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Nuclear IRS-1 and Cancer

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Abstract

The family of insulin receptor substrates (IRS) consists of four proteins (IRS-1 - IRS-4), which were initially characterized as typical cytosolic adaptor proteins involved in insulin receptor (IR) and insulin-like growth factor I receptor (IGF-IR) signaling. The first cloned and characterized member of the IRS family, IRS-1, has predicted molecular weight of 132 kDa, however, as a result of its extensive serine phosphorylation it separates on a SDS gel as a band of approximately 160–185 kDa. In addition to its metabolic and growth-promoting functions, IRS-1 is also suspected to play a role in malignant transformation. The mechanism by which IRS-1 supports tumor growth is not fully understood, and the argument that IRS-1 merely amplifies the signal from the IGF-1R and/or IR requires further investigation. Almost a decade ago, we reported the presence of nuclear IRS-1 in medulloblastoma clinical samples, which express viral oncoprotein, large T-antigen of human polyomavirus JC (JCV T-antigen). This first demonstration of nuclear IRS-1 was confirmed in several other laboratories. The nuclear IRS-1 was also detected by cells expressing the SV40 T-antigen, v-Src, in immortalized fibroblasts stimulated with IGF-I, in hepatocytes, 32D cells, and in an osteosarcoma cell line. More recently, nuclear IRS-1 was detected in breast cancer cells in association with estrogen receptor alpha (ERα), and in JC virus negative medulloblastoma cells expressing ERβ, further implicating nuclear IRS-1 in cellular transformation. Here, we discuss how nuclear IRS-1 acting on DNA repair fidelity, transcriptional activity, and cell growth can support tumor development and progression.

IRS-1 Structure and Function

IRS-1 was long considered an example of a typical cytosolic molecule involved in signal transduction from two prominent membrane tyrosine kinases, insulin receptor (IR) and insulin-like growth factor I receptor (IGF-IR) (Myers et al., 1993). The molecular structure of IRS-1 has been well characterized revealing two conserved regions within N-terminal portion of the protein (Myers and White, 1996) and (Fig.1). The first one is designated PH due to its similarity to a pleckstrin homology (PH) domain; and the second, PTB, shows similarities to a putative phosphotyrosine-binding (PTB) domain present in Shc and other proteins (Sun et al., 1992). The PH domain contains a positively charged binding pocket that mediates interaction with phospholipids and with proteins containing an acidic motif (Burks et al., 1997; Burks et al., 1998; Myers et al., 1995). The PTB domain has the ability of recognizing phosphorylated tyrosine residues within NPXY motives, providing a mechanism of coupling IRS-1 with Tyr950 or Tyr960 in the juxtamembrane region of the IGF-IR and IR, respectively (Craparo et al., 1995; Eck et al., 1996; Wolf et al., 1995). Over

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20 tyrosine phosphorylation sites on the IRS-1 docking molecule can recruit proteins equipped with src-homology (SH2) domain, and several enzymes and adapter proteins have been confirmed as partners in IRS-1 mediated signaling cascade (Fig.1). These include Grb-2 (Myers et al., 1994; Valverde et al., 2001), PI-3 kinase (Myers et al., 1992), SHP2 phosphatase (Myers et al., 1996), Fyn (Sun et al., 1996), Nck (Lee et al., 1993), and Crk (Beitner-Johnson et al., 1996). Other proteins such as polyomavirus large T-antigens (Fei et al., 1995), 14-3-3 (Ogihara et al., 1997), integrins (Reiss et al., 2001; Vuori and Ruoslahti, 1994; Wang et al., 2007), and estrogen receptors (Morelli et al., 2004; Sisci et al., 2007a; Surmacz and Bartucci, 2004; Urbanska et al., 2009) that may contribute to malignant transformation, associate with IRS-1 through interaction/s that seem to be independent from IRS-1 tyrosine phosphorylation. Interestingly, the binding and cytoplasmic retention of the DNA repair protein, Rad51, by hypo-phosphorylated IRS-1 was inhibited in cells stimulated with IGF-I, strongly implicating the IGF-IR/IRS-1 signaling axis in homologous recombination directed DNA repair (Gualco et al., 2009b; Reiss et al., 2006; Trojanek et al., 2006b; Trojanek et al., 2003).

Another important aspect of IRS-1 regulation is its phosphorylation of serine residues, which in contrast to IRS-1 tyrosine phosphorylation is thought to inactivate some of the most relevant functions of this docking molecule (Myers and White, 1996; Sun et al., 1992), including TNFα–mediated development of insulin resistance (Hotamisligil et al., 1996; Peraldi et al., 1996). The best characterized serine residues of IRS-1 are: Ser307 (murine)/ Ser312 (humans), which become phosphorylated as a result of PI3K, PKC, JNK and TNFα activation; Ser612, phosphorylated by MAPK; the cluster of serines Ser632, 662, and 731, which upon phosphorylation by Akt/mTOR pathway may lead to IRS-1 degradation; and Ser789 which following AMPK mediated phosphorylation, may result in enhanced association of IRS-1 with IR during exercise and starvation (Schmitz-Peiffer and Whitehead, 2003; Shaw, 2006; White, 2002). Interestingly, elevated serine phosphorylation of IRS-1 has been found to facilitate its interaction with integrins (Reiss et al., 2001; Wang et al., 2006; Wang et al., 2007), however, the same serine phosphorylation strongly inhibited the binding between IRS-1 and JCV T-antigen (Lassak et al., 2002).

