein and a member of the superfamily of the receptors with tyrosin kinase activity. Unlike the other members it is a heterodimer of two disulphide bond monomers, each consisting of α- and β-chain. Insulin binding place of the molecule is on the α-subunit which has a molecule weight of 135kDa. In contrast the β-subunit (95kDa) has a short extracytoplasmic part and a long intracytoplasmic tail with tyrosin kinase activity. The IR is bivalent; the affinity to the first insulin molecule bond is greater than to the second one (4).

The two α-chains form a ligand binding tunnel. The amino acid sequence responsible for the insulin binding is from 240 to 250 residues - Thr-Cys-Pro-Pro-Pro-Tyr-Tyr-His-Phe-Gln-Asp (8). The insulin molecule binds to the receptor through electrostatic interactions. When the insulin molecule comes into the ligand binding tunnel, it leads to conformational changes in the receptor and the α-chains get nearer to one another and so do the β-chains. Thus the intracytoplasmic tails become close enough to phosphorilate the appropriate parts. This inner autophosphorilation of the IR activates it and makes possible the initiation of a cascade of intracellular kinases and a signal transdusing (10).

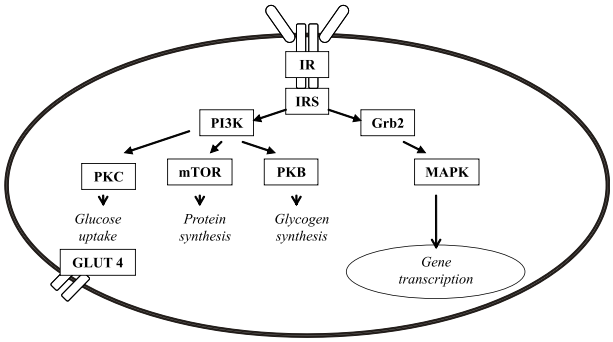

Fig.1. Signal delivery pathways of the IR. IR - insulin receptor; IRS - insulin receptor substrate; PI3K - phosphor-inositol-3-kinase; PKC- protein-kinase C; mTOR - mammalian target of rapamycin; PKB - protein-kinase B; Grb-2 - growth factors adaptor protein; MAPK - mitogen activated kinase.

The main substrates of the phosphorilation are IRS-1, 2, 3 and 4 (insulin receptor substrate). IRSs are other tyrosin

Address for correspondence:
Feodora Ivanova, Laboratory of Clinical Immunology, University hospital "St. Marina", Varna 9010, "Hr. Smirnenski"str. 1, BULGARIA,
E-mail: feodora@mail.bg

kinases and their substrates - PI3K, Fyn, HSP-2, take part in the further delivery of the insulin signal into the cell. It is considered that IRS-1 and 2 have major role in the glucose metabolism in the hepatocytes, IRS-1 and 3 - in the adipocytes, and IRS-2 has a crucial role in the signal delivery in the β-cells of the pancreas. PI3K phosphorilates further some serin-treonin kinases, which mediate effects like glucose assimilation, glyconeogenesis, lipogenesis, protein synthesis, cell survival (fig.1) (7).

Genetics

The sequence of the IR is cloned in 1985 and the structure of exones is described in 1989 by Seino et al. The gene locus is on the short arm of 19. chromosome -19p13.1. There are two isoforms of the IR, which differ by twelve amino acid residues in the C-terminus of the α-chain, encoded by exon 11. These two forms are marked as 11+ and 11- and do not have any considerable functional differences (3).

Physiological regulation of the expression of IR by the intracytoplasmic glucose level

The IR delivers the signal from insulin for increased uptake and utilization of glucose in the cell. The high level of intracytoplasmic glucose exerts feed back inhibition on this process leading to decreasing the expression of IR and thus to diminishing the glucose uptake. Such lessening of the IRs is observed in the peripheral tissues and in the β-cell of pancreas. The high level of intracytoplasmic glucose and the low level of IRs increase the production and secretion of insulin (3).

