Minireview: Nuclear Insulin and Insulin-like Growth Factor-1 Receptors: A Novel Paradigm in Signal Transduction

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The specificity of the insulin receptor (InsR) and insulin-like growth factor-1 receptor (IGF1R) signaling pathways has been the focus of significant debate over the past few years. Recent evidence showing nuclear import and a direct transcriptional role for both InsR and IGF1R adds a new layer of complexity to this dialog. Hence, in addition to the classical roles associated with cell-surface receptors (eg, ligand binding, autophosphorylation of the tyrosine kinase domain, activation of insulin receptor substrate 1 (IRS-1) and additional substrates, protein-protein interactions with membrane and cytoplasm components), new data are consistent with nuclear (genomic) role(s) for both InsR and IGF1R. The present review provides a brief overview of the physical and functional similarities and differences between InsR and IGF1R and describes data from a number of laboratories providing evidence for a new layer of signaling regulation (ie, the ability of InsR and IGF1R to translocate to the cell nucleus and to elicit genomic activities usually associated with transcription factors). The ability of InsR and IGF1R to function as transcription factors, although poorly understood, constitutes a new paradigm in signal transduction. Although research on the role of nuclear InsR/IGF1R is still in its infancy, we believe that this rapidly developing area may have a major basic and translational impact on the fields of metabolism, diabetes, and cancer. **(*Endocrinology* 154: 1672–1679, 2013)**

The insulin/insulin-like growth factors (IGFs) constitute a network of ligands, cell-surface receptors, and binding proteins involved in the regulation of multiple physiological and pathological processes. Insulin/IGFs play key developmental and metabolic roles at every stage of life, from early ontogeny until old age. Although the insulin receptor (InsR) and IGF-1 receptor (IGF1R) share the majority of their downstream cytoplasmic mediators, most experimental and clinical evidence is consistent with the notion that InsR activation (mainly by insulin) leads primarily to metabolic activities, whereas IGF1R activation (mainly by IGF-1 or IGF-2) leads to proliferative and differentiative events. These views have been challenged in recent years due, in part, to a number of scientific and technological breakthroughs, including the availability of animal models with organ-specific disruptions of particular ligands or receptors (1). The complexity of the insulin/IGF-1 axis, along with a striking overlap in the spectrum of activities elicited by InsR and IGF1R, make these analyses extremely difficult, however.

InsR and IGF1R belong to a family of transmembrane tyrosine kinase–containing receptors. In their mature form, they present as heterotetramers composed of 2 extracellular α-subunits and 2 transmembrane β-subunits (2). Both receptors show a high degree of homology (84% in the tyrosine kinase domain, 45%–65% in the ligand binding domain, and above 50% in overall amino acid sequence). In addition, the receptors display a remarkable similarity in genomic organization. Thus, the 12 exons (out of 21) of the *IGF1R* gene are identical in size with the homologous exons of *InsR*, the main difference being that the *IGF1R* gene does not contain an equivalent of the

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Abbreviations: EGFR, epidermal growth factor receptor; ER, estrogen receptor; FGFR, fibroblast growth factor receptor; IGF1R, IGF-1 receptor; InsR, insulin receptor; IRS, insulin receptor substrate; SUMO, small ubiquitin-like modifier; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

alternatively spliced exon 11 of the *InsR* gene. This splicing event leads to the generation of 2 InsR isoforms, InsR-A and Ins-B, which lack or contain, respectively, exon 11 (3). These isoforms are differentially expressed during development, with InsR-A predominantly expressed in fetal tissues and InsR-B predominately expressed in adult tissues, particularly liver, muscle, and adipocytes (3, 4). The IGF1R displays an opposite pattern of expression, being absent in liver and present at low levels in adipose tissue and at high levels in brain (5). In addition, and consistent with its potent antiapoptotic, prosurvival role, the IGF1R is overexpressed in most tumors and malignant cells.