Nuclear Localization of IRS-1

Although the mechanism by which IRS-1 translocates to the nucleus is not fully understood, IRS-1 could employ its own putative nuclear localization signals (NLS) (Fig.1). One such a possibility was discussed in the report describing nuclear translocation of IRS-1 following ectopic expression of IGF-IR and IRS-1 in 32D cells, which resulted in IL-3 independence and tumor formation by these mouse hematopoietic cells (Prisco et al., 2002). One putative NLS site stretches between amino acids 14–28 within the PH domain of the IRS-1, where 8 out of 15 amino acids are basic (Prisco et al., 2002), and the other (KKWRHK) proposed in the original paper of Keller *et al.* describing isolation of mouse IRS-1 cDNA, also resides within the PH domain (Keller et al., 1993). The presence of these sequences may explain translocation of IRS-1 to the nucleus following IGF-1-induced activation of IGF-IR (Tu et al., 2002), or insulin/IGF-2 - induced activation of IR, but only isoform A (IR-A) (Wu et al., 2003). Interestingly, IR-A lacks 12 amino acids from the C-terminus and is expressed preferentially in fetal and transformed tissues, where it is suspected to activate growth-promoting signals *via* IGF-2-mediated activation (Lawrence et al., 2007).

In addition to the described putative NLS sequences, PH domains of IRS-1 and IRS-2 interact with a nuclear acidic protein, nucleolin (Burks et al., 1998), which could imply that chaperon proteins are involved in the nuclear translocation of IRS proteins. Indeed, detection of nuclear IRS-1 in cells expressing JCV T-antigen (Lassak et al., 2002), SV40 T-antigen (Prisco et al., 2002), ERα (Morelli et al., 2004) and ERβ (Urbanska et al., 2009)

indicates that IRS-1 may require other proteins equipped with NLS for its effective shuttling to the nucleus. The first characterization of the interaction between IRS-1 and JCV T-antigen, using a GST pull-down assay and a collection of the overlapping IRS-1 truncation mutants, revealed that this viral oncoprotein binds to the stretch of amino acids 212–300 within the N-terminal fragment of IRS-1 (Lassak et al., 2002), which includes a portion of PTB domain, and represents an overlap between two N-terminal fragments of IRS-1, which both are capable of pulling down JCV T-antigen. In addition, the IRS-1-JCV T-antigen interaction is independent from IRS-1 tyrosine phosphorylation and is strongly inhibited by IRS-1 serine phosphorylation (Lassak et al., 2002).

We have also identified that the region between amino acids 412–628 within the C-terminal portion of JCV T-antigen pulls down IRS-1 (Khalili et al., 2003). Although this region is far from the site where T-antigens interact with pRB proteins, it could overlap with the sites responsible for the p53 binding (Sullivan and Pipas, 2002). Therefore, in view of a potential role of nuclear IRS-1 in cellular transformation, it will be critically important to know if indeed IRS-1 interaction with T-antigens interferes with T-antigen –mediated p53 inactivation. Interestingly, Merkel cell polyomavirus, which is most firmly established as an etiological agent in Merkel cell carcinoma (Feng et al., 2008; Kassem et al., 2008; Viscidi and Shah, 2008), viral DNA is chromosomally integrated in the manner that T-antigen is disrupted. The resulting truncated T-antigen loses the ability to support viral replication, but it retains the ability of bind pRb (Harrison et al., 2011; Houben et al., 2011; Shuda et al., 2008). Further experiments are required to determine if such a truncated large T-antigen found in Merkel cell carcinoma can mediate IRS-1 nuclear translocation.

Two additional proteins with a strong nuclear affinity and with the ability of binding IRS-1 are estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ). The first indication of a possible functional interplay between IRS-1 and ERα was demonstrated in the course of *vitro* breast cancer studies, which provide initial evidence that estrogens could regulate IRS-1 expression and stability (Morelli et al., 2003; Oesterreich et al., 2001). More recently, a direct nuclear binding between IRS-1 and ERα has been demonstrated (Morelli et al., 2004) and characterized (Sisci et al., 2007a) in breast cancer cells. In addition, molecular evaluation of the interaction revealed that ERα has two putative binding sites on the IRS-1 molecule. The first is located within the first 200 amino acids spanning entire PH and part of PTB domain, and the second is located within the C-terminal portion of IRS-1 (Sisci et al., 2007a).

In medulloblastomas, which develop in the cerebellum, and are considered the most common intracranial tumors of the childhood (Reiss, 2002), nuclear IRS-1 was found first in association with JCV T-antigen (Lassak et al., 2002), and later with ERβ (Urbanska et al., 2009). Our recent studies demonstrate that ERβ-IRS-1 interaction involves exclusively the C-terminal domain of IRS-1 between amino acids 931 and 1233 (Urbanska et al., 2009). However, in contrast to ERα-IRS-1 complex, we did not observe ERβ binding to the N-terminal portion of IRS-1, which on the other hand, represents the prominent binding site for JCV T-antigen (Lassak et al., 2002).