Methods for IR analysis

The researching of the IR started with the radiological assays in the 70's. These methods use insulin conjugated with ^{125}I and not conjugated insulin. The radioactive emission of the samples is measured and insulin binding sites are calculated (6). The modern radiological methods apply monoclonal antibodies for detecting IR.

There are some assays for visualizing cell surface molecules, which use colloidal gold (cAu) as a marker. These techniques imply absorption of colloidal gold on some proteins (for example insulin) and the binding of the latest to some cells is demonstrated through transmission electron microscopy (9).

Polyclonal and monoclonal antibodies against the IR, IRS, and PKB are used by immunoprecipitation and Western Blot techniques for quantitative and qualitative identification of these proteins (7). The antibodies, conjugated with an appropriate dye, make it possible to detect the IRs through immunohistochemistry and immunofluorescence.

The monoclonal antibodies are used for allocating the IR in different tissues in the human organism and this receptor is appointed as CD220 in the Cluster of Differentiation (VII Workshop, 2001).

There are different clones of monoclonal antibodies specific for α-chain (83-7, 83-14, 47-9, MA-10, MA-5, MA-20, B6), as well as for the β-chain (CT-3, 18-44) of the IR.

The flowcytometry is a technique appropriate for evaluating the expression of particular cell surface molecules in the single cell suspension. Through some standardized procedures the number of IRs on each cell could be measured. The flowcytometry is useful also for detecting the tyrosin kinases and their activity, thus for functional characterization of the IR. (Tabl. 1.)

Tabl. 1. Number of IRs, detected on different cells.

Cells	Number of IR per cell	References
Adipocytes and Hepatocytes	200 000	Rhodes C.J., M. White. Molecular insights into insulin action and secretion. European Journal of Clinical Investigation, 32 (Suppl. 3), (2002) 3-13
Lymphocytes	2 200	Olefsky J., G.M. Reaven. Decreased insulin binding to lymphocytes from diabetic subjects. The journal of clinical investigation, vol. 54, 1974, 1323-1328
Monocytes	15 000	Olefsky J. et al. Insulin binding in diabetes. Diabetes, 26, 1977
Monocytes	700 - 22 000	Борисова И. Рецептори за пептидни хормони, научен обзор, МА, ЦНИМЗ, София 1985
Erythrocytes	20 - 350	
Granulocytes	100	
Platelets	570	

Crystalographic and spectrographic methods are applied for studying the quaternary structure of the IR. In addition to these data, the three dimensional view and the atomic organization of the complex insulin - IR are characterized through scanning transmissional electronic micrography (STEM). This method determines the order, centre of gravity and the rotation of the separated domains (10).

The molecular techniques are widely used for detecting of some mutation in the genes of IR and the second messengers.

Implication of the expression of the IR in the pathogenesis of some diseases

In the human organism the β-cells of the pancreas organize and start functioning about the 25. gestation week and after that the level of insulin increases. The defects in the development, caused by deficiency of insulin or IR, appear in the same time of age of the fetus.

Pathophysiological mechanisms of diabetes mellitus type 2 are connected with defects of insulin secretion as well as peripheral insulin resistance. The insulin resistance has a crucial role and precedes the clinical manifestation with some years. It is conditioned by low levels of expression, lowered affinity and dysfunction of the IR. Massimo

Federichi et al. show that muscle cells from patients with diabetes mellitus type 2 express significantly less IRs and have triple lower capacity for binding insulin compared to healthy people (1).

Leprechaunismus represents the heaviest form of insulin resistance, caused by mutation or absence of IR. It is characterized by retardation in the time of birth and no putting on weight. Heavy postprandial hyperglycemia and fasting hypoglycemia in the presence of hyperinsulinemia is observed. It takes years before the β-cells of the pancreas decompensate (5).

CONCLUSION

Knowing the mechanisms of regulation of expression and function of the IR is crucial for understanding the pathogenesis of diseases like diabetes mellitus type 2, obesities, syndrome X, as well as other processes associated with metabolism and growth.

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