InsR-mediated and IGF1R-mediated Signal Transduction

Ligand binding induces conformational changes in the structures of the InsR and IGF1R and activates their intrinsic tyrosine kinase activity. In terms of ligand specificity, insulin and the IGFs bind with high affinity to their specific receptor and with lower affinity to the noncognate receptor, with the exception of IGF-2, which also binds InsR-A with high affinity (4). A number of recent articles provide an updated picture of the InsR/IGF1R-mediated signaling events (6, 7). A cardinal (*and* still unanswered) question has been how activation of InsR or IGF1R leads to distinct (sometimes opposing) biological events, despite the fact that both receptors share the majority of their downstream cytoplasmatic mediators. As mentioned above, InsR is a vital mediator of metabolic responses, whereas IGF1R is primarily involved in mitogenesis, differentiation, and antiapoptotic activities. Although some of the variation can be attributed to different hormone-receptor affinities or divergent tissue distribution or subcellular localization, variation can also be explained by differences in the internalization of the receptors, or structural differences in the β-subunit, specifically in the C-terminus, which may lead to specific activation of particular substrates and signaling pathways.

Finally, there is evidence for the existence of hybrid receptors (InsR-IGF1R), composed of InsR and IGF1R hemireceptors, in some tissues (8, 9). A cross-talk between insulin, IGFs, and their receptors appears to be a relatively common event in many organs and systems. Hence, the role of InsR in mitogenesis and cell motility may provide the foundation for its involvement in cancer development and progression (4). On the other hand, IGF-1 exhibits important metabolic effects. For example, in vivo infusion of recombinant IGF-1 leads to an acute decrease in circulating glucose values (10).

Regulation of *InsR* and *IGF1R* Gene Expression

Control of *InsR* and *IGF1R* gene expression is mainly attained at the level of transcription (4, 5, 11). Similarly to the protein structures, the regulatory regions of both genes display a large degree of homology, containing TATA-less, GC-rich, "initiator" type of promoters. Comprehensive analyses have established that transcription of the *IGF1R* promoter is dependent on a number of stimulatory zinc-finger proteins, including Sp1 and Krüppel-like factors (eg, KLF6) (12). In addition, the promoter was identified as a downstream target for tumor suppressor action, and multiple antioncogenes (eg, p53, breast cancer gene-1, and von-Hippel Lindau) were shown to inhibit *IGF1R* transcription (13–15). DNA affinity chromatography linked to mass spectroscopy analysis led recently to the identification of the entire collection of *IGF1R* promoter-binding proteins in estrogen receptor (ER)-positive and ER-negative breast cancer cells (12). Interactions between stimulatory and inhibitory transcription factors play an important role in *IGF1R* regulation and, consequently, were postulated to have a major impact on the proliferative status of the cell (11, 16). Finally, a number of transcription factors involved in *InsR* gene regulation have been identified, including nuclear proteins HT-FIR, IRNF-I, IRNF-II, Sp1, and HMGA1 (4).

Early Evidence for Nuclear Localization of InsR and IGF1R

Initial evidence for insulin binding to nuclei was provided by Goldfine and Smith in 1976, who demonstrated rapid and reversible binding of labeled insulin to purified nuclei from rat liver (17). A series of subsequent articles by Goldfine and associates identified the nuclear envelope as the major site of insulin binding in nuclei and established that intracellular binding sites are immunologically distinct from those on the plasma membrane (18, 19). Similar observations were reported by Horvat (20) and Bergeron et al (21) in 1978, showing specific binding of insulin and growth hormone in Golgi fractions isolated from liver.

In 1987, Podlecki et al demonstrated that InsR translocates to the nucleus after internalization (22). Later, in 1996, immunofluorescence analyses conducted by Chen et al revealed that IGF1R is also present in the nucleus and that stilbene estrogen doubled nuclear levels of IGF1R (23). In 2003, Sun et al reported that insulin receptor substrate 1 (IRS)-1, IRS-2, and IRS-3 translocate to the nucleus and nucleoli of several types of cells following induction by an activated IGF1R or certain oncogenes (24).