Nuclear Localization of IRS-2 and IRS-3

IRS-3 is a shorter version of IRS-1, and is expressed in rodents but not in humans (Bjornholm et al., 2002). The nuclear presence of IRS-3 was demonstrated in the Takahashi lab (Kabuta et al., 2002) about the same time when nuclear IRS-1 was discovered (Lassak et al., 2002). The region of IRS-3 necessary for its nuclear localization was cloned between amino acids 192 and 223, the region that represents the fragment of the PTB domain that is unique for IRS-3 (Kabuta et al., 2002). In the same experimental setting the authors did not

observe nuclear IRS-1, IRS-2 or IRS-4 despite the fact that COS-7 cells used in this study are expected to express SV40 T-antigen. Recent analysis of nuclear IRS-3, by the same group, revealed that importin-β binds IRS-3, which was sufficient for nuclear translocation of the complex (Kabuta et al., 2008). Since IRS-1 and IRS-2 failed to bind importin-β, the IRS-3 - importin-β interaction seems to represent a unique mechanism of nuclear translocation within the IRS family.

IRS-2 can also translocate to the nucleus. IRS-1 was found in the nuclei of mouse embryo fibroblasts stimulated with IGF-I, however in contrast to IRS-1, IRS-2 translocation was not mediated by SV40 T-antigen, and required instead the presence of a functionally active IGF-IR (Sun et al., 2003). Inactivating mutations in the tyrosine kinase domain of the IGF-IR prevented nuclear translocation of IRS-2, which implicated tyrosine kinase activity of the IGF-IR in this process (Sun et al., 2003). In addition, nuclear IRS-2 has been shown to form nucleolar complexes with the upstream binding factor (UBF-1), a key regulator of RNA polymerase I activity involved in biosynthesis of ribosomal RNAs (Sun et al., 2003). In a different study, nuclear IRS-2 was found in association with NFκB in breast cancer cells, which supported the recruitment of IRS-2 to the cyclin D1 promoter (Wu et al., 2010). Interestingly, antisense against IRS-2 inhibited IGF-I-induced PI3K and NFkB activities and repressed proliferation of both BT20 (IRS-1 negative) and MCF-7 (IRS-1 positive) breast cancer cells (Wu et al., 2010). Since MCF-7 cells have been previously shown to possess nuclear IRS-1 in association with ERα, and nuclear IRS-1 also binds cyclin D1 promoter (Chen et al., 2005), further experiments are required to clarify a possible distinct role for nuclear IRS-1 and nuclear IRS-2 in this cellular model. In this respect, a different role for IRS-1 and IRS-2 has been already proposed by Nagle et al. (Nagle et al., 2004) who demonstrated that breast cancer cells which are IRS-1 negative but express IRS-2 are highly metastatic, however, their invasive potential is lost when IRS-2 is downregulated. In a different scenario, reintroduction of IRS-1 to IRS-1-negative prostate cancer cells, LNCaP, triggered cell aggregation and inhibited their invasiveness (Reiss et al., 2000; Reiss et al., 2001). Therefore, at least in breast and prostate cancer cells, the expression of IRS-2 seems to associate with higher, and IRS-1 with lower tumor cell invasiveness. Further studies are required to determine if nuclear translocation of IRS-1 and/or IRS-2 can contribute to the process of malignant transformation in general, and to tumor invasiveness in particular.

Nuclear IRS-1 and DNA Repair

Nuclear IRS-1 was initially detected in association with polyomavirus JC (Lassak et al., 2002; Reiss et al., 2006) and SV40 T-antigens (Tu et al., 2002), which are viral oncoproteins known to trigger abnormal cell proliferation and cause genomic instability. Polyomaviruses, including human JCV and BKV, and their simian counterpart, SV40, are small non-enveloped viruses with a single copy of double-stranded DNA. Their oncogenic potential is closely associated with the activation of so-called “early genome” of the virus, which forces infected cells to re-enter the S phase of the cell cycle, providing cellular DNA replication machinery for viral replication. The early genome of SV40 and JCV transcribes a common precursor RNA, which is differentially spliced yielding several viral products among which large tumor antigen (T-antigen), and small tumor antigen (t-antigen), predominate (Corallini et al., 1987; Khalili et al., 1999; Khalili and Stoner, 2001; Reiss and Khalili, 2003; Reiss Krzysztof, 2010; Wang et al., 2004; White et al., 2005). Polyomaviruses infect humans, monkeys, rodents, and birds with a restricted host and tissue specificity, and the infection of cells in which the virus does not fully replicate may lead to a partial expression of the viral genome (Imperiale, 2000; Imperiale, 2001). When SV40 or JCV T-antigen is expressed in the cells, it binds and inactivates two major negative regulators of the cell cycle, p53 and pRb (Kao et al., 1993; Krynska et al., 1997; Saenz-Robles et al., 2001). Although these canonical interactions with host proteins initiate DNA replication, they do not explain why

cells expressing T-antigen are often characterized by genomic instability and undergo malignant transformation. Multiple studies have already demonstrated chromosomal instability with no consistent patterns and with many new karyotypes emerging at each consecutive passage of the T-antigen expressing cells (Hunter and Gurney, 1994; Kappler et al., 1999; Ramel et al., 1995; Ricciardiello et al., 2003; Woods et al., 1994). A large variety of chromosomal defects suggest that T-antigens may affect stability of the genome at a very basic level. For instance, one explanation could be unfaithful DNA repair of double strand breaks (DSBs) in cells that are actively replicating DNA. To ensure uninterrupted DNA replication and to avoid apoptosis, at least one of the two prominent DNA repair mechanisms: homologous recombination directed DNA repair (HRR) or non-homologous end joining (NHEJ), has to be activated (Hoeijmakers, 2001).

