IGF-IR in neuroprotection and brain tumors

Elisa Gualco¹, Jin Ying Wang¹, Luis Del Valle¹, Katarzyna Urbanska¹², Francesca Peruzzi¹, Kamel Khalili¹, Shohreh Amini¹, Krzysztof Reiss¹

¹Center for Neurovirology, Department of Neuroscience, Temple University School of Medicine, Philadelphia, PA 19122; ²Department of Cell Biology, Faculty of Biotechnology, Jagiellonian University, Krakow, Poland

TABLE OF CONTENTS

1. Abstract
2. Introduction: IGF-IR signaling system
3. IGF-IR in the brain
4. IGF-IR in neurodegenerative disorders
  4.1 IGF-I - TNFα signaling interplay in HIV associated dementia (HAD).
    4.1.1 IGF-I and TNFα in non-apoptotic neuronal injuries
    4.1.2 Functional Interplay between IGF-I, TNFα and the family of a disintegrin and metalloproteases (ADAMs) in neuronal injury and regeneration
    4.1.3 Contribution of HIV and diabetes to the development of HAD
    4.1.4. IGF-IR and reactive oxygen species (ROS) metabolism
  4.2. IGF-IR and Alzheimer’s disease (AD)
5. IGF-IR and brain tumors
  5.1. IGF-I and cellular transformation
  5.2. IGF-IR in medulloblastoma
  5.3. IGF-IR in DNA repair and genomic integrity
  5.4. Nuclear Translocation of IRS-1 and cancer
6. Acknowledgement
7. References

1. ABSTRACT

The IGF-IR is a multifunctional tyrosine kinase receptor involved in several biological processes including cell proliferation, differentiation, DNA repair, and cell survival. In the brain IGF-I plays a critical role during embryonic and early postnatal development. In the mature brain, IGF-I binding sites have been found in different regions of the brain, and multiple reports confirmed a strong neuroprotective action of the IGF-IR against different pro-apoptotic insults. When the IGF-IR signaling system is insufficiently deployed, either by low level of expression in elderly individuals, or by the inhibition associated with inflammatory cytokines, neuronal function and survival could be compromised. The examples of such CNS pathologies include HIV associated dementia, diabetic neuropathies, and Alzheimer’s disease. On the other hand, elevated expression activity of the IGF-IR may support uncontrolled cell proliferation and protection from apoptosis. Probably the best example of the IGF-IR involvement in brain tumors is medulloblastomas in which functional cooperation between viral oncoprotein, JC virus large T-antigen, and IGF-IR has been recently established. Therefore, better understanding of the beneficial and potentially harmful aspects of the IGF-IR can be critical for the development of new clinical regimens against neurodegenerative disorders and brain tumors.

2. INTRODUCTION: IGF-IR SIGNALING SYSTEM

The insulin-like growth factor I receptor (IGF-IR) is a membrane associated multifunctional tyrosine kinase (TK), which plays a critical role in a number of basic biological events including cell proliferation, differentiation, DNA repair, and protection from apoptosis (1-5). The IGF-IR gene is located on chromosome 15q26 and encodes a single polypeptide of about 70% amino acid homology with insulin receptor (IR) (6). Following post-translational modifications, which include proteolytic cleavage of the pro-receptor and its glycosilation, the mature IGF-IR is a heterotetradimer consisting of two extracellular α subunits, which contain cysteine-reach ligand-binding pocket, and two β subunits with extracellular and transmembrane domains, and cytoplasmatic region containing tyrosine kinase domain and C-terminal domain (7) (Figure 1). There are three natural ligands, IGF-I, IGF-2 and insulin, which are capable of binding and activating the IGF-IR. IGF-I binds the receptor with the highest affinity (Kd= 1nM); and the bindings of IGF-2 (Kd= 15-20nM) and insulin (Kd= 100nM) are much less effective (8, 9). IGF-1 is a single chain polypeptide, structurally related to insulin, which is synthesized primarily by the liver in response to growth hormone (GH). IGF-I is produced also locally by a large number of tissues including the brain, in which GH –
mediated control of IGF-I synthesis is much less evident (10). IGF-I blood levels range between 10 to 500 ng/ml, and more than 90% of this circulating IGF-I is associated with high affinity IGF binding proteins (IGFBP 1-6), which prolong IGF-I half-life by preventing its proteolysis, and modulate IGF-I availability for the receptors (11). The ligand binding leads to the clustering and subsequent autophosphorylation of multiple IGF-IR molecules on tyrosine residues in the kinase domain, followed by the phosphorylation of juxtamembrane tyrosines and C-terminal serines (12). This initial activation is followed by the recruitment of multiple signaling molecules, such as insulin-receptor substrates (IRS-1 – IRS-4), Src homology domain C-terminal adaptor family members (Shc) (13), and 14-3-3 proteins (14), which link the activated IGF-IR with diverse intracellular pathways, leading to the activation of DNA replication, DNA repair, and the induction of multiple anti-apoptotic signals (1, 2, 15-17) (Figure 2). From IRS-1 that binds to Tyr 950 in the β-subunit of the IGF-IR, derive both the Ras-MAP and PI-3 kinase-Akt/PKB pathways (18-20). Another substrate of the IGF-IR has been identified in mice with a targeted disruption of the IRS-1 genes, and designated as IRS-2 (21). More recently, IRS-3 (22), and IRS-4 (23) have also been cloned providing an additional interface between the IGF-IR and intracellular signaling molecules. Besides IRS and She families, the IGF-IR has other direct substrates including, GRB10 (24), Crk (25), PI-3 kinase (26) Syphosphatase (27), and C-terminal Src kinase (CSK) (28). Several laboratories have established that one of the most prominent pathways to protect cells from apoptosis via IGF-IR is the recruitment of PI-3 kinase, and subsequent activation of the serine/threonine kinase, Akt/PKB (29-32).

