IGF1/IGF1R MITOGENIC PATHWAYS AND IMPLICATIONS IN GB THERAPY

Oana Alexandru[1], Cornelia Zaharia[1], Valerica Tudoric[1], D.O. Alexandru[2]
[1]Department of Neurology,University of Medicine and Pharmacy, Craiova
[2]Department of Informatics and Biostatistics, University of Medicine and Pharmacy, Craiova

ABSTRACT

Glioblastoma (GB), the most frequent human brain cancer commonly exhibit abberant proliferation and diminished apoptosis.The neoplastic transformation of the glial cells is often related to an overexpression of growth factors and upregulation of their signaling pathways. Insulin-like growth factor 1 (IGF1) and insulin-like growth factor receptor 1 (IGF1R) are often abnormally expressed in GB and seem to be interesting therapeutic targets for GB therapy. This mini-review will focus on IGF1/IGF1R mitogenic pathways. It will also present some inhibitors and antisense targeting IGF1, its receptor and their signaling pathways for GB treatment.
Key words: insulin-like growth factor, IGF1R, mitogenic pathways, GB, therapy

INTRODUCTION

Brain tumors account for 85% to 90% of all primary central nervous system (CNS) tumors. Worldwide approximately 176,000 new cases of brain and other CNS tumors were diagnosed in the year 2000, with an estimated mortality of 128,000. GB is accounting more than 50% of all brain tumor cases and 20% of all intracranial tumors. The treatment considered for GB is surgery, but chemotherapy alone or combined with radiation are also considerable tools in treating the remaining tumor or in preventing metastases (1).

Like most tumors, glioma cells aquire the ability to synthesize growth factors and present abnormalities of signal transduction pathways. IGF1 is an important signaling protein in all cancer cells (GB included) and it is generally acknowledged that its receptor is involved in transformation, mitogenesis, differentiation and protection from apoptosis. Therefore IGF1R is a promising molecular target for cancer prevention and treatment (2-4).

THE IGF AXIS

The IGF family involves the ligands (insulin, IGF1 and IGF2), associated receptors (IGF1R, IGF2R and insulin receptor) and insulin-like growth factor binding proteins (IGFBPs, BP1 to BP6). IGF1 and 2 have 60% amino acid homology compared with proinsulin.

IGF1 also known as somatomedin C derives principally from the liver, and is a 7,7 kd polypeptide composed of 70 amino acids. IGF1 binds with high affinity to IGF1R and with low affinity to the insulin receptor and IGF2R. He plays a very important role in fetal, normal and neoplastic tissue differentiation. The ligand is particularly stimulating neurogenesis and myelinization (5-8).

IGF2 is a fetal growth factor, a 7,5-kd polypeptide composed of 67 amino acids.

IGF2 binds with high affinity to IGF2R and with low affinity to IGF1R and insulin receptor (9-11).

The IGF1R and the insulin receptor (1R) have a tetrameric structure consisting of two identical a-subunits and two identical β-subunits with extracellular, transmembrane and intracellular domains (Figure 1). The receptors are 70% homologous at the amino acid level but they present differences in signaling and functions. The function for 1R in health and disease is well known while IGF1R plays an important role in childhood growth. It's also demonstrated that exists hybrids between IGF1R and 1R (6, 12).

Binding of IGF1 to IGF1R activates the receptor's tyrosine kinase activity, leading to activation of distinct signaling pathways like: mitogen-activated protein kinase (MAPK) cascade and phosphatidylinositol 3-kinase-Akt (PI3K-Akt) pathway. Insulin binding induces 1R activation leading to glucose uptake and inhibition of gluconeogenesis in the liver (12).

The IGF2R is a monomeric transmembrane protein that does not have tyrosine kinase domain and shows no evidence of signaling capability. IGF2R binds IGF2 and proteins that contain a manose-6-phosphate moiety. The receptor mediates endocitosis