Nuclear translocation of IRS-2 was seen only in cells expressing a wild-type IGF1R. Furthermore, an intact IGF1R tyrosine kinase domain constitutes an essential requisite for nuclear translocation of both IRS-1 and IRS-2, as shown by the fact that mutations in the IGF1R tyrosine kinase domain abrogated IRS translocation (24). Finally, additional cytoplasmatic mediators involved in InsR/IGF1R signal transduction, including PI3K, AKT, and MAPKs, were also shown to translocate to the nucleus (25).

Nuclear Translocation of InsR and IGF1R: Potential Mechanisms

Although accumulating evidence indicates that intact, or proteolytically cleaved, fragments of InsR (26) and IGF1R (26–29) translocate to the nucleus, the mechanism(s) responsible for nuclear import remains virtually unknown. InsR and IGF1R are present in the perinuclear and nucleolar area of the nucleus in a small ubiquitin-like modifier (SUMO)-ylated form. Receptor SUMOylation takes place in a ligand-dependent fashion and seems to be a crucial requisite for nuclear translocation (27, 29). Of interest, SUMOylation sites on lysine residues within the tyrosine kinase domain are conserved among a variety of homologs from different species. Mutagenesis of these sites arrested nuclear translocation and gene activation. The theoretical possibility that IGF1R may directly shuttle to the nucleus from the endoplasmic reticulum/Golgi apparatus (ie, *not* from the cell surface) was discarded by careful analyses conducted by Deng et al (29). Furthermore, studies confirmed that ligand-mediated InsR and IGF1R phosphorylation are essential for nuclear trafficking.

Proteosomal, lysosomal, and endocytic pathways, which are mainly involved in InsR/IGF1R degradation, are also operative in nuclear translocation of the receptors (30). In this context, use of the clathrin-dependent endocytosis inhibitor *dansylcadaverine* abrogated IGF1R nuclear import (28). A study by Sjostrom et al (31) revealed that RanBP2, a SUMO E3 ligase located at the nuclear pore complex, binds IGF1R, and that expression of RanBP2 increases IGF1R SUMOylation and nuclear IGF1R. Importin-β, an important player in nuclear translocation, was also shown to coimmunoprecipitate with IGF1R. Finally, use of an in situ proximity ligase assay established that IGF1R colocalizes with α-tubulin, providing support to the hypothesis that the receptor is translocated to nucleus along microtubules (31).

IGF1R Displays Specific DNA-binding Capacity

Electrophoretic mobility shift assays using a series of randomly synthesized biotin-labeled oligonucleotides, in combination with an IGF1R antibody in supershift assays, allowed Sehat et al (27) to establish that IGF1R physically interacts with double-stranded DNA. The capacity of IGF1R to interact, either directly or indirectly, with DNA was investigated at a genome-wide level by means of chromatin immunoprecipitation-seq assays. The majority (~80%) of IGF1R-enriched regions were found to be intergenic (ie, distal from any annotated gene), whereas ~6% of these regions were located in introns and ~6% were located in exons. Hence, data are consistent with the notion that IGF1R may bind to enhancer regions and function as a transcriptional activator. So far, no studies have documented the specific genomic DNA sequences bound by nuclear InsR.

Do InsR and IGF1R Function as Transcription Factors?

The finding that IGF1R is actively translocated to the nucleus, where it binds putative enhancer sites in gDNA in a specific manner and drives transcription of target genes (27), prompted investigators to explore in depth the putative roles of InsR and IGF1R as nonclassical transcription factors. A recent study has shown that nuclear IGF1R binds transcription factor LEF-1, a key regulator of the Wnt signaling cascade, and acts as a coactivator of LEF-1/TCF target genes (32). In addition, results of DNA affinity chromatography from our laboratory have demonstrated that transcription factor LEF-1/TCF binds to the *IGF1R* promoter region (12). Combined, these results point to a complex bidirectional regulatory loop whereby transcription factor IGF1R is capable of controlling Wnt cascade action, whereas, in parallel, LEF-1/TCF modulates *IGF1R* gene expression (Figure 1). Finally, nuclear IGF1R was shown to bind a LEF-1 binding site in the *cyclin D1* promoter region, a key player in cell-cycle progression (32). The biological significance of this intricate regulatory network is still unclear.