What makes the IGF-IR different from other receptors is that at least three PI-3 kinase molecules can be recruited by one activated molecule of the IGF-IR. The PI-3 kinase binds directly to pY1316 residue of the C-terminal domain of the ligand activated IGF-IR (27), and two additional PI3 kinase molecules bind pY608 and pY939 of activated IRS-1 (33). Another unique property of the IGF-IR is that at least four signaling branches may account for IGF-I mediated protection from apoptosis. Two of which depend on Akt activation, and result in the phosphorylation dependent inactivation of Bad (17, 34-36) and pro-caspase 9 (37). Two other pathways seem to be Akt independent, and involve either the activation of the Ras-MAP kinases pathway, which result in Raf phosphorylation and its translocation to mitochondria (17, 34-36), or NFκB– mediated activation of inhibitors of apoptosis (IAPs) (38). Because of these multiple growth promoting and pro-survival properties, the IGF-IR signaling system gained a lot of attention as a potential neurotrophic factor, and has been intensively studied in the peripheral and central nervous system disorders. Here we review recent literature on the role of IGF-IR signaling system in the CNS, and discuss how our continuously growing knowledge about this powerful receptor could contribute to the battle against neurodegenerative disorders and brain tumors.

3. IGF-IR IN THE BRAIN

In the brain IGF-IR and two of its ligands, IGF-I and IGF-2, are highly expressed during embryonic and early postnatal development, but decrease substantially during adolescence and adult life (39). In the mature brain, IGF-I binding sites and IGF-IR mRNA are still found in the
choroid plexus, circumventricular areas, and the superior olivary nuclei (39). In the cerebellar cortex, the IGF-IR is expressed in granular and Purkinje neurons, where IGF-I is mitogenic in vitro, and protects neurons from low potassium induced apoptosis (39-41). Since apoptotic death of neuronal cells accompanies degenerative disorders of the CNS, it is not a surprise that the IGF-I signaling system became an important candidate for neuroprotection. Indeed, Gluckman et al. reported the protective effects of IGF-I in ischemic injuries of the CNS (42), and D'Mello et al. found that IGF-I inhibited low potassium-induced apoptosis of cerebellar granule neurons induced by low level of potassium (43). Dudek et al. showed that IGF-I-mediated protection from apoptosis is controlled by PI3-kinase activation of Akt, rather than PI3-kinase activation of p70S6 kinase in primary cultures of cerebellar neurons (44). These early findings were followed by a myriad of reports providing overwhelming evidence for the role of IGF-I in neuroprotection. For instance, IGF-I protected neurons from oxidative stress (45), high glucose (46), glutamate-induced apoptosis (47), and from TNFα-mediated degeneration of neuronal processes (48). IGF-I has been also shown to protect hippocampal and cortical neurons from N-methyl-D-aspartate (NMDA)-induced and
nitric oxide -induced apoptosis (49). A unique aspect of the IGF system in the brain is the presence of a truncated form of IGF-I (des-IGF-I), which lacks the first 3 amino acids. Probably the most relevant biologically feature of this truncation is that des-IGF-I does not bind IGF binding proteins, and therefore could be more active as a local auto- or para-crine regulator of cell proliferation and cell survival in normal and transformed brain cells (50).

Different transgenic animal models overexpressing IGF-I confirmed the importance of the IGF system in CNS. It has been reported for instance that transgenic mice overexpressing the growth hormone grew to larger size than control mice (51), a phenomenon which could be associated with the fact that growth hormone induces expression of IGF-I in the liver, which is the major source of plasma IGF-I. Mathews and co-workers detected that transgenic mice overexpressing IGF-I have an increased brain weight, slightly larger than the increase in total body weight (52). In a transgenic mice, in which IGF-I construct was engineered to be expressed in the adult heart, the overproduction of IGF-I was sufficient to cause an 85% increase in IGF-I plasma levels and a significant increase in brain weight (53).

In contrast, transgenic mice with targeted disruption of the IGF-IR gene (IGF-IR-/−) have reduced brain size and altered brain structures, including reduced myelination due to decreased proliferation and maturation of oligodendrocytes, which associates with the attenuation of axonal growth, and a marked decrease in the density of neural cells mostly observed in the spinal cord and brainstem (54). Our recent immunohistochemical analysis of the IGF-IR-/− embryos indicated significantly higher levels of apoptotic cells detected in the brain and in the dorsal root ganglia, which was accompanied by much lower levels of anti-apoptotic protein, Survivin (Figure 3). In addition to the lower levels of Survivin, the brain and dorsal root ganglia of the knockout mice were smaller and poorly differentiated (Figure 3). This lower degree of differentiation in the IGF-IR-/− embryos correlated with less abundant expression of the neuronal marker, class III β-tubulin, and higher levels of expression of the early marker of neural progenitors, nestin, in comparison to bigger and better differentiated brains from age-matching non-transgenic littermates (Figure 3).

In a different transgenic model, gene ablation of IGF-I has revealed deficits in neuronal and oligodendrocytic populations in olfactory bulb, dentate gyrus and striatum, and in the cochlear ganglion neurons (55). These major anatomical differences were likely caused by lower rate of cell proliferation, compromised cells survival, and less effective neuronal differentiation caused by the absence of IGF-I during embryonic and postnatal development. In addition to these gross structural brain abnormalities, the IGF-I null mice displayed also changes in metabolic brain function, such as reduced glucose uptake, which is the major source of energy for neurons (56).

Several studies provided evidence that IGF-I and IGF-2 are able to increase the number of neural cells, including neurons, astrocytes, and oligodendrocytes, in vivo and in vitro, mostly by increasing proliferation of their precursors (57-60). IGF-I actions on the cell cycle progression of neural progenitors seem to depend on signaling cooperation with other growth factors. For instance, IGF-I by itself had only minimal effects on DNA replication of oligodendrocyte progenitors; however, in synergy with FGF-2, IGF-I strongly activated G1 – S phase progression and cell proliferation (61). In addition to its mitogenic properties, IGF-I can also direct differentiation process towards neuronal and oligodendrocytic lineages. It has been reported for instance that during development IGF-I promoted differentiation of neural progenitors towards neurons (62), and affected the fate of multipotent adult neural progenitors by directing their differentiation towards oligodendrocytes (63). In other reports, neural progenitors from embryonic and adult mouse striatum, which normally proliferate in response to epidermal growth factor (EGF) (64), differentiated preferentially into the neurons following IGF-I stimulation in a dose dependent manner. Further experiments demonstrated that the observed increase in the number of neurons produced by the IGF-I was not mediated by an increase of cell survival or cell proliferation, but rather depended upon induction of the differentiation program (57, 62).