We have demonstrated that cells expressing JCV T-antigen are characterized by impaired HRR, which resulted in the accumulation of mutations in cells replicating DNA (Trojanek et al., 2006a; Trojanek et al., 2006b). In this process, JCV T-antigen did not interact directly with the HRR complex, but instead it utilized IRS-1 (Fig.2). Following T-antigen-mediated nuclear translocation (Lassak et al., 2002), IRS-1 has been found in complex with Rad51, which is the main enzymatic component of HRR (Thacker, 1999). Importantly, this T-antigen-induced inhibition of HRR did not function in cells lacking IRS-1, and was reproduced in the absence of T-antigen by the mutant IRS-1 equipped with an artificial nuclear localization signal. As a result of this interaction between nuclear IRS-1 and Rad51 HRR was significantly repressed, however, enzymatically-induced DNA strand breaks were still repaired most likely by NHEJ (Trojanek et al., 2006a). This compensatory action of NHEJ was however associated with the accumulation of spontaneous mutations detected at the sites of damaged DNA (Trojanek et al., 2006a) (Fig.2). Other examples of T-antigen-mediated interference with DNA repair include SV40 T-antigen interference with MRE11 nuclear foci formation (Digweed et al., 2002), and T-antigen binding to another DNA repair protein, Nbs1, which forms an early DNA repair complex with MRE11 and Rad50 (Wu et al., 2004). In these studies however the involvement of nuclear IRS-1 was not evaluated.

Recently, we have reported the presence of nuclear IRS-1 in medulloblastoma cells negative for JCV T-antigen in which nuclear IRS-1 was found in complex with ERβ (Urbanska et al., 2009) and (Fig.3). Following cisplatin-induced DNA damage, nuclear IRS-1 localized at the sites of damaged DNA where it interacted again with Rad51. In medulloblastoma cells, engineered to express reporter plasmid for homologous recombination (Pierce et al., 1999; Trojanek et al., 2003), the ER antagonist, ICI 182,780, and an IRS-1 mutant (931–1233) lacking the ERβ binding site, both decreased the content of nuclear IRS-1 and stimulated DNA repair by homologous recombination (Urbanska et al., 2009). These encouraging results brought however one unexpected side effect, which should be seriously considered in view of estrogen receptor-related anticancer strategies. Although our further experiments with ICI182,780 confirmed the expected decrease in the accumulation of nuclear IRS-1, the accompanied increase in DNA repair by HRR resulted in the development of cisplatin resistance in medulloblastoma cells. This could be clinically relevant since the use of ICI182,780 has already been suggested as a supplementary treatment for medulloblastoma (Belcher et al., 2009).

Nuclear IRS-1 and Cell Growth

The involvement of the IGF-IR-IRS-1 signaling axis in controlling cell size has been well documented in a wide variety of organisms including Drosophila (Bohni et al., 1999) and mice (Pete et al., 1999), and was confirmed in cell culture (Valentinis et al., 2000). For example, transgenic mice knockouts for the IGF-IR gene (Baker et al., 1993; Gualco et al., 2009a), or IRS-1 knockouts (Araki et al., 1994) are characterized by severe growth

retardation. In a similar manner, deletion of a Drosophila homolog of IRS-1, a protein referred as Chico, decreased size of the fly about 50% by affecting both cell size and cell number (Bohni et al., 1999). Conversely, targeted disruption of the IGF-2 receptor (IGF-2R), which is thought to sequester IGF-2 during fetal development, increased overall IGF-2 availability, which led to over-stimulation of the IGF-IR/IRS-1 signaling axis, and resulted in the development of abnormally large embryos (Ludwig et al., 1996). Despite of these multiple examples, we still do not fully understand how IGF-I or insulin-activated IRS proteins contribute to the increase in size. One explanation has been provided by the observation that nuclear/nucleolar IRS-1 and IRS-2 interact with the upstream binding factor 1 (UBF-1), which is a key regulator of RNA polymerase-I involved in biosynthesis of ribosomal RNA (rRNA) (Drakas et al., 2004; Tu et al., 2002). There are several important observations, which emerged from this finding: (i) IGF-1 stimulation induces phosphorylation of the C-terminal portion of UBF, and the presence of IRS-1 increases markedly this phosphorylation; (ii) antibody against IRS-1 precipitates UBF-1 exclusively from the nuclear fraction; (iii) both IRS-1 and IRS-2 are capable of binding UBF-1 (ref); (iv) nuclear translocation of IRS-1 in 32D cells correlated well with a marked increase in rRNA synthesis; (v) in the nucleus, IRS-1 co-precipitated with PI3-K; and (vi) IRS-1 bound PI-3K directly phosphorylated UBF-1. Collectively, these data strongly indicate that the IGF-I-mediated determination of cell size depends, at least partially, on the activation of PI3-K by nuclear IRS-1, which results in the phosphorylation-dependent activation of UBF-1 and subsequent activation of rRNA synthesis. Since the increase in cell size is also important during cell proliferation when cells must double in size between the G1 and G2 phase of the cell cycle, this could represent a new function for nuclear IRS-1 in supporting abnormal cell proliferation.