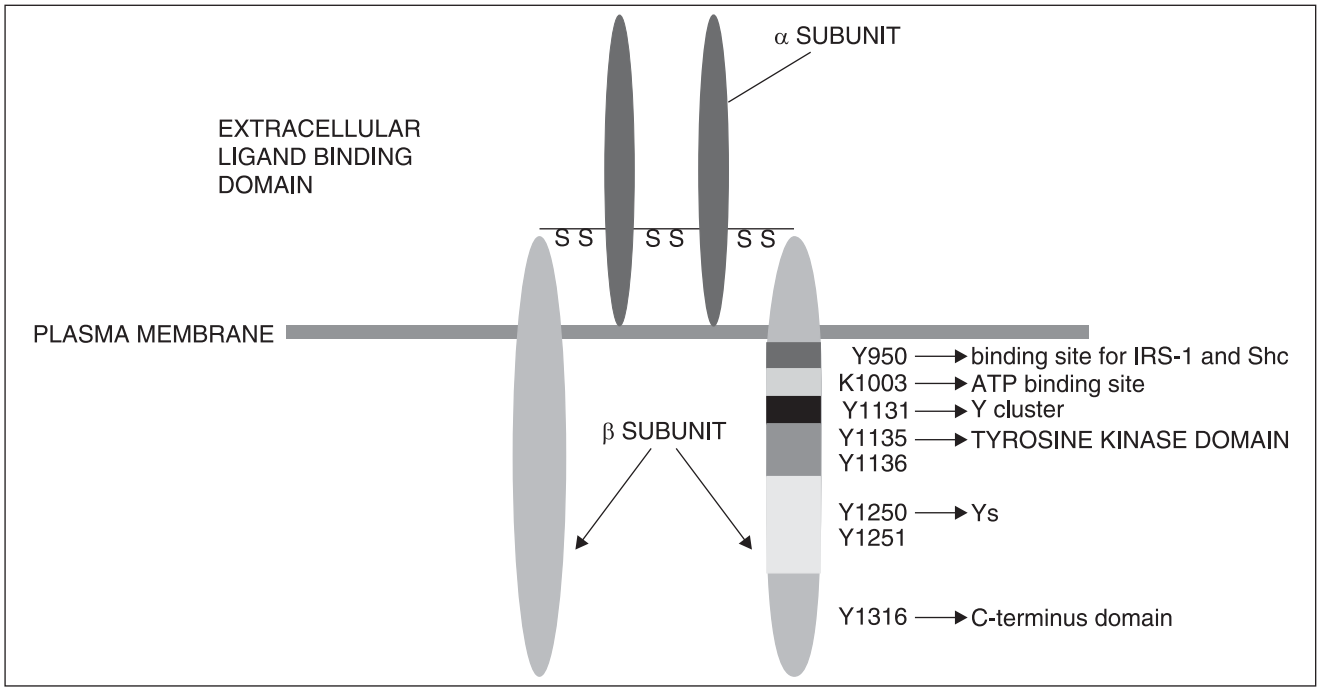

Figure 1
IGF1R: The IGF1 receptor has a tetrameric structure consisting of 2 identical subunits and 2 identical β subunits with extracellular, transmembrane and intracellular domains. (modified after Baserga et al., 1997)