Taken together, presented results from neuronal cell cultures, clinical samples and from transgenic animal models clearly indicate that impairment of the IGF-IR signaling system compromises the development and maintenance of different neural components in the brain, and may contribute to the pathology of the CNS.

4. IGF-IR IN NEURODEGENERATIVE DISORDERS

4.1. IGF-I - TNFα signaling interplay and HIV associated dementia (HAD)

Despite of the use of highly active antiretroviral therapy (HAART) HIV-1 infection of the brain is associated with various manifestations of neurological disorders ranging from mild cognitive and motor impairments to clear dementia (65-68). The major productive reservoir of HIV-1 infection in the brain are perivascular macrophages and, to a lesser extent, microglia (69, 70). HIV-1 can also infect astrocytes, and there are sporadic reports of HIV-1 detection in oligodendrocytes and neurons, however in these three cell types the infection is not productive (71, 72). Pathological changes associated with the presence of HIV-1 in the brain involve mainly the white matter and are characterized by perivascular cuffs of lymphocytes, parenchymal microglial nodules, multinucleated giant cells, and reactive astrocytes, which define HIV encephalitis (HIVE). Interestingly, pathological changes in the gray matter are less pronounced, and neuronal apoptosis is considered as a rare event. HIV-1 - activated macrophages, microglia and astrocytes have been shown to release proinflammatory cytokines such as tumor necrosis factor-α (TNFα), transforming growth factor-β (TGF-β), monocyte chemo-attractant protein-1 (MCP-1), and interleukin-1β (IL-1β), all of which are suspected of
IGF-IR in neuroprotection and brain tumors

Figure 3. Immunohistochemical characterization of the IGF-IR knockout embryos. The knockout embryos are smaller in size and volume and show decrease expression of Survivin particularly in the brain, spinal cord and dorsal root ganglia (DRG) (montages). At higher magnification, both the brain and DRG show lower levels of Survivin which correlated with increased number of apoptotic cells. In the knockout mice, a significant number of cells undergoing apoptosis is detected in both the brain and DRG, compared with the wild type mice in which no apoptotic cells were detected by TUNEL assay. In addition to the lower levels of Survivin and its expected effect on cell survival, the brain and DRG of knockout mice is smaller and poorly differentiated. This degree of differentiation correlates with lower levels of Class III β-Tubulin and higher levels of the earlier marker, Nestin, compared to brains of the wild type mice, expressing higher levels of βIII-Tubulin and less nestin, suggesting a higher degree of differentiation.

altering neuronal function and survival (66, 73-75). In addition to cytokine-mediated neurotoxicity, HIV may also involve the failure of neuroprotective mechanisms. In particular, the IGF-IR signaling system, which is known to play a significant role in neuroprotection, may be insufficiently deployed. There are several findings that point to a role for IGF-I in neuro-AIDS. Decreased levels of IGF-I together with growth hormone resistance have been found to be contributing factors in AIDS wasting syndrome (AWS); and the neuronal expression of IGF-I
IGF-IR in neuroprotection and brain tumors

receptor (IGF-IR) appears to be insufficient as a compensatory mechanism to counteract neuronal degeneration (76, 77). In addition, reduced levels of serum IGF-I have been observed in HIV-infected children, especially those with Failure to Thrive (FTT), (78, 79). As IGF-I is a principal mediator of the action of human growth hormone, its role in anabolic effects has prompted studies on IGF-I levels in HIV-infected patients, and the use of both IGF-I and growth hormone in the treatment of cachectic patients (80-83). Although some improvements in body mass have been noted, results of some of these studies suggest partial resistance to growth hormone and IGF-I therapies in the setting of HIV wasting syndrome (78). Decreased levels of IGF-I in the CNS, or alternatively development of IGF-I/insulin resistance may compromise neuronal survival during HIV infection. In this respect, TNFα inhibits some of the known aspects of the IGF-IR and insulin receptor (IR) in diabetes (84, 85), and TNFα has been shown to inhibit IGF-I-stimulated protein synthesis in myoblasts (78), and compromise neuronal survival (48, 86). Therefore, one could speculate that the interaction between TNFα and IGF-I signal transduction pathways may represent an important component, which could determine neuronal fate in the paradigm of HIVE.

4.1.1. IGF-I and TNFα in non-apoptotic neuronal injury

Although the molecular mechanisms involved in IGF-IR–mediated neuro-protection are associated with a strong anti-apoptotic potential of this tyrosine kinase receptor, several reports, including our recent studies suggested that neuronal damage may happen in the absence of apoptosis (87), and that IGF-I could be still protective (48, 86). This includes neuronal damage often observed in the presence of TNFα (75, 88), and possibly, TNFα – mediated serine phosphorylation of IRS-1 (89, 90). The process of IRS-1 phosphorylation on serine residues has been associated with the inactivation of IRS-1 function as a signaling molecule, which when phosphorylated on multiple tyrosine residues amplifies and diversifies the signal from insulin and IGF-I receptors (89, 91). Several serine kinases have been implicated in the process of IRS-1 inactivation. For instance, c-Jun N-terminal kinase (JNK) has been identified to mediate IRS-1 phosphorylation at Ser328 and Ser330 (89, 92). Other studies demonstrated that PKCζ (93), MAP kinases p42/p44 and p38 (94), and PK3-kinase (95, 96) could be also involved, further stressing the importance of IRS-1 phosphorylation during neuronal outgrowth and neuronal injury.