Nuclear IRS-1 and Gene Expression

Although a direct interaction between nuclear IRS-1 and double stranded DNA has not been reported, several recent publications suggest that nuclear IRS-1 could participate in modulating transcriptional activity of genes involved in cell growth and cell proliferation (Chen et al., 2005; Wu et al., 2008). The first study suggesting nuclear IRS-1 as a transcriptional modulator demonstrated its' interaction with the upstream binding factor 1 (UBF-1), a key regulator of RNA polymerase-I involved in biosynthesis of rRNA (Tu et al., 2002). Importantly, the IRS-1/UBF-1 complex localizes preferentially in the nucleolus, which is known to contain multiple tandem copies of the ribosomal DNA (rDNA). Importantly, the UBF-1/IRS-1 nucleolar complex activated the expression from rDNA promoters, which resulted in elevated rRNA biosynthesis, leading to overall increase in protein synthesis (Chen et al., 2005; Drakas et al., 2004; Wu et al., 2005). Another example of nuclear IRS-1 working as a transcriptional modulator is its cytosolic and nuclear binding to β-catenin (Chen et al., 2005). The IRS-1/β-catenin complex has been found to interact with the c-myc and cyclin D1 promoters, and the binding was associated with elevated transcriptional activity from these two growth-control genes (Chen et al., 2005). IRS-1 has been also found to form complexes with ERα (Morelli et al., 2004) and with androgen receptor (AR) (Lanzino et al., 2009). Both complexes translocate to the nucleus, and have been shown to interact with the promoter regions containing ER and AR responsive elements, respectively. In case of ERα the binding of IRS-1 was associated with a significant decrease in transcriptional activity (Morelli et al., 2004), and the IRS-1/AR complex was found to be stimulatory (Lanzino et al., 2009). Collectively, these findings suggest a new role for nuclear IRS-1 in IGF-IR/IRS-1 signaling axis, which in addition to its canonical signaling effects on cell growth and cell proliferation, propose nuclear IRS-1 as a transcriptional modulator. In particular, the findings that nuclear IRS-1 can function as AR and ER transcriptional modulator could indicate its involvement in the development and progression of breast and prostate cancer.

Nuclear IRS-1 in Cancer Clinical Samples

Nuclear IRS-1 was detected for the first time in archival clinical samples of medulloblastoma (Lassak et al., 2002). The original study of 17 medulloblastoma biopsies revealed the presence of IRS-1 in the cytoplasm of 6 cases, while another 6 cases showed both cytosolic and prominently nuclear IRS-1. Interestingly, all cases of cytoplasmic IRS-1 were negative for T-antigen expression, while all cases in which IRS-1 was located in the nucleus were T-antigen positive. Furthermore, T-antigen and IRS-1 seem to colocalize the nuclei of tumor cells (Lassak et al., 2002). Subsequent immunohistochemical studies performed on an additional 20 cases of medulloblastoma, corroborated the nuclear localization of IRS-1 in 3 T-antigen positive cases, however 6 samples exhibiting nuclear IRS-1 were T-antigen negative. In these 8 samples, the nuclear IRS-1 was found in complex with another nuclear protein, estrogen receptor beta (ERβ), which was later confirmed *in vitro* (Urbanska et al., 2009) and (Fig. 3). Since nuclear IRS-1 was detected in classic, neuroblastic, and desmoplastic medulloblastomas, which differ in respect to their invasiveness and behavior (Kim et al., 2011; Rossi et al., 2008), we were not able to assign any prognostic values associated with nuclear IRS-1, ERβ or with JCV T-antigen (Lassak et al., 2002; Urbanska et al., 2009). In addition, to medulloblastoma clinical samples our preliminary observations from Glioblastomas also indicate the presence of nuclear IRS- in association with JCV T-antigen and/or with ERβ, (Fig. 4). However, more intensive studies are required to determine if nuclear IRS-1 could have any prognostic or diagnostic values for these malignant Glial tumors.

In contrast to observations made with medulloblastoma, nuclear IRS-1 detected in breast cancer biopsies correlated well with more differentiated and less metastatic phenotype (Sisci et al., 2007b). In the breast cancer cells and in biopsies nuclear IRS-1 was found in association with ERα (Morelli et al., 2003; Morelli et al., 2004; Sisci et al., 2007a; Sisci et al., 2007b). Nuclear IRS-1 was detected in 1.6% of control normal mammary epithelium and in 20% of benign tumors. In ductal carcinoma, both nuclear IRS-1 and ERα negatively correlated with tumor grade, size, mitotic index and lymph node involvement (Sisci et al., 2007b). Recently, nuclear IRS-1 has been shown to be a good predictor for tamoxifen-response in patients with early breast cancer (Migliaccio et al., 2010). In this study, tissue array from over thousand patients diagnosed with stage 1 and 2 breast cancer reviled positive correlation between nuclear IRS-1 and ERα, and between nuclear IRS-1 and progesterone receptor, and nuclear IRS-1 *per se* showed negative correlation with lymph node involvement. On the other hand, cytosolic IRS-1 did not correlate with ERα, but showed positive correlation with tumor size and S-phase fraction. Importantly, tamoxifen-treated patients with the tumor cells showing nuclear IRS-1 had both better recurrence–free survival and overall survival (Migliaccio et al., 2010).

Conclusions

In this review, we have presented the experimental work from multiple laboratories, which indicate that IRS proteins, in addition to their canonical function as cytosolic signal transduction molecules, can be shuttled to the nucleus, and that nuclear presence of IRS-1, may contribute to the process of malignant transformation. As illustrated in Fig.5, several nuclear proteins have been implicated in the process of IRS-1 translocation. They include: JC virus T-antigen; SV40 T-antigen; v-Src; Estrogen receptor α; and Estrogen receptor β. A different mechanism has been also proposed, which involves nuclear acidic protein, nucleolin, ant its' binding to the PH domain of IRS-1 and IRS-2. Also within the PH-domain of IRS-1 there are two putative nuclear localization signals (NLS), which possibly contributed to nuclear translocation of IRS-1 in cells stimulated with IGF-I. Finally, nuclear translocation of IRS-3 was mediated by importin-β, which has been shown binding to the unique sequence found exclusively on the PTB domain of IRS-3.