Transcription Factors InsR and IGF1R Bind to and Regulate *IGF1R* Promoter DNA

The ability of nuclear InsR/IGF1R to govern *IGF1R* gene transcription was recently investigated in cultured breast cancer cells expressing or lacking the ER. Although

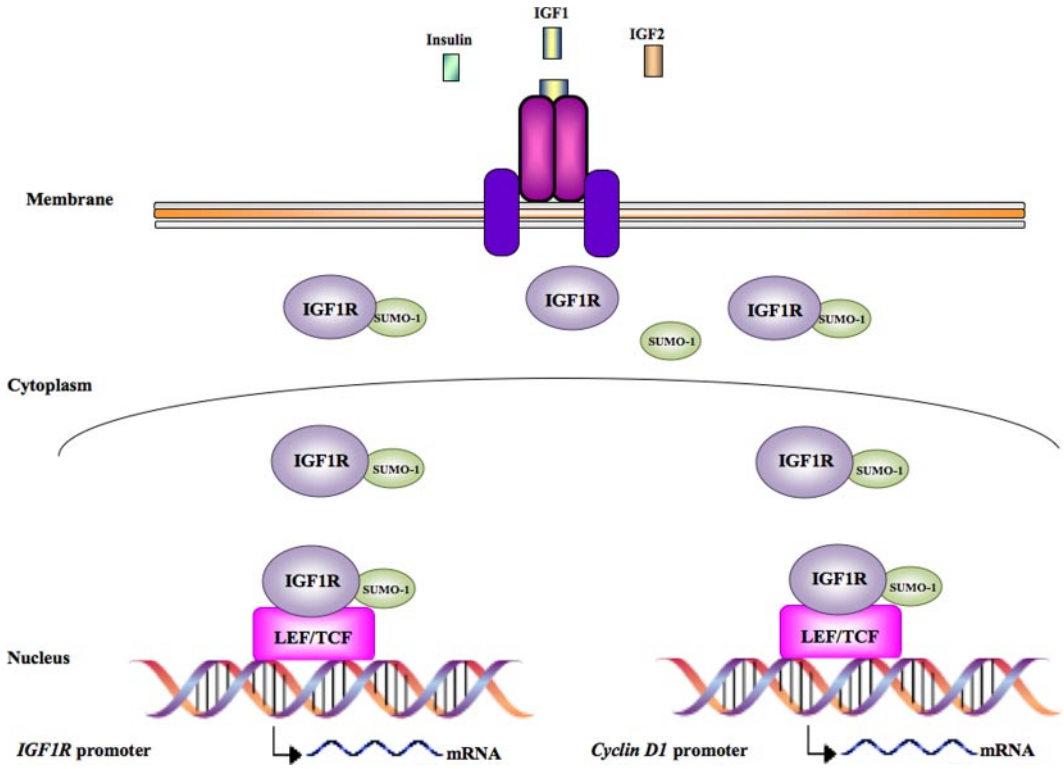

Figure 1. Schematic diagram of the transcriptional role of nuclear IGF1R. The emerging picture suggests that, following ligand binding and IGF1R phosphorylation at the cell-surface level, the receptor is translocated from the cytoplasm to the nucleus in a SUMO-dependent manner. Three lysine residues have been identified in the intracellular domain of the IGF1R that are responsible for its specific interaction with SUMO. The SUMOylated IGF1R binds to a LEF/TCF binding site in the *cyclin D1* (and additional) promoters, with ensuing target gene activation. Nuclear IGF1R is also able to autoregulate expression of its own gene, perhaps following association with LEF-TCF, which has been identified as an *IGF1R* promoter-binding protein, leading to an increase in *IGF1R* promoter activity and IGF1R biosynthesis.