Only a few reports have implicated serine phosphorylation of IRS-1 in the process of TNFα–mediated neuronal damage (90, 97). One such study demonstrated that cerebellar granule neurons lost the ability of responding to IGF-I survival signal when treated with low doses of TNFα. IRS-2 serine phosphorylation, and a loss of signaling connection between IGF-IR and PI3-kinase were suspected for this TNFα–mediated IGF-I resistance. We have demonstrated that differentiated PC12 neurons, which overexpress ectopic IGF-IR, were able to maintain neuronal processes in the presence of TNFα much better than parental PC12 neurons (48). Although initially, we did not observe any significant changes in the phosphorylation status of cytosolic IRS-1 and IRS-2, our results suggested that TNFα–mediated degeneration of neuronal processes was linked with the interaction between IRS-1 and αβ1 integrin (86). Such an interaction has been already proposed by Vuori and Rueslahti, who first demonstrated the binding between IRS-1 and αβ3 integrin in cells cultured on vitronectin (98). More recently, reciprocal interactions between the insulin receptor - IRS-1 signaling axis and αβ1- integrin showed both the improvement of insulin-mediated attachment to fibronectin, and increased IRS-1 phosphorylation (99). In addition, extensive studies on cell adhesion and motility of LNCAp prostate cancer cells demonstrated the formation of IRS-1–αβ1 integrin complex under conditions in which IRS-1 is predominantly phosphorylated on serine residues (33). The association between β1-integrin and IGF-IR has also been demonstrated in myeloma cells (100), and several other reports confirmed the presence of a functional cross-talk between integrins and the IGF-IR signaling system (101-103). Finally, our recent study demonstrates an inverse relationship between neuronal stability and TNFα–mediated serine phosphorylation of IRS-1, and subsequent binding of pS-IRS-1 to αβ1-integrin in the membrane rafts of differentiated PC12 neurons (104) (Figure 4).

4.1.2. Functional interplay between TNFα, IGF-I and the family of a disintegrin and metalloproteases (ADAMs) in neuronal injury and regeneration

Activation of proinflammatory cytokines including TNFα and their effects on nervous system have been repeatedly demonstrated in vitro (48, 86, 105, 106), and in neurodegenerative disorders such as HAD (75, 107, 108), Multiple Sclerosis, Alzheimer’s disease, and neuronal injuries that develop after ischemia and type 2 diabetes (105, 109-111). TNFα is synthesized as a trimeric type II membrane-associated precursor (112). Upon cleavage, the mature form of TNFα is released from the cell surface and can either enter the blood stream or can stay in the extracellular environment (113). Recently, a considerable effort has been made to identify the TNFα convertase/sheddase. ADAM17 (a disintegrin and metalloprotease 17, also referred as TNFα converting enzyme or TACE) is presently considered as the major sheddase for TNFα (114, 115). A targeted deletion of ADAM17 in transgenic mice confirmed its critical role in the shedding of TNFα (116). In addition, other members of the family, ADAM9 and ADAM10, which are abundantly expressed in the nervous system, have been also confirmed as efficient TNFα convertases (117). These neuronal ADAMs, are also responsible for cleavage–mediated activation of Notch and its ligand Delta (117-120); cleavage of collagen IV, and amyloid precursor protein (APP) - decreasing formation of the toxic β-amyloid (117); and, what is closely related with the topic of this review, tissue release of IGF-I by cleaving IGF-I binding proteins (121, 122). Importantly, ADAM10 is also known to participate in axonal outgrowth, and when eliminated by a single knockout, leads to severe developmental changes including underdevelopment of the CNS (117).
Figure 4. Functional implications of the pS-IRS-1 -β1-integrin membrane complex. Panel A: Double immunolabeling of IRS-1 and β1-integrin in differentiated PC12 neurons. Fluorescent images were collected from inverted fluorescent microscope equipped with motorized Z-axis and deconvolution software (SlideBook4). Anti-β1-integrin mouse monoclonal antibody and anti-mouse FITC-conjugated secondary antibody (green fluorescence), as well as anti-IRS-1, rabbit polyclonal antibody, and anti-rabbit rhodamine-conjugated secondary antibody (red fluorescence), were utilized. Note the presence of a strong co-localization between IRS-1 and β1-integrin detected in neuronal processes after TNFα treatment and much less of the co-localization after IGF-I treatment (yellow fluorescence). The percentage of co-localization between IRS-1 and β1-integrin is indicated with standard deviation (n=3). Original magnification x1000. Panel B: The pS-IRS-1 – β1-integrin complex formation in membrane rafts of differentiated neuronal cells impairs the binding between integrins and collagen and is thought to compromise stability of neuronal processes. This protein – protein interaction is facilitated by TNFα which triggers accumulation of pS-IRS-1 in membrane rafts and is attenuated by IGF-I, which facilitates tyrosine phosphorylation of IRS-1, impairs formation of the complex and supports neuronal survival.
4.1.3. Contribution of HIV and diabetes to the development of HAD

Deregulation of glucose homeostasis has been recently considered as a serious risk factor for cognitive impairment among HIV –infected individuals (123, 124). This impairment of the mental status became even more important in view of recent findings that protease inhibitors, which are the major components of highly active antiretroviral therapy (HAART), may cause glucose intolerance and often lead to the development of type 2 diabetes mellitus (124, 125). Two potential mechanisms are associated with this unexpected metabolic impairment: (i) direct interaction between protease inhibitors and glucose transporter GLUT4 (126); and (ii) interference with cellular retinoic acid binding protein type I (CRABP-I), which in normal circumstances is expected to support PPARγ–mediated downregulation of free fatty acids and TNFα (127, 128). As a result of this HIV –associated metabolic abnormality, insulin resistance and type-2 diabetes may develop, leading to weight loss, atypical fat distribution (125), and later to serious organ damage, which includes eyes, kidneys, peripheral nervous system and the brain (129). The slowly progressing alterations in cerebral function and structure that occur in diabetes are often referred as diabetic encephalopathy. The clinical manifestation of diabetic encephalopathy includes changes in cognitive function including moderate impairment of verbal memory and mental speed (130). These pathologic changes are very similar to those observed in HIV associated dementia; and in fact, the incidence of dementia doubles in elderly individuals who are both diabetic and HIV positive (124, 131).

