Insulin-like growth factor-binding protein-3 (IGFBP-3) induces apoptosis by its ability to bind insulin-like growth factors (IGFs) as well as its IGF-independent effects involving binding to other molecules including the retinoid X receptor-α (RXRα). Here we describe that in response to IGFBP-3, the RXRα binding partner nuclear receptor Nur77 rapidly undergoes translocation from the nucleus to the mitochondria, initiating an apoptotic cascade resulting in caspase activation within 6 h. This translocation is a type 1 IGF receptor-signaling independent event as IGFBP-3 induces Nur77 translocation in R-cells. IGFBP-3 and Nur77 are additive in inducing apoptosis. GFP-Nur77 transfection into RXRα wild-type and knockout mouse embryonic fibroblasts and subsequent treatment with IGFBP-3 show that RXRα is required for IGFBP-3-induced Nur77 translocation and apoptosis. Addition of IGFBP-3 to 22RV1 cell lysates enhanced the ability of GST-RXRα to “pull down” Nur77, and overexpression of IGFBP-3 enhanced the accumulation of mitochondrial RXRα. This unique nongenotropic nuclear pathway supports an emerging role for IGFBP-3 as a novel, multicompartmental signaling molecule involved in induction of apoptosis in malignant cells.

Over the past decade, multiple lines of investigation have validated insulin-like growth factor-binding protein-3 (IGFBP-3) as an inducer of cellular apoptosis, effects that can be unrelated to its IGF binding (1). Most importantly, several groups have now reported successful in vivo treatment of cancer models with IGFBP-3, either as a single agent or in combination with chemotherapeutic agents (2–4). However, the molecular mechanisms by which IGFBP-3 induces apoptosis remain largely unknown at present.

Several novel IGFBP-3 binding partners have been recently identified that may participate in its IGF-independent proapoptotic effects (1). We and others demonstrated that retinoid X receptor-α (RXRα) is a binding partner for IGFBP-3 (5, 6) and that RXRα is required for IGFBP-3 apoptotic effects (5). Indeed, IGFBP-3 potentiates RXRα-mediated signaling while inhibiting signaling via other RXRα heterodimeric partners (5–7). Our discovery of IGFBP-3 binding to RXRα suggested that its apoptotic effects might involve an RXRα-dependent transcriptional mechanism. Most published reports have evaluated IGFBP-3-induced apoptosis at 24–72 h (8–11), consistent with a transcriptional mechanism. However, we have recently described apoptosis activation by IGFBP-3 (as evidenced by caspase activation and histone associated DNA fragmentation ELISA) as early as 1–6 h after IGFBP-3 exposure, suggesting a mechanism that does not require de novo gene transcription (12, 13).

The orphan nuclear receptor Nur77 (also known as NGFI-B (14) and TR3 (15)) is a nuclear receptor transcription factor and is an important regulator of apoptosis in different cells (16). It is a member of the orphan steroid receptor family, which also includes Nor1 and Nurr1. This family is essential for apoptosis of self-reactive immature thymocytes following stimulation of the T-cell receptor (17, 18). In response to synthetic apoptotic stimuli, Nur77 translocates from the nucleus to the mitochondria to induce cytochrome c release and apoptosis in leukemia (19), lung (20), ovary (21), stomach (22), colon (23), and prostate cancer cells (24). Subcellular localization of Nur77 is important for its biologic function. In the nucleus, it functions as a transcription factor to mediate cell proliferation events. Targeted to the mitochondria, it takes on a novel role as a mediator of apoptosis, not unlike the role played at the mitochondria by another transcription factor, p53 (25). Most importantly, Nur77 can also heterodimerize with RXRα (26) and participate in its transcriptional activities (26–28). The mitogenic effect of Nur77 requires its DNA binding and transactivation functions in the nucleus, whereas both are dispensable for the apoptotic effects of Nur77 at the mitochondria (29).