In the nucleus, IRS-1 affects several basic control mechanisms, which when dysregulated, may support malignant transformation (Fig.5). In particular, nuclear IRS-1 was found to inhibited homologous recombination directed DNA repair (HRR) *via* its direct binding with Rad51, which resulted in the accumulation of spontaneous mutations. Another potential cancer related function of nuclear IRS-1 is its' association with UBF1, which activated ribosomal RNA biosynthesis, stimulated overall protein synthesis, and contributed to the increase in cell size during G1 – G2/M progression. Also by modulating gene transcription, nuclear IRS-1 stimulated cyclin D1 and c-myc promoter activities, in this case however nuclear IRS-1 was found in complex with β-catenin. Collectively, presented data indicate that nuclear IRS-1, by limiting DNA repair fidelity and by forcing cell growth and DNA replication, could link this signaling molecule to malignant transformation.

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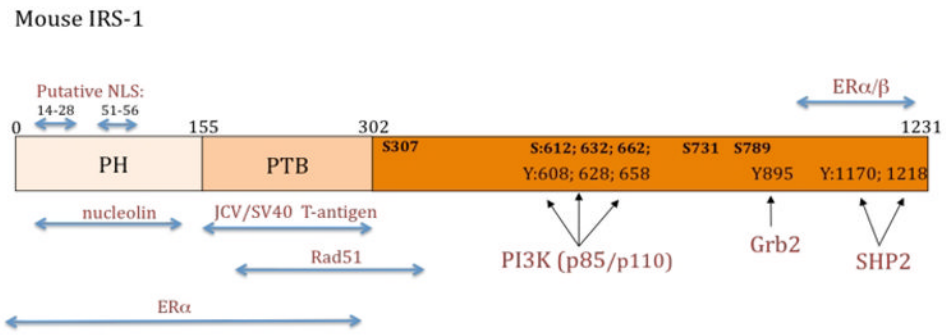

Figure 1. Schematic diagram of mouse IRS-1 protein

There are two major functional domains within the N-terminus portion of IRS-1: pleckstrin homology domain (PH), spanning between amino acids 0–155, and phosphotyrozine binding domain (PTB) located between amino acids 155–302. Black arrows indicate exact binding sites for PI3 kinase, Grab2 and SHP2 at indicated tyrosine residues (Y). Functionally relevant serine residues (S) and their corresponding amino acid positions are also indicated. Blue arrows indicate putative binding regions for proteins, which are suspected to translocate IRS-1 to the nucleus, including nucleolin, polyomavirus T-antigens (JCV and SV40), estrogen receptor α (ERα) and estrogen receptor β (ERβ). Other indicated sites include the binding between IRS-1 and DNA repair protein, Rad51, and positions of putative nuclear localization signals (NLS).

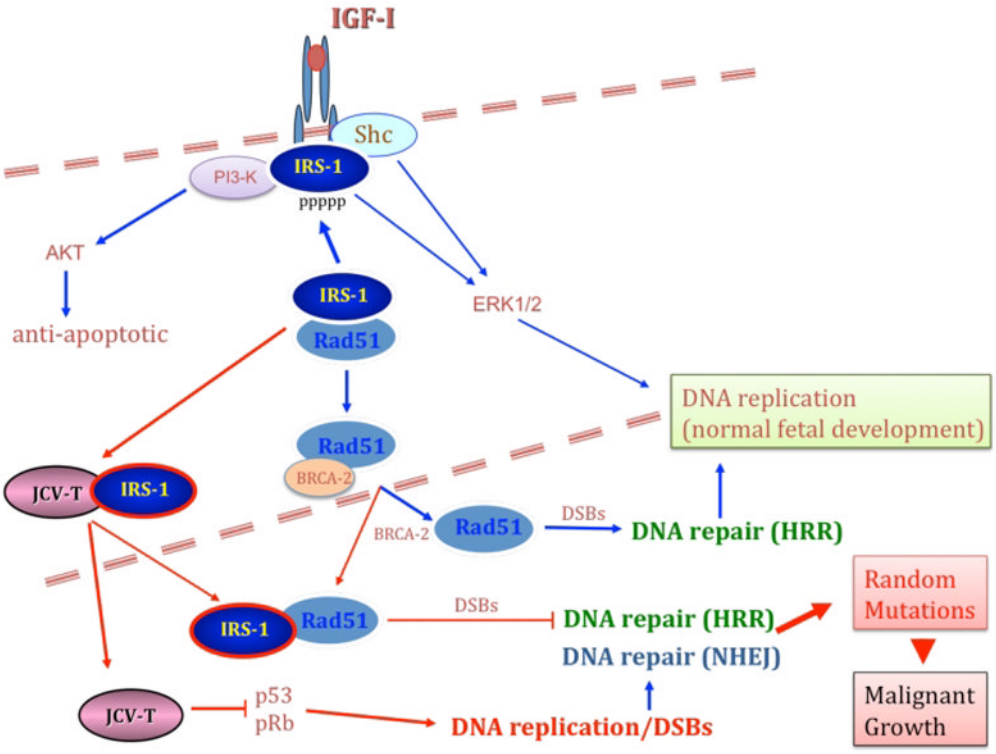

Figure 2. The IGF-IR-IRS-1-JCV T-antigen signaling interplay: effects on cell proliferation cell survival and DNA repair fidelity