4.1.4. IGF-IR and reactive oxygen species (ROS)

Hyperglycemia and diabetes mellitus are associated with the increase in reactive oxygen species (ROS) production at the cellular level (132, 133), which plays an important role in the development of diabetic complications (134, 135). ROS dependent signals have been also linked to defects in genomic maintenance and accelerated aging (136). We have shown recently a strong IGF-IR antioxidant phenotype in mesangial cells maintained at high glucose concentration (132, 137). This novel IGF-IR function was closely coupled with the improvement of cell survival. These observations are in agreement with the studies in which longevity in mice was directly linked to increased resistance to oxidative stress, in which the adaptor protein p66Shc, one of the IGF-IR signaling molecules, have emerged as major genetic determinants of longevity and oxidative stress, in mammals (138, 139). In this respect, our preliminary experiments indicate that both high glucose (25mM for 16 hours) and prolonged exposure to TNFα (50nM for 3-5 days) elevated the accumulation of ROS in differentiated neuronal cultures of PC12 cells. The accumulated ROS were found in neuronal processes and in perinuclear cytoplasm, and paralleled with gradual loss of neuronal processes. In the presence of IGF-I, however, differentiated PC12 neurons did not accumulate ROS above the control levels, and were partially protected from TNFα–mediated retraction of neuronal processes. On the other hand, multiple reports in the literature strongly indicate that IGF-IR is associated with accelerated aging, and that reduced IGF-I expression promotes longevity (139-142). In contrast to the enhanced longevity, age related decrease in IGF-I has been associated with increased risk of cardiovascular disease (143, 144), premature arteriosclerosis and diabetes (145), as well as neurodegenerative disorders (146-148).

Our search for a potential mechanism involved in this IGF-IR –mediated antioxidant function pointed to the p66Shc – FOXO3a signaling axis. This is in a strong agreement with multiple reports, which support a dominant role for wild type p66Shc protein in the intracellular pathways that are involved in generation of mitochondrial ROS, and the conversion of oxidative stress to apoptosis (149-151). Recently, better understanding of p66Shc has emerged, based on evidence that the p66Shc protein functionally interacts with the mammalian Forkhead homolog, FOXO3a (150). In the proposed mechanism, ROS induced phosphorylation of p66Shc at a critical SH2 Ser-36 residue, leads to the phosphorylation dependent inactivation of FOXO3a, and associated transcriptional repression of one of the most prominent anti-ROS enzymes, manganese superoxide dismutase (MnSOD) (152, 153). Therefore, answer to the question, how activated IGF-IR affects functional interplay between FOXO3a and p66shc could be very helpful in clarifying our understanding of the complicated relationship between IGF-IR signaling system, ROS metabolism, and aging.

4.2. IGF-I and Alzheimer’s disease (AD)

A strong line of experimental evidence supports the role of IGF-I in neuronal outgrowth, survival and differentiation (154-156), and in modulation of neuronal excitability and synaptic plasticity (157, 158). Therefore, it is conceivable that IGF-IR signaling pathways may be dysregulated, or inefficiently deployed in neurodegenerative disorders. AD is a progressive neurodegenerative disease associated with cognitive and behavioral dysfunction, occurring predominantly later in life, and characterized by cell loss with increased activation of the pro-apoptotic genes and signaling pathways, impaired energy metabolism, mitochondrial dysfunction, and chronic oxidative stress (159, 160). Neurofibrillary tangles and senile plaque accumulation in the hippocampus and neocortex are the main pathological features of AD. Senile plaques are manly build by amyloid β-peptide (Aβ), which is formed by γ-secretase –mediated cleavage of the amyloid precursor protein (APP) (161, 162). Although most of the secreted fragments of APP have positive effects on cell survival, neuronal outgrowth, and synaptic plasticity (163); the accumulation of Aβ in the brain is associated with neuronal damage, and represents a high risk factor in the development of AD (164).

A considerable line of evidence supports the involvement of abnormal IGF-I and insulin function in Aβ deposition (155). It has been demonstrated for instance that IGF-I and insulin increase the level of APP through the activation of PI3-K signaling pathway (165), and that IGF-I promotes the cleavage of APP through the stimulation of α-secretases, and in this way could contribute to the reduction of the harmful Aβ (166). In addition, IGF-I appears to
stimulate elimination of brain amyloids through a complex process that includes stimulation of Aβ release and its subsequent clearance from the brain parenchyma (166).

In AD transgenic mouse model, IGF-I was demonstrated to induce clearance of Aβ from the brain by upregulating levels of transport proteins such as albumin and transthyretin (166); and it has been suggested that disrupted IGF-I signaling in cells involved in blood brain barrier (BBB) may contribute to amyloidosis (167). In this process, choroid plexum epithelium has been shown to represent the primary target of IGF-I-mediated clearance of brain Aβ, and disruption of the IGF-IR signaling in the choroid plexus was sufficient to trigger pathological changes similar to those found in AD brains including brain amyloidosis and Tau-hyperphosphorylation (168). These observations have been indirectly supported by the fact that the choroid plexus in AD patients shows amyloid deposits and tangles (169).

As a consequence of the reduced IGF-I signaling at the BBB, brain Aβ clearance is diminished. Because Aβ has been also shown to antagonize insulin and IGF-I receptor binding in neurons (170), even more severe deficiencies in the IGF-I and insulin signaling may develop, further compromising neuronal survival and function. Therefore, the loss of sensitivity to IGF-I, and partial BBB dysfunction, which occur during normal aging, can contribute to the development of AD amyloidosis (171). This hypothesis was supported by the observation that AD patients have lower levels of transthyretin, an amyloid carrier in cerebro-spinal fluid (CSF) compared to normal aged individuals (172).

Although all these findings strongly suggest the involvement of IGF-I-resistance at the BBB as a pathogenic event in AD, a direct cause of this resistance remains to be clarified. In this respect, alterations in IGF-BPs, and activated cytokine linked to inflammatory responses at the BBB, could attenuate IGF-I signaling in this critically important compartment of the brain (173-175).