Because the nuclear receptor RXRα is an intracellular binding partner for IGFBP-3, we hypothesized that IGFBP-3 would modify RXRα/Nur77 heterodimeric DNA binding, shifting this heterodimer from a DNA binding state to one that targets mitochondria. Mitochondrial translocation of RXRα/Nur77 would then result in the release of cytoplasmic cytochrome c, activation of intracellular caspases, and induction of apoptosis.

Here we report evidence that IGFBP-3 is a rapid biological signal molecule for RXRα/Nur77 translocation. Our results reveal a new interaction between the nuclear receptor and IGFBP-3 superfamilies and identify IGFBP-3 as a unique signal modulator of both traditional and novel nuclear receptor roles at the junction of cellular proliferation and apoptosis.
IGFBP-3 Translocates RXRα/Nur77

EXPERIMENTAL PROCEDURES

Materials—Celtrix (Mountain View, CA) provided recombinant human IGFBP-3. IGF-1 was a generous gift from Amersham Biosciences. The commercial antibodies used are as follows: anti-human IGFBP-3 from DSL (Webster, TX); anti-Nur77 from Geneka Biotechnology (Mountain View, CA); anti-RXRα from Santa Cruz Biotechnology (Santa Cruz, CA); anti-cytochrome c from Pharmingen; and anti-β-actin from Sigma. PMP70 Antibody and cathepsin S antibodies were from Zymed Laboratories Inc. (South San Francisco) and R&D System (Minneapolis, MN), respectively. For the Western immunoblot utilizing the R-MEFs, polyclonal rabbit anti-Nur77 antibody (Harlan Biosciences, Indianapolis, IN) was generated against two specific N-terminal peptides (Geneka Biotechnology, South San Francisco). Nur77 banding from the human Nur77 peptide sequence. Sera were purified on a protein A column (Amersham Biosciences) and verified by Western blotting. Nur77 banding pattern was confirmed using CCRF-CEM nuclear extract (Active Motif, Carlsbad, CA). SDS-PAGE reagents, TWEEN, and fat-free milk were purchased from Bio-Rad. ECL reagents were from Amersham Bio-science (Piscataway, NJ), and verification of Western blotting using anti-Nur77 antibody. The experiments were repeated three times.

Cell Culture—22RV1 cells, A172 cells, CCRF-CEM, and F9 embryonal carcinoma cells from ATCC (Manassas, VA), and F9 RXRα cells from Invitrogen. All other chemicals were from Sigma. Studies to provide evidence that the absorbance values generated by the neutralizing antibody IR3 (13). We therefore confirmed these rapid effects in a variety of cell lines. MEFs exhibited a 70% increase in apoptosis as evidenced by fluorometric assessment of caspase 3/7 activity (Fig. 1A) as early as 2 h post-treatment. This induction was maximal to nearly 2.5-fold over baseline at 6 h. Similarly, in the human glioblastoma cell line A172, an almost 2-fold increase in apoptosis was detected as early as 1 h (Fig. 1B). In the 22RV1 prostate cancer cell line (Fig. 1C), a significant 32% increase in caspase activation was induced by the addition of IGFBP-3. This subse-quently rose to a 40 and 51% increase over serum-free levels at 6 and 24 h, respectively. These results confirm that IGFBP-3 induction of apoptosis, assessed in multiple cell lines, is a rapid event. Experiments were repeated three times.

IGFBP-3 Induces Rapid Nucleomitochondrial Translocation of Nur77—To investigate whether Nur77 translocation could mediate the pro-apoptotic effects of IGFBP-3 in CaP cells, we first established that IGFBP-3 leads to nucleomitochondrial translocation of Nur77. We performed a time course of IGFBP-3 treatment and observed the subcellular localization of Nur77 by indirect immunofluorescence confocal microscopy in 22RV1 CaP cells. Within 15 min there was strong cytoplasmic appearance of red-staining Nur77 compared with minimal cytoplasmic labeling at time 0 (Fig. 2A). The faint nuclear staining seen at time 0 may reflect inaccessibility of anti-Nur77 antibody to the protein secondary to RXR heterodimerization or Nur77 homodimerization. The cytoplasmic Nur77 presence