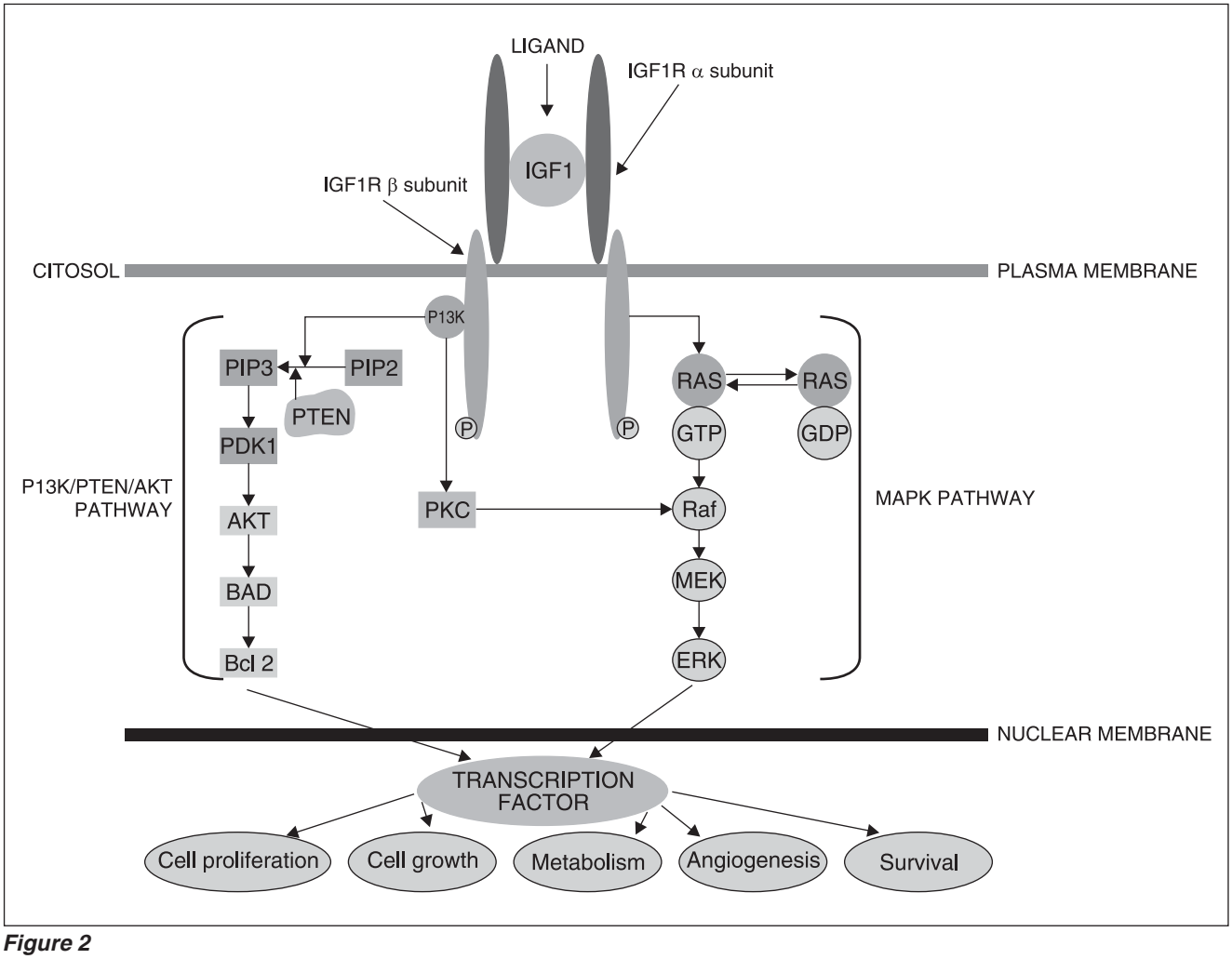

Figure 2
Mithogenic pathways that are altered in glioblastomas (modified after Rich JN and Bigner DD, 2004)

and degradation of IGF2. Therefore this receptor has been described as a „sink“ for IGF-2 (13-15).

The binding proteins (IGFBPs) are produced by the liver. There are at least six IGF binding proteins with sizes from 22 to 31 kDa. They have growth regulatory actions that are probably independent of their capacity to bind IGF1. In general they inhibit interaction of IGF's with the IGF1R (16, 17).

IGF1/IGF1R MITOGENIC PATHWAYS IN GB

GB are heterogeneous tumors which frequently target elements in specific cellular processes. The tumors are characterized by active cellular proliferation which gives to tumor cells a survival advantage over surrounding non-neoplastic tissues. The presence of growth factor ligands (such as IGF1) and their receptors (such as IGF1R) is frequent in GB. Growth factors pathways regulate: apoptotic resistance, motility, invasion and neo-angiogenesis beyond cellular proliferation (18, 19).

In normal cells the ligand, IGF1 bind to his specific cell surface receptor, IGF1R, forms a signaling complex leading to the phosphorylation of specific receptor residues. In this way the receptor becomes activated. The phosphorylation induces conformational changes which enable binding of intracellular effectors that propagate the pathway signal. Growth factor pathway activity is terminated by rapid receptor inactivation, internalization and degradation.

GB activate receptor pathways by:

a. overexpression of ligands and/or receptors;
b. mutation of receptors;
c. activation of intracellular transducers;
d. loss of negative regulators (20).

The phosphatidylinositol 3-kinase (PI3K)/PTEN/AKT pathway is activated by a number of different signals including activation of IGF1R. PI3K phosphorylates the 3'hydroxyl group of the inositol ring of phosphatidylinositol (4, 5) biphosphate (PIP2) resulting phosphatidylinositol (3, 4, 5) trisphosphate (PIP3). PIP3 acts like a second messenger recruiting proteins with pleckstrin homology (PH) domains (like AKT) to the cell membrane where they can be activated. AKT is a protein with PH domain that regulates the cell cycle, metabolism and apoptosis. Activated AKT phosphorylates and blocks a variety of proapoptotic proteins including: BAD, caspase-9, GSK-3 beta kinase and forkhead transcription factors. Furthermore AKT induces the expression of antiapoptotic proteins such as Bcl2 (Figure 1).