InsR and IGF1R translocate to the nucleus of *both* ER-positive and ER-depleted cells, nuclear receptors were shown to bind to the *IGF1R* promoter *only* in ER-negative cells (26). The inability of nuclear IGF1R to bind to its cognate promoter in ER-positive cells stems from the fact that a series of GC boxes (Sp1 binding sites) in the proximal promoter are occupied by ERα, hence preventing or diminishing IGF1R binding. Of interest, transcription factors InsR and IGF1R display diametrically opposite activities in the context of *IGF1R* regulation. Thus, whereas nuclear IGF1R stimulates *IGF1R* gene expression, nuclear InsR inhibits *IGF1R* promoter activity in both ER-positive and ER-depleted cells. This novel mechanism of *IGF1R* autoregulation is described in more detail in Figure 2.

Is Nuclear Translocation a Common Event Among Tyrosine Kinase Receptors?

Several tyrosine kinase–containing receptors, including members of the epidermal growth factor (EGFR), ErbB, fibroblast growth factor (FGFR), MET, vascular endothelial growth factor (VEGF) (VEGFR1), and TrkA/nerve growth factor receptor families, were reported to travel to the nucleus and to function as transcription factors (33, 34). For example, nuclear EGFR was shown to promote DNA repair, replication, and radio-resistance and to act as a transcriptional coactivator in *cyclin D1* gene regulation (35). Transactivation of the *cyclin D1* promoter was also stimulated by an intact nuclear ErbB-1 receptor (36). Likewise, evidence was provided showing that EGFR and E2F1 associate at the *B-Myb* promoter to drive its expression during the G_1/S phase of the cell cycle (36). Nuclear HER2 has been established to associate with multiple genomic targets in vivo, including the cyclooxygenase enzyme (*COX-2*) gene promoter, and to stimulate transcription (34). Moreover, an ErbB-4 receptor fragment, in complex with TAB2 and N-CorR, translocates to the nucleus and acts as a transcriptional repressor of genes that promote the formation of astrocytes (33).

Additional evidence in support of the notion that nuclear translocation constitutes a common theme in tyrosine kinase receptor action was provided by studies showing that an intact VEGFR is present in the nucleus following addition of VEGF. Pigment epithelium-derived factor reduced VEGF-induced angiogenesis and the nuclear level of intact VEGFR (33). An additional example is