RESULTS

IGFBP-3 Induces Rapid Induction of Apoptosis—We have observed previously that in A172 glioblastoma cells, IGFBP-3-induced caspase activation reaches its maximum at 1–6 h, after which it decreases (12). Likewise, in human macrovascular umbilical vein endothelial cells, the vascular endothelial growth factor-induced survival of the human umbilical vein endothelial cells is inhibited by IGFBP-3, via the induction of apoptosis in a type 1 IGF receptor-independent manner utilizing the neutralizing antibody aR3 (13). We therefore confirmed these rapid effects in a variety of cell lines. MEFs exhibited a 70% increase in apoptosis as evidenced by fluorometric assessment of caspase 3/7 activity (Fig. 1A) as early as 2 h post-treatment. This induction was maximal to nearly 2.5-fold over baseline at 6 h. Similarly, in the human glioblastoma cell line A172, an almost 2-fold increase in apoptosis was detected as early as 1 h (Fig. 1B). In the 22RV1 prostate cancer cell line (Fig. 1C), a significant 32% increase in caspase activation was induced by the addition of IGFBP-3. This subsequently rose to a 40 and 51% increase over serum-free levels at 6 and 24 h, respectively. These results confirm that IGFBP-3 induction of apoptosis, assessed in multiple cell lines, is a rapid event. Experiments were repeated three times.

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Subcellular Fractionation Procedures—Nu-CLEAR protein extraction kit™ was from Sigma. Subcellular fractions were isolated according to the manufacturer’s protocol. The Apoalert™ cell fractionation kit (Clontec) was used to isolate a mitochondrial fraction from the cytoplasm of cells. The purity of the fraction was assessed by immunoblot for PMP70 (peroxiosomal) and cathepsin S (lysosomal) contamination.

Transient Transfections—Cells (2 × 10^6) were seeded in 96-well culture plates. Reagents were appropriately scaled up to 6-well plates for transfections that were followed by mitochondrial isolation and subsequent Western immunoblotting. Transfections were done with Lipofectamine PLUS Reagent as directed by the manufacturer (Invitrogen). Typically, 50 ng of β-galactosidase expression vector (pSV-β-gal, Promega, Madison, WI) and 50 ng of expression vector containing IGFBP-3 and/or Nur77 were mixed with carrier DNA to give 0.2 μg of total DNA per well. After 24–48 h of transfection, caspase activity was quantitated and normalized for transfection efficiency to measurements of aliquots of co-transfected β-galactosidase gene activity (β-galactosidase enzyme assay system, Promega).

G2/M Pull-down—The GST-RXXα fusion vector encoding the full-length RXRα molecule and was the generous gift of Dr. D. J. Mangelsdorf and has been described previously (32). GST-RXXα fusion protein was produced in GST-RXXα-transformed Esherichia coli DH5 cells, which were lysed and loaded on glutathione-Sepharose 4B beads (Sigma). Ten μg of purified GST-RXXα bound to beads was incubated with 500 μg of cell lysate, with or without 200 ng of recombinant IGFBP-3 protein, and then separated by centrifugation. The bound proteins were analyzed by nonreducing SDS-PAGE followed by Western blotting using anti-Nur77 antibody. The experiments were repeated three times.

Densitometric and Statistical Analysis—Densitometric measurement of autoradiographs was performed by using computer-scanned densitometry. All experiments were repeated at least three times. Means ± S.D. are shown. Statistical analyses were performed using analysis of variance utilizing InStat (GraphPad, San Diego). Differences were considered statistically significant when p < 0.005, denoted by ** as shown in the figures.
Rapid Mitochondrial Translocation of Nur77 by IGFBP-3 Oc- 
curs via a Type 1 Receptor Independent Mechanism—The pos-
sibility that IGFBP-3 acts to induce apoptosis independently of IGFs and IGF receptors was investigated by testing the ability of IGFBP-3 to induce apoptosis in the IGF receptor-negative (R−) embryonic fibroblast cells derived from an IGF-1R knock-
out mouse (30). This effect was mediated in part by a type 1 IGF receptor independent mechanism as IGFBP-3 was still able to induce apoptosis, with a 32% increase over base line at 2 h, that was maximal at 6 h in type 1 IGF receptor-disrupted MEFs in a fragmented DNA/histone ELISA (Fig. 3A). These cells have been shown previously to neither bind nor respond to IGFs. IGFBP-3-induced mitochondrial translocation of Nur77 in this unique system was further demonstrated by immuno-
blotting analysis, which showed accumulation of Nur77 in the mitochondrial fraction (Fig. 3B). To demonstrate the purity of the mitochondrial fraction, expression of mitochondrial-specific protein Hsp60 and nuclear-specific protein poly(ADP-ribosyl) polymerase (PARP) is shown as well as immunoblots to PMP70 and cathepsin S to show purity of the mitochondrial fraction.