In GB phosphatidylinositol 3-kinase pathway is commonly active and therefore the levels of PIP3 are higher then normal. PIP3 levels are also increased by the loss of the negative regulator phosphatase and tensin homologue deleted on chromosome ten (PTEN). PTEN is a cytoplasmic dual-specificity phosphatase that acts on several proteins and functions as a negative regulator of PIP3 levels reciprocal to PI3K. The loss of PTEN has been associated to a higher AKT activity, tumor angiogenesis and invasion. PTEN loss was found in de novo glioblastomas. Increased PIP3 levels induces AKT activation which contribute to the suppression of apoptosis in the presence of apoptotic stimuli. Therefore the development of PI3K/PTEN/AKT pathway inhibitors is a promising therapeutic target for GB (20-24).

The Ras/Raf/MEK/ERK pathway also known as mitogen-activated protein kinase (MAPK) pathway is activated by small-G protein Ras family members. After IGF1 binding to IGF1R the receptor becomes activated and phosphorylated. Phosphorylated receptor tyrosne residues permit the binding of proteins with src homology 2 (SH2) domains such as Grb2, that act as signal transducers or adaptor modules. These proteins recruit Sos-1, an activator of Ras proteins. Sos-1 function as Ras guanine-nucleotide exchange factors (GEF). The GEF binds to the SH2 domains of Grb2 and mediates the exchange of GDP in GTP, activating Ras which is initially synthesized as an inert cytosolic polypeptide (proRas) and then farnesylated by farnesyl transferase (FTase). The activation of Ras-GTP stimulates several effectors such as: Raf-1, Rac, Rho, MEKK, PI3K and phospholipase C promoting cell proliferation and survival (Figure 2).

In GB the activation of Ras/Raf/MEK/ERK signaling pathway is due to overexpression of receptor tyrosine kinases such as IGF1R. In consequence targeted therapeutic strategies such as: antisense oligonucleotides, FTase inhibitors, Raf inhibitors, MEK inhibitors and so on had developed in order to inhibit Ras pathways (21, 25, 26).

IGF1/IGF1R INHIBITORS FOR GB THERAPY

The IGF1/IGF1R are targeted to design new inhibitors capable to block ligands effects through binding:

a. to IGF1;
b. to the extracellular domain of IGF1R;
c. to intracellular sites of IGF1R. They are represented: monoclonal antibodies, chemical compounds, small molecule inhibitors (27).

Some inhibitors such as: anti-receptor antibodies and small molecule inhibitors specific of IGF1R tyrosine kinase domain have been developed but yet they are not been evaluated in clinical trials. Also for more specificity co-targeting 1R must be taken into account (28, 29).

Other promising targets are: the co-inhibition of IGF1R and EGF-R which decreased apoptosis in GB cell lines and the use of etoposide (30).

ANTI-GENE TO IGF1/IGF1R FOR GB THERAPY

It is known that the presence of the IGF1 gene in the mature brain is a signal for neoplastic processes. Therefore the development of anti-gene therapies is a promising target for GB therapy.

The anti-genes can be classified in three categories:

a. the antisense molecules;
b. the triple helix(TH)-forming oligomers;
c. the sense oligodeoxynucleotides which is a relatively recent finding (31-33).

The experimental studies using gene therapy have shown efficiency therefore the first clinical trials for the treatment of GB was conducted in the 1990's. Today the clinical trials focuse on IGF1 and his receptor. The anti-gene to IGF1R focused on: antisense therapies that interrupt the anti-apoptotic effect of IGF1 and down-regulation of IGF1R which leads to inhibition of tumor growth. The anti-gene to IGF1 focused on IGF1 antisense and TH approaches. Some clinical trials with promising results were conducted using antisense IGF1 (34-36).

CONCLUSIONS

The advances in the understanding the molecular biology underlying the GB pathogenesis have revealed abnormalities of common cellular pathways and functions. IGF1/IGF1R play important roles in the proliferation, transformation and motility of GB cells. Therefore this fact is being exploited to develop potential therapeutic targets in order to inhibit IGF1/IGF1R actions.

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