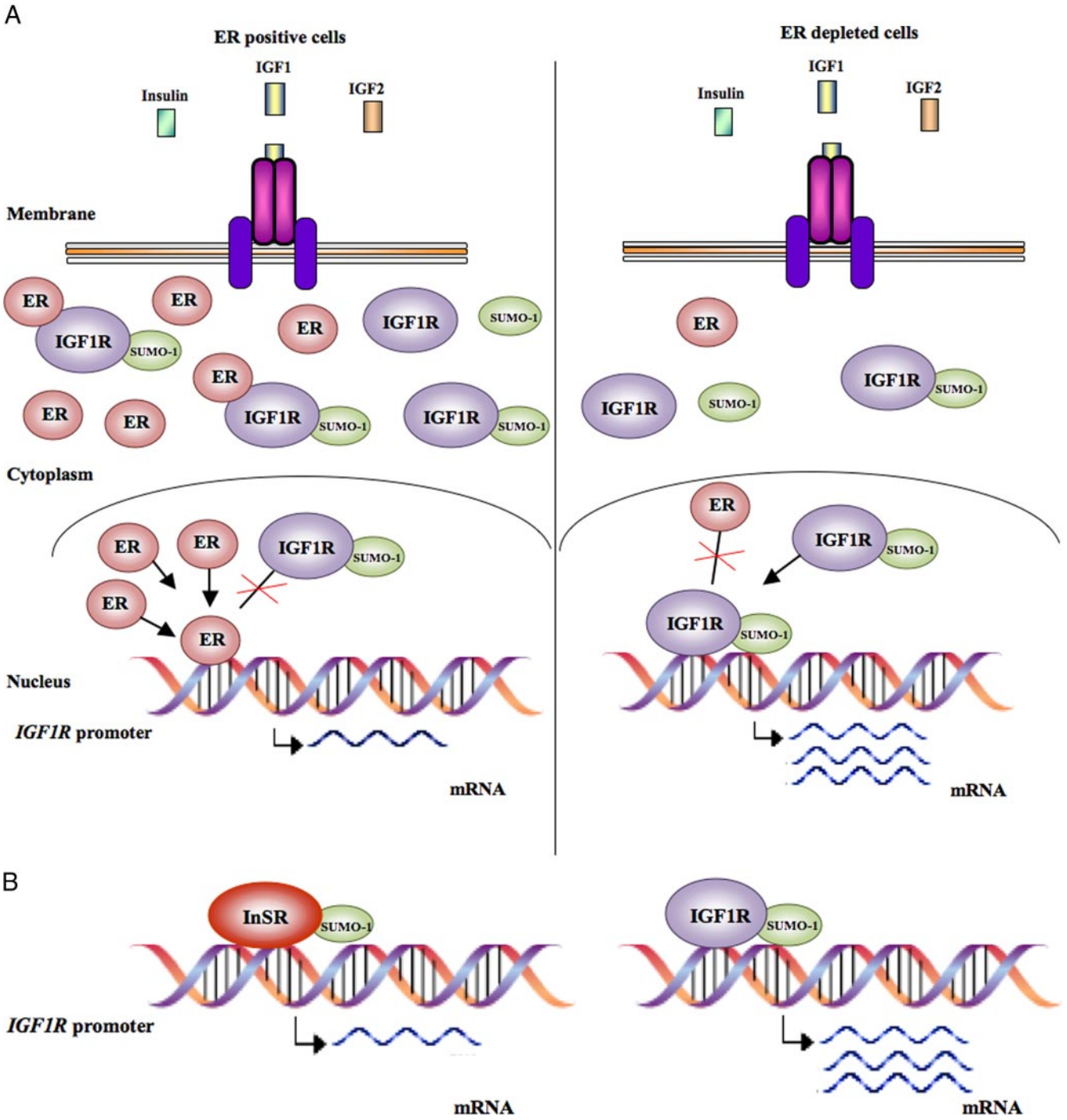

Figure 2. Proposed mechanism of *IGF1R* gene autoregulation by nuclear InsR/IGF1R in breast cancer cells. (A) Effect of ER status on the capacity of nuclear IGF1R to autoregulate *IGF1R* expression. EMSA, chromatin immunoprecipitation, and DNA affinity chromatography assays have identified the ERα as a potent *IGF1R* promoter-binding transcriptional activator. IGF1R levels are markedly reduced in ER-depleted, in comparison to ER-positive, cells. Although IGF1R translocates to nucleus in *both* ER-positive and ER-depleted breast cancer cells, nuclear SUMOylated IGF1R binds its cognate promoter *only* in ER-depleted cells. This differential binding is most probably due to the fact that GC-rich binding elements in the *IGF1R* promoter are occupied by ER in ER-positive cells, hence preventing nuclear IGF1R to associate with DNA. (B) Differential regulation of *IGF1R* gene expression by nuclear InsR and IGF1R. InsR, similarly to IGF1R, is able to translocate to the nucleus in a SUMO-dependent fashion and to bind to the *IGF1R* promoter. However, although IGF1R enhanced *IGF1R* promoter activity, InsR diminished *IGF1R* promoter activity. The clinical implications of the differential regulation of the *IGF1R* gene by transcription factors InsR and IGF1R are yet to be investigated.

provided by cell-surface receptor FGFR1, which was shown to form a complex with orphan nuclear receptor Nurr1 and to participate in postmitotic dopaminergic neuron development (37). Specifically, Nurr1 and FGFR1 bind to a common region in the tyrosine hydroxylase promoter (the rate-limiting enzyme in dopamine synthesis)

and enhance gene action. This nuclear partnership constitutes a new mechanism for tyrosine hydroxylase gene regulation in dopaminergic neurons. An additional case for tyrosine kinase receptor nuclear import was provided by Rakowicz-Szulczynska et al (38), who showed that the nerve growth factor-liganded TrkA/nerve growth factor was associated with chromatin. Finally, Matteucci et al (39) demonstrated that nuclear MET fragments function as transcription factors in invasive breast cancer. In summary, the examples described here provide convincing evidence that nuclear translocation constitutes a generalized phenomenon among tyrosine kinase receptors, and that nuclear import may impinge on a wide array of biological processes.

Basic and Clinical Implications of Nuclear InsR and IGF1R Localization

The capacity of transcription factors InsR and IGF1R to bind DNA in a sequence-specific manner and to control transcription of genes involved in cell-cycle progression, including autoregulation of the *IGF1R* gene, suggests that this novel mechanism of action may confer on these (and other) cell-surface receptors the ability to modulate growth and developmental events at an additional, wider level (ie, a genomic control level). In breast cancer cells, for example, nuclear InsR/IGF1R have been suggested to be involved in a switch in oncogene-induced transformation at advance stages of the disease. In addition, the finding that nuclear IGF1R complexes with LEF-1/TCF at the human *cyclin D1* promoter, leading to an increase in cyclin D1 levels, suggests that nuclear IGF1R may be responsible for aberrant cell-cycle progression, hence contributing to neoplastic transformation. The impact of nuclear InsR/IGF1R on the control of metabolic processes has not yet been exhaustively investigated.

Finally, the significance and implications of nuclear InsR/IGF1R in terms of prognosis and clinical correlates are still controversial. Aleksic et al (28) reported the presence of nuclear IGF1R in primary renal cancer cells, formalin-fixed tumors, preinvasive lesions of the breast, and rapidly proliferating nonmalignant tissues. In this study, nuclear IGF1R was associated with poor prognosis in renal cancer. Asmane et al (40) conducted an immunohistochemical analysis of nuclear IGF1R in patients with unresectable or metastatic soft tissue sarcomas, Ewing sarcoma, and osteosarcoma treated with monoclonal antibodies against IGF1R. In contrast to the previous study, exclusive intranuclear immunoreactivity for IGF1R (in comparison to cytoplasmic, or nuclear + cytoplasmic, localization) was significantly associated with a better progression-free survival and overall survival. Hence, these results suggest that nuclear IGF1R may serve as a prognostic biomarker for successful therapy. Finally, an IGF1R fragment was detected in nuclei of orbital fibroblasts derived from Graves disease, an autoimmune syndrome, but not in healthy fibroblasts. Nuclear translocation required an active ADAM17, a membrane-associated metalloprotease. This previously unrecognized behavior of IGF1R exclusively in Graves disease was suggested to play a role in the pathogenesis of this disorder (41). In summary, research on the role of nuclear InsR/IGF1R on cancer initiation and progression is expected to expand in the near future and may lead to new diagnostic and prognostic opportunities. In particular, it will be important to identify potential correlations with additional biomarkers, including ER and LEF-1/TCF. As mentioned above, understanding the complex interplay of these specific transcription factors with nuclear IGF1R will provide clues regarding the interactions between the Wnt and IGF1R pathways in cancer. The availability of relevant technologies, including imaging and genome-wide platforms, makes these analyses feasible.

Conclusions

Despite the fact that nuclear insulin binding was first described almost 40 years ago, the field remained out of the main focus for many years and was regarded by many researchers as a "curiosity." Nuclear insulin binding was considered a "nondogmatic" concept, leading certain investigators to cast doubts about the biological importance of these unquestionably different signaling pathways. It seems, in addition, that a number of technical issues might have confounded the interpretation of early data. As mentioned above, the availability of powerful analytic tools nowadays is expected to advance this area of investigation.

In summary, comprehensive biochemical, molecular, and morphological analyses provide unambiguous evidence that InsR and IGF1R translocate to the cell nucleus in different types of cells and tissues. Receptor SUMOylation seems to be critical for nuclear import, although it is not obvious that this modification is an essential prerequisite for the translocation process. Following nuclear import, InsR/IGF1R, directly or in association with other proteins, bind to promoters and enhancer elements at specific target genes and control expression in a tightly regulated fashion. In this sense, nuclear InsR and IGF1R fall within the functional definition of transcription factors, providing an additional layer of biological regulation at a genome-wide level. Finally, preliminary evidence from a

number of laboratories suggests that nuclear InsR/IGF1R constitute potential biomarkers for several types of cancer. Future studies will shed light on the biological and clinical relevance of this novel paradigm in signal transduction.

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