The impairment of IGF-I signaling system observed in the brain of AD patients has been also associated with the pathological development of neurofibrillary tangles (NFT), which contain microtubule-associated protein, Tau, in the form of filamentous aggregates. The Tau was originally discovered as a heat stable protein that facilitates assembly of the microtubules in vitro (176). Further studies demonstrated that Tau phosphorylation is a physiological process required for cytoskeleton assembly and stabilization. The physiological roles of Tau involve regulation of neurite extension (177), axonal transport, and microtubules stability and dynamics (178, 179). In neurodegenerative diseases in which Tau pathology has been observed, Tau is hyper-phosphorylated (180), and this aberrant phosphorylation characterized Tau proteins isolated from AD brains (181).

The kinases, which are responsible for physiological phosphorylation of Tau include Erks, and cyclin dependent kinase 5 (Cdk-5), both of which are known to be activated by IGF-I and insulin (182-184). On the other hand, impaired insulin or IGF-I signaling may result in hyper-phosphorylation of Tau due to the reduced activation of PI3–kinase/Akt signaling axis, which results in the release of the inhibition of multifunctional serine/threonine kinase GSK3β. Unlike other protein kinases, GSK3β is normally constitutively active, and is primarily regulated through the phosphorylation dependent inactivation (185). IGF-I and insulin have been shown to mediate this phosphorylation dependent GSK3β inactivation (186, 187), and possibly contribute to the attenuation of Tau hyper-phosphorylation.

Although the mechanisms of increased GSK3β activation in AD can be readily explained on the basis of impaired IGF-1/insulin signaling, coexisting increased levels of Erks (188), Akt (189, 190) and Cdk-5 (191) found in AD brains should compensate for this abnormal Tau phosphorylation. However, GSK3β can also be activated in response to the accumulation of oxidative stress (192, 193), which is commonly seen in the brains of AD patients (194, 195). Therefore, impaired IGF-I/insulin signaling systems can synergize with oxidative stress in the process of GSK3β activation, leading to the aberrant Tau phosphorylation, and the formation of neurofibrillary tangles. Importantly, the potential role of attenuated IGF-I responses in AD is supported by the evidence that IGF-I treatment resulted in enhanced cognitive performance, increased levels of synaptic proteins, and reduced astrogliosis associated with Aβ plaques (196). In addition, Mini-Mental State Examination scores and serum levels of IGF-I in AD have been evaluated, and show that lower levels of serum IGF-I correlate well with cognitive impairments (146).

5. IGF-IR AND BRAIN TUMORS

5.1. IGF-I in cellular transformation

The relevance of IGF-IR in normal growth and development has been best demonstrated by the studies in mice with targeted disruption of the IGF-IR gene (54). These mice are 45% of the size of wild type littermates at birth, and die shortly due to severe organ hypoplasia (54). In vivo, mouse embryo fibroblasts (MEF) derived from the IGF-IR (-/-) embryos (R- cells) have been shown to grow more slowly than wild-type fibroblasts in monolayer cultures, and were unable to proliferate under anchorage-independent conditions (197). Cell growth in anchorage-independence indicates transformation in vitro and tumorigenicity in vivo; and interestingly, R- cells demonstrated unusual resistance to cellular transformation by a number of oncogenic proteins, including SV40 large T-antigen, activated RAS, overexpression of epidermal growth factor receptor (EGFR) (198), and by human polyomavirus JC large T-antigen (199).

In close relation to these multiple observations, downregulation of the IGF-IR expression (200), or inhibition of its function (201, 202), results in the reversion of the transformed phenotype and massive apoptosis, especially when the cells were cultured in anchorage-independence (202). Accordingly, the effects of IGF-IR
IGF-IR in neuroprotection and brain tumors

inhibition were much more pronounced against metastatic cancer in comparison to the inhibition of the primary tumor growth (203, 204). Similarly, downregulation of the IGF-IR had only modest effects on cell growth in monolayer. The obtained growth inhibition ranged between 10 and 15%, suggesting that the IGF-IR is not an absolute requirement for cell growth in monolayer; however its function in supporting anchorage-independent growth and survival are much more apparent (202, 205, 206).

IGF-IR activation or overexpression is associated with an increased propensity for invasion and metastasis by inducing tyrosine phosphorylation of IRS-1 that can influence the interaction between E-cadherin and β-catenin, enhancing β-catenin transcriptional activity and disconnecting E-cadherin from the actin cytoskeleton (207). The loss of E-cadherin expression or function is well recognized as causing disruption of cell–cell contacts and release of invasive tumor cells from primary epithelial tumors (208). Similarly, tumor cell motility and invasive potential are influenced by the crosstalk between the IGF-IR signaling axis and integrins (209), and by IGF-induced secretion of matrix metalloproteinases (210). The importance of these findings was supported by the experiments in which overexpressed IGF-IR conferred metastatic phenotype in a murine model of pancreatic cancer (211), and by the studies in murine hemopoietic and human prostate cancer cells that express low levels of the IGF-IR and lack IRS-1 (212, 213).