Additive Apoptotic Effects of Overexpression of IGFBP-3 and Nur77—To determine whether mitochondrial targeting of Nur77 by IGFBP-3 plays a role in regulating the release of cytochrome c from mitochondria into cytosol, the location of cytochrome c was examined during the course of IGFBP-3 treatment. Immunoblotting of a cytoplasmic fraction that was depleted of mitochondria showed that the addition of 1 μg/ml of IGFBP-3 caused a greater than 3-fold increase in the appearance of cytoplasmic cytochrome c at 30 min, which increased to almost 6-fold at 60 min and was sustained at 180 min (Fig. 4A).

The release of cytoplasmic cytochrome c is directly upstream of caspase activation in the mitochondrial pathway of apoptosis (33). Overexpression of Nur77 in thymocytes induces massive apoptosis (34). We co-expressed by transient transfection both IGFBP-3 and Nur77 in mammalian expression vectors to as-

FIG. 1. Rapid activation of apoptosis by IGFBP-3. A, time course apoptosis induction of mouse embryonic fibroblasts (MEFs) after treatment with 1 μg/ml of IGFBP-3. Apoptosis induction was quantitated by fluorometric measurement of activated caspase 3/7. Values are represented as percent of serum-free (SF). B, time course of human glioblastoma line A172 apoptosis induction after treatment with 1 μg/ml of IGFBP-3. Apoptosis induction was quantitated by fluorometric measurement of activated caspase 3/7. Values are represented as percent of serum-free. C, time course of human prostate cancer line 22RV1 apoptosis induction after treatment with 1 μg/ml of IGFBP-3. Apoptosis induction was quantitated by fluorometric measurement of activated caspase 3/7. Values are represented as percent of serum-free. **, p < 0.005 relative to serum-free conditions.

FIG. 2. IGFBP-3 induces nucleomi-
tochondrial translocation of Nur77. A, indirect immunofluorescent confocal microscopy of 22RV1 cells after treat-
manship with IGFBP-3. Nur77 is labeled in blue. Note rapid appearance of cytoplasmic Nur77. SFM, serum-free medium. B, Western immunoblot of subcellular fractions of 22RV1 prostate cancer cells after treat-
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trols and show purity of the nuclear frac-
tion. C, 22RV1 mitochondrial fraction iso-
lated after treatment with 1 μg /ml IGFBP-3. Membrane was probed with PARP, PMP70, and cathepsin S to show purity of the mitochondrial fraction.

Note that cytochrome c was readily detectable in the cytoplasmic fraction as it was in the nuclear fraction, indicating that Nur77 localization in the cytoplasm is an early event.

Additive Apoptotic Effects of Overexpression of IGFBP-3 and Nur77—To determine whether mitochondrial targeting of Nur77 by IGFBP-3 plays a role in regulating the release of cytochrome c from mitochondria into cytosol, the location of cytochrome c was examined during the course of IGFBP-3 treatment. Immunoblotting of a cytoplasmic fraction that was depleted of mitochondria showed that the addition of 1 μg/ml of IGFBP-3 caused a greater than 3-fold increase in the appearance of cytoplasmic cytochrome c at 30 min, which increased to almost 6-fold at 60 min and was sustained at 180 min (Fig. 4A).