Here we propose a sequence of events in which ligand activated IGF-IR triggers multiple signaling responses leading to synchronized activation of cell proliferation (SHC or IRS-1-mediated activation of Ras-MAP kinase pathways); protection from apoptosis (IRS-1 induced activation of Akt); and DNA repair by homologous recombination. The IGF-I-mediated phosphorylation of IRS-1 seems to play a critical role in this model. In the absence of IGF-I a fraction of hypo-phosphorylated IRS-1 accumulates in the perinuclear region in complex with Rad51 (Trojanek et al., 2003). Following IGF-I stimulation, activated IGF-IR phosphorylates IRS-1 on multiple tyrosine residues, decreasing the affinity of IRS-1 to Rad51, and engages IRS-1 in multiple signaling events supporting IGF-I-induced cell proliferation and cell survival (Reiss et al., 1998; Trojanek et al., 2006b; Trojanek et al., 2003). If at the same time DNA double strands (DSBs) are formed (either naturally or by genotoxic treatment), the cell can repair them in a faithful manner, by Rad51-supported homologous recombination directed DNA repair (HRR), or less faithfully, by non-homologous end joining (NHEJ). In the presence of JCV T-antigen cells can proliferation because of p53, pRb inactivation. In parallel, JCV T-antigen translocates IRS-1 to the nucleus (Lassak et al., 2002), thus creating a condition in which IRS-1 can bind Rad51 in the subcellular compartment in which Rad51 is expected to support HRR. Therefore, if JCV T-antigen expressing cells experience extensive DNA damage, the resulting DNA double strand breaks (DSBs) can either trigger apoptosis, or if NHEJ will compensate for the impaired HRR, spontaneous mutations can accumulate in the surviving cells. These mutations when accumulate may provide the cells with growth and survival advantage, which could result in the selection of clone/s initiating tumor development and progression.

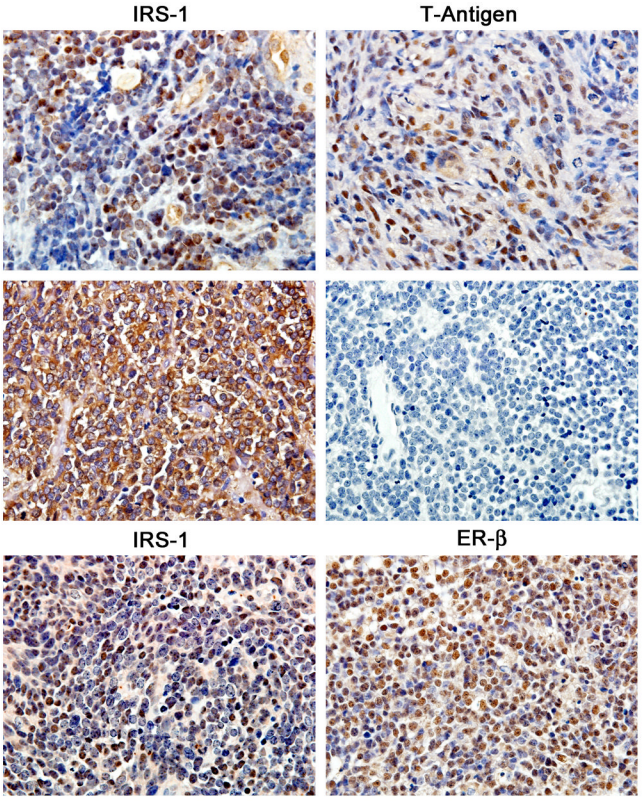

Figure 3. Nuclear translocation of IRS-1 in Medulloblastomas

Immunohistochemistry for the IGF-IR/IR docking molecule, IRS-1, shows a prominent nuclear localization in clinical samples of medulloblastomas that express the JCV early oncoprotein, large T-antigen (upper panels). In T-antigen negative samples, IRS-1 remains in the cytoplasm of neoplastic cells (middle panels). In some tumors that lack T-antigen expression IRS-1 is still located to the nucleus in association with Estrogen Receptor beta (lower panels). Original magnification for all panels 600x.

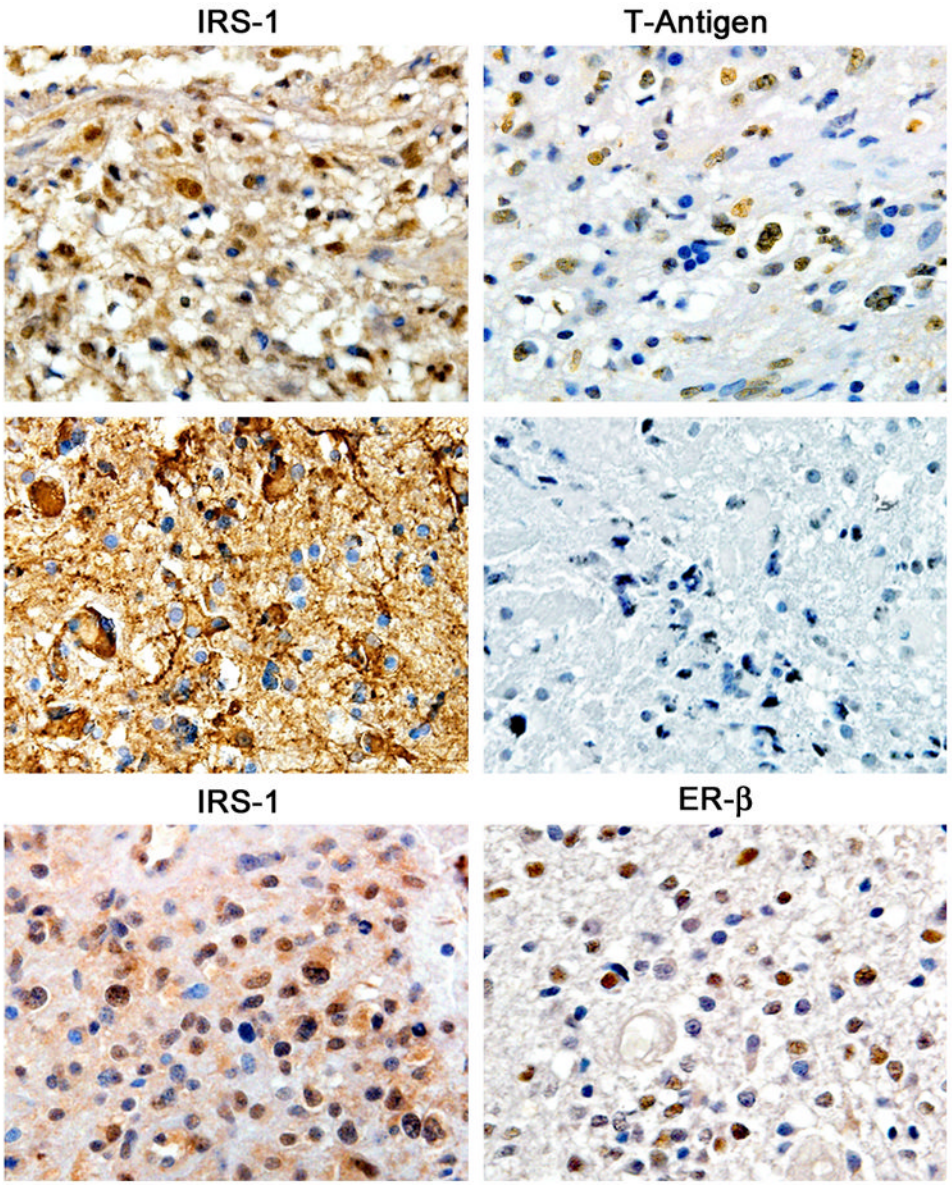

Figure 4. Nuclear translocation of IRS-1 in Glioblastomas

In a similar patter of expression, IRS-1 is detected by immunohistochemistry in the cytoplasm and nuclei of neoplastic cells in cases of Glioblastoma multiforme that are positive for JCV T-Antigen (upper panels). However, tumors that lack T-antigen demonstrate exclusive cytoplasmic IRS-1 (middle panels). Finally, IRS-1 can be translocated to the nucleus in the presence of Estrogen Receptor beta (lower panels). All panels original magnification 600x.

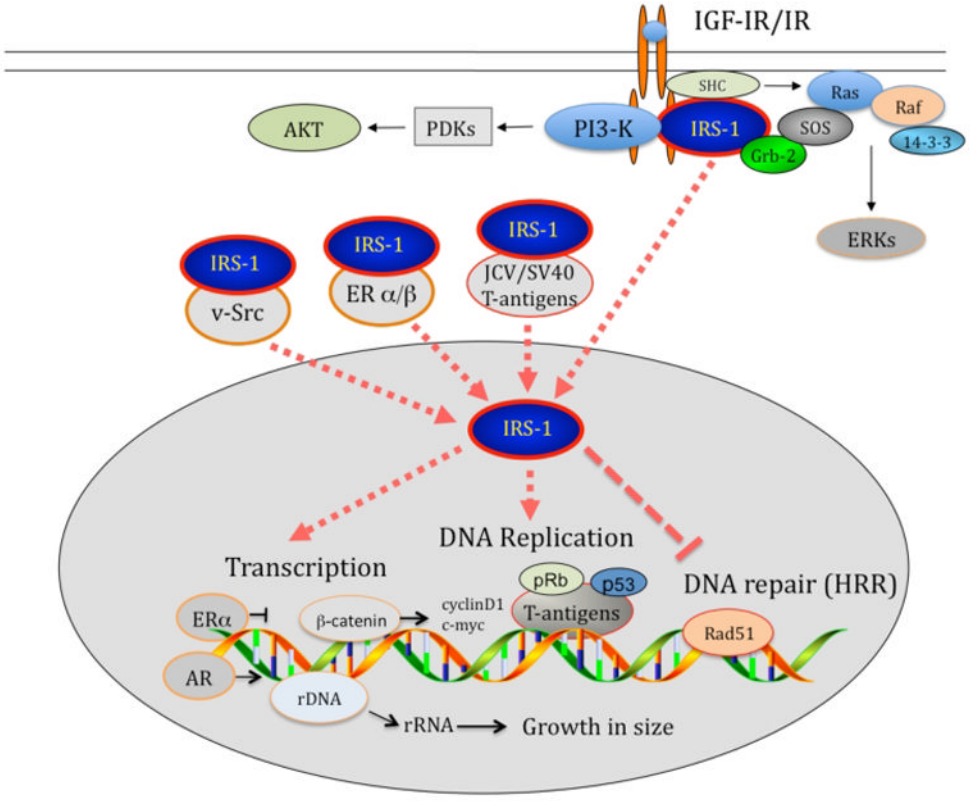

Figure 5.
Schematic illustration summarizing mechanisms involved in IRS-1 nuclear translocation and basic cellular processes affected by nuclear IRS-1.