5.2. IGF-IR in medulloblastoma

Although brain tumors secrete IGF-I and IGF-2, and the activity of IGF-IR signaling system has been detected in glioblastomas (107-109), neuroblastomas (58), astrocytomas and meningiomas (1), the most compelling evidence of a direct involvement of the IGF-IR comes from medulloblastomas (214-218), which are malignant cerebellar tumors of the childhood. Medulloblastomas represent nearly 25% of all pediatric intracranial neoplasms. These highly malignant tumors arise from the cerebellum and affect mainly children between ages five and fifteen. Although the etiology of medulloblastomas has not been fully elucidated, several reports show that the IGF-IR signaling system is highly active in medulloblastoma cell lines (214, 215), in medulloblastoma animal models (215, 217-220) and in medulloblastoma clinical samples (199, 216). First, Kurilhana et al. detected IGF-1 binding sites in one medulloblastoma specimen (221), and later, the presence of IGF-IR protein and its transcript have been confirmed by Western blot and RT-PCR, respectively (214). The results from our laboratory illustrated that medulloblastoma cell lines and over twenty medulloblastoma biopsies were characterized by an abundant presence of the IGF-IR, and its major signaling molecule, IRS-1 (215, 216 and Figure 5). Medulloblastoma cell lines and medulloblastoma biopsies were strongly positive when immunolabeled with anti-pY1316-IGF-IR antibody, which recognizes phosphorylated (active) form of the IGF-IR (222). In addition, growth and survival of medulloblastoma cell lines cultured in anchorage-independence were strongly dependent on the presence of exogenous IGF-I (215, 216). In this condition, different strategies aiming against the IGF-IR function were very efficient in eliminating medulloblastoma cells in vitro and in experimental animals (215, 217, 223). Other examples of the IGF-IR involvement include strong synergy between Sonic hedgehog/Patched and the IGF-IR signaling systems, which resulted in a significant increase in medulloblastoma tumor formation in RCAS/tv-a transgenic mice (218); and the detection of nuclear IRS-1 in medulloblastoma cell lines and medulloblastoma biopsies positive for the human polyomavirus JC (224).

Growing evidence is accumulating in support of a viral component as a potential etiologic factor in medulloblastomas (216, 225). In this respect, human neurotropic polyomavirus JC encodes within its early genome a regulatory protein, JC T-antigen. JC T-antigen beside its critical role in the viral cycle has transforming properties in vitro (226, 227) and is tumorigenic in experimental animals (228-230). Recent studies revealed association of JCV genome with spontaneous medulloblastomas in humans, and the detection of JC virus in human tumor cells (216, 231). This may raise serious epidemiological concerns, since more than 80% of human population becomes asymptomatically infected by this ubiquitous polyomavirus (232). Since the presence of IGF-IR has been repeatedly shown as a strong requirement for T-antigen–mediated cellular transformation (233), it encouraged investigations of a potential functional interaction between the IGF-IR system and JC virus in the paradigm of medulloblastoma. Several important findings have been established in the course of these studies: (i) insulin receptor substrate 1 (IRS-1), which is the major signaling molecule for the IGF-IR, translocates to the nucleus in the presence of the JC T-antigen (224); (ii) nuclear IRS-1 was detected in T-antigen positive medulloblastoma cell lines, and in T-antigen positive biopsies from patients diagnosed with medulloblastoma (224 and Figure 5); (iii) IRS-1 domain responsible for direct JC T-antigen binding was localized within the N-terminal portion of IRS-1 molecule, and the binding was independent from IRS-1 tyrosine phosphorylation and was strongly inhibited by IRS-1 serine phosphorylation (224); (iv) competition for the IRS-1 – T-antigen binding by a dominant negative mutant of IRS-1 (PH/PTB domain), inhibited growth and survival of JC T-antigen –transformed cells in anchorage-independent culture conditions (224). These multiple finding suggest that not only IGF-IR, but also its major signaling partner, IRS-1, can be considered as a potential therapeutic target especially in those medulloblastoma cases which express viral oncoprotein, JC virus T-antigen.

5.3. IGF-IR in DNA repair and genomic integrity

During transformation, an increase in the cell’s propensity to accumulate mutations may develop as a result of dysregulation of normal mechanisms controlling faithful and unfaithful DNA repair. Scattered reports indicate that the IGF-IR may have functions affecting DNA repair. These include enhanced radioresistance proportional to the IGF-IR protein level in both mouse embryo fibroblasts and breast tumor cells (234), enhanced DNA repair via the IGF-
I activated p38 signaling pathway in response to UV-mediated DNA damage (235, 236), and delayed UVB-induced apoptosis via IGF-I-mediated activation of Akt, resulting in enhanced repair of DNA cyclobutane thymidine dimers in keratinocytes (237). In contrast, one report notes a delay in DNA repair of potentially lethal radiation damage observed following IGF-I and insulin treatments of A549 cells (238).

Another demonstration of a potential involvement of the IGF-IR in DNA repair has been provided by experimental studies focused on the effects of IGF-I stimulation on DNA repair of double-strand breaks (DSBs). DSBs are usually formed after cell exposure to ionizing radiation, endogenous free radicals, anticancer drugs including cisplatin and mitomycin C, and can be inflicted spontaneously during DNA replication, when replication forks encounter other DNA lesions, including single strand breaks and intra-strand cross-links (239-242). Importantly, even a small number of DSBs can initiate a strong pro-apoptotic signal when damaged DNA is left unrepaired. Therefore, in addition to anti-apoptotic pathways, cell survival relies also on the efficiency of DNA repair (243). Early events in the detection of DSBs include activation of protein kinases ATM (ataxia telangiectasia mutated), ATR (ATM -related), and DNA-PK, which all have been shown to phosphorylate histone H2AX (γH2AX) within mega-base pair regions surrounding DSBs, “attracting” different components of the DNA repair machinery (244, 245). To prevent DNA damage-induced apoptosis, DNA breaks must be repaired. In cells replicating DNA, homologous recombination DNA repair (HRR) seems to predominate, while quiescent cells utilize non-homologous end joining NHEJ (240). The choice between DNA repair mechanisms can be controlled, at least partially, by the availability of DNA template. Cells that proliferate have an advantage of using newly synthesized template supplied in the process of DNA synthesis. Cells arrested in G1 could potentially utilize HRR; however the homology search is much more complicated and requires the access to the template on the homologous chromosome. Alternatively, quiescent cells may simply link DNA ends without any template using DNA binding Ku70/Ku80 complex and DNA-PK, followed by DNA ligation with XRCC4-ligase 4 (246, 247). This quick fix, however, may cost the cell a gain or loss of several base pairs. Therefore DNA repair by NHEJ can be considered as an error prone mechanism, which may lead to the accumulation of spontaneous mutations after DNA damage (240-242).