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was maintained throughout this 2-h time course, although some Nur77 reappeared in a nuclear location by the end of 2 h.

To confirm that IGFBP-3 is a biologic Nur77 translocation signal, we also assessed relative Nur77 concentrations in nu-

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**Fig. 3. Rapid mitochondrial accumulation of Nur77 by IGFBP-3 occurs via a type 1 receptor independent mechanism.**

A, type 1 IGF receptor-disrupted mouse embryonic fibroblasts were treated with IGFBP-3 (1 μg/ml) for the indicated times, and apoptosis was measured in a fragmented DNA/histone ELISA. B, the mitochondrial fraction was analyzed for expression of Nur77 by Western blotting. We reported previously that the nuclear receptor RXR/α/H9251 cells are a nuclear binding partner for IGFBP-3 and is required for IGFBP-3-induced apoptosis (5). Also, in response to nerve growth factor, nuclear RXR/α/H11002/α/H9251 cells were treated with 1 μg/ml IGFBP-3 overnight, and nuclear distribution consistent with its function as a transcription factor at basal conditions (Fig. 5A). Treatment with 1 μg/ml of IGFBP-3 resulted in the rapid appearance of extranuclear GFP-Nur77 observed within 15 min. This effect was not seen in the sister RXR/α/H11002/α/H9251 line, as IGFBP-3 treatment had no effect on the translocation of nuclear GFP-Nur77. In addition, transfection of the Nur77 overexpression vector into F9 RXR/α/H11002/α/H9251 cells induced a marked increase in caspase activation, whereas transfection into the sister F9 RXR/α/H11002/α/H9251 line failed to induce any significant increase in caspase activity (Fig. 5C). Indeed, addition of 200 ng of IGFBP-3 to 500 μg of 22RV1 cell lysate demonstrated that IGFBP-3 enhanced the ability of GST-RXRα to “pull down” Nur77 (Fig. 5D), indicating that IGFBP-3 augments the ability of RXRα/Nur77 to physically associate. In fact, isolation of mitochondria from cells transfected with IGFBP-3 revealed a 3-fold increase in mitochondrial RXRα (Fig. 5E) that was not seen in cells transfected with control expression vector, demonstrating that IGFBP-3 leads to the co-export of RXRα and Nur77. Together, our results demonstrate that the mechanism of IGFBP-3-induced Nur77 translocation, like IGFBP-3-induced apoptosis, requires RXRα and involves co-migration of RXRα/Nur77 heterodimers to mitochondria.

**DISCUSSION**

Multiple lines of *in vitro*, *in vivo*, and clinical evidence point to IGFBP-3 as an anti-cancer molecule (37). *In vitro*, IGFBP-3 has been shown to induce apoptosis in a variety of cancer models via both IGF-dependent and IGF-independent mechanisms (1). *In vivo*, recent publications report efficacy either as a single agent or in chemotherapy combinations in non-small cell lung cancer (2) and colon cancer (3). IGFBP-3 gene expression is commonly lost in human cancer cell lines and xenografts as detected in DNA microarray analysis of cancerous cells compared with noncancerous cells (38). Decreased IGFBP-3 expression is associated with prostate cancer progression, demonstrating more frequent loss of expression in advanced disease, in both human (39) and mouse (40) models. In addition, recent evidence demonstrates that methylation of the IGFBP-3 promoter is one mechanism by which the silencing of IGFBP-3 expression in cancer cells is achieved (41).

Despite promising pre-clinical evidence using IGFBP-3 as a cancer therapy (2–4), controversy remains as to the complex role of IGFBP-3 in various tumors. IGFBP-3 modulates cellular proliferation with dual actions that either enhance IGFs or inhibit their actions as well as actions that are independent of its binding to IGFs (1). Evidence for this duality has been reported in renal cell (42, 43), lung (44, 45), breast, and other cancers (46). Most interestingly, an outcome prediction model for prostate cancer was established utilizing HoxC6 and IGFBP-3 expression, as IGFBP-3 was positively associated with Gleason score (47). However, recent expression profiling of HoxC6 small interfering RNA transfections and HoxC6 overexpression identified IGFBP-3 as a potential proapoptotic repression target of HoxC6 in prostate cancer (48). Clearly, more work needs to be done to examine the role of IGFBP-3 in cellular proliferation and apoptosis.

We have demonstrated recently (49) in a prostate cancer model, the requirement for IGFBP-3 secretion and re-uptake by endocytic pathways (specifically caveolin- and transferrin receptor-mediated) for apoptosis induced by transforming growth factor-β. After internalization, IGFBP-3 rapidly localizes to the nucleus where it interacts with RXRα and other factors (1). Nuclear import is a nuclear localization signal-dependent process and is mediated by importin-β factor (50).

Our observation that IGFBP-3 translocates Nur77 has several important implications. First, because IGFBP-3 is a biological signal *versus* the previously described chemical apoptosis inducers (*i.e.* calcium ionophores, etoposide, rexinoids) (24), this implies that the normal prostate epithelial cell has an endogenous signal (IGFBP-3), which can induce a programmed cell death cascade upon cancer surveillance. In fact, we have
reported recently that EWS/FLI-1, an abnormal transcription factor resulting from oncogenic fusion in Ewing’s tumor, binds the IGFBP-3 promoter \textit{in vitro} and \textit{in vivo} and represses its activity. Moreover, IGFBP-3 silencing can partially rescue the apoptotic phenotype caused by EWS/FLI-1 inactivation. IGFBP-3-induced Ewing cell apoptosis relies on both IGF-1-dependent and -independent pathways. These findings therefore identify the repression of IGFBP-3 as a key event in the development of Ewing’s sarcoma (51).

IGFBP-3 mediates the effects of multiple anti-proliferative and pro-apoptotic biological agents including transforming growth factor-\(\beta\) (52), tumor necrosis factor-\(\alpha\) (53), retinoids (54), p53 (55), and 1,25-dihydroxyvitamin D3 (56). In addition, IGFBP-3 gene expression is commonly lost in human prostate cancer cell lines and xenografts and was detected in DNA microarray analysis of normal compared with cancerous cells (38). Decreased IGFBP-3 expression is associated with prostate cancer progression, demonstrating more frequent loss of expression in advanced disease in both human and mouse models (37–39). Low IGFBP-3 levels in prostate cancer imply impairment of RXR\(\alpha/Nur77\) translocation and subsequent apoptosis of cancerous cells.

We have shown that expression of IGFBP-3 and Nur77 together are additive in their pro-apoptotic effects. The importance of both these genes that are inactivated on the cellular path to immortalization is supported by the fact that IGFBP-3 binding and proteolysis are the targets of the E7 protein encoded by human papillomavirus type 15, one of the few viral genes that can immortalize primary human cells and thereby override cellular senescence (57), and that Nur77 is inactivated by the Epstein-Barr virus transactivator EBNA2, essential for the immortalization of B-cells (58). These small, lean, viral genomes would presumably selectively inactivate critical apoptosis-inducing host proteins that would hinder the viral program of self-propagation. Additionally, two recent papers (36, 59) have now described a nongenotropic carrier function of RXR\(\alpha\) to transport Nur77 to the mitochondria to initiate a mitochondria-dependent apoptotic pathway.
Finally, we have described a novel interface between the nuclear receptor superfamily and the growth and survival-regulating IGFBP-3 axis. Beyond its initial description as a serum carrier for the growth-promoting IGFs, IGFBP-3 has emerged as a multifunctional, intrinsic, and IGF-independent signaling protein that mediates important autocrine and paracrine regulation of growth and homeostasis in a variety of tissues (1). Although we have described previously IGFBP-3 binding to the nuclear receptor RXRα and supershifting RXR-RXRα complexes in electrophoretic mobility shift assays, modulating traditional nuclear receptor roles as transcription factors via modulation of signaling via the RXRE and presumably taking on a co-activator/co-repressor role in the nucleus (5–7), we currently describe IGFBP-3 as a modulator of novel nuclear receptor roles as extra-nuclear mediators of cellular processes. Furthermore, both