The major enzymatic component of HRR in eukaryotic cells is Rad51. Rad51 is a structural and functional homologue of bacterial RecA recombinase (248-250). Following detection of DSBs, the breast cancer susceptibility gene product (BRCA2) is suspected to mediate translocation of Rad51 to the sites of damaged DNA (251, 252). In parallel, the ATM -activated 5’-3’ endonuclease complex (Rad50 - MRE11 - NBS1) exposes both 3’ ends of DNA at the break (253, 254). The ends are initially protected by the ssDNA binding protein RPA,
IGF-IR in neuroprotection and brain tumors

which is subsequently replaced by Rad51 in a process that involves the initial binding of Rad52 into the ssDNA – RPA complex (255, 256). As a result, newly formed Rad51 nucleoprotein filaments are directly involved in homology search and strand invasion, which in eukaryotic cells require ATPase activity from Rad54 (248, 257, 258).

Our recent studies indicate that activated IGF-IR enhances HRR, by a mechanism that controls translocation of Rad51 to the sites of damaged DNA (nuclear foci) (1). This effect involves a direct cytosolic interaction between Rad51 and IRS-1. The binding is direct, occurs within the perinuclear region of the cell, involves the N-terminal portion of IRS-1, and was attenuated by IGF-I–mediated IRS-1 tyrosine phosphorylation. Importantly, cells with low levels of the IGF-IR, or cells expressing IGF-IR mutants that fail to phosphorylate IRS-1, retain Rad51 within the perinuclear compartment, and show significantly less DNA repair by HRR (1). These results indicate a novel function for the IGF-IR/IRS-1 signaling axis, which involves intracellular trafficking of Rad51 from cytosol to the sites of damaged DNA - a crucial step in the process of DNA repair by homologous recombination.

5.4. Nuclear translocation of IRS-1 and cancer

IRS-1 has been considered for a long time as a typical cytosolic protein implicated in insulin receptor and IGF-I receptor signaling (259, 260). Beside its metabolic and growth promoting function, IRS-1 has been also suspected to play a role in malignant transformation, presumably by amplifying the IGF-IR signal. The first convincing evidence indicating transforming potential of IRS-1 was demonstrated in R-cells, which are mouse embryo fibroblasts from mice with targeted disruption of the IRS-1 gene. The R- cells, which are resistant to SV40 T-antigen – mediated transformation (261), acquired transformed phenotype following co-transfection with IRS-1 and T-antigen containing expression vectors (261, 262). In a similar manner, T-antigen from human polyomavirus JC (JCV T-antigen) also required the presence of IGF-IR/IRS-1 signaling axis for transformation (199). Interestingly, we have found nuclear IRS-1 in cells expressing JCV T-antigen (224), and in JCV T-antigen positive medulloblastoma clinical samples (199, 216, 224 and Figure 5). This first demonstration of nuclear IRS-1 was further confirmed by others. For instance, nuclear IRS-1 have been found in cells transfected with v-src, SV40 T-antigen, in fibroblasts stimulated with IGF-I (263, 264), in hepatocytes (265), in 32D cells (266), and in osteoblasts (267). In other reports, nuclear IRS-1 was detected in breast cancer cells in association with estrogen receptor alpha (ERα) (268, 269). Although the mechanism by which IRS-1 is translocated to the nucleus is not well established, it could utilize its own putative nuclear localization signal (263). On the other hand, abundant presence of nuclear IRS-1 in cells expressing proteins with high affinity to the nucleus, such as JCV T-antigen (224), SV40 T-antigen (264), and ERα (268) may indicate that IRS-1 requires association with other proteins for its efficient nuclear translocation. Biological relevance of nuclear IRS-1 has been studied quite extensively during last several years. In fibroblasts stimulated with IGF-I, nuclear IRS-1 has been found in association with upstream binding factor (UBF1 - regulator of RNA polymerase I), which coincided with increased rRNA synthesis (270). In addition, a direct connection between IRS-1 and homologous recombination dependent DNA repair (HRR) have been recently proposed (1). This new signaling interplay involves peri-nuclear binding between hypo-phosphorylated IRS-1 and the major enzymatic component of HRR, Rad51. IGF-I–mediated IRS-1 tyrosine phosphorylation attenuated IRS-1 binding to Rad51 allowing efficient translocation of Rad51 to the sites of damaged DNA, which lead to the increased contribution of HRR in DNA repair of double strand breaks. In JCV T-antigen positive cell however, IRS-1 translocates to the nucleus, where it has been found in the complex with Rad51 at the sites of damaged DNA (271, 272). Importantly, the presence of nuclear IRS-1-Rad51 complexes was strongly associated with the inhibition of HRR and with the increased incidence of spontaneous mutations in cells expressing JCV T-antigen (271).

6. ACKNOWLEDGMENTS

We would like to acknowledge Jessica Otte for her technical assistance. This work was supported by grants from NIH: RO1CA095518 to KR, RO1 MH71162 to FP, and PO1 NS36466 to KK, LDV, and KR.

7. REFERENCES


IGF-IR in neuroprotection and brain tumors


IGF-IR in neuroprotection and brain tumors


IGF-IR in neuroprotection and brain tumors


IGF-IR in neuroprotection and brain tumors


IGF-IR in neuroprotection and brain tumors


IGF-IR in neuroprotection and brain tumors


IGF-IR in neuroprotection and brain tumors


IGF-IR in neuroprotection and brain tumors


IGF-IR in neuroprotection and brain tumors


235. Heron-Milhavet, L., M. Karas, C. M. Goldsmith, B. J. Baum & D. LeRoith: Insulin-like growth factor-I (IGF-I) receptor activation rescues UV-damaged cells through a


IGF-IR in neuroprotection and brain tumors


**Key Words**: IGF-IR, Neuroprotection, Brain Tumors, Review

**Send correspondence to**: Krzysztof Reiss, Temple University School of Medicine, Department of Neuroscience, Center for Neurovirology, Biology Life Sciences Bldg. Room 230, 1900 North 12th Street, Philadelphia, PA 19122, Tel: 215-204-6345; Fax: 215-204-0679; E-mail: kreiss@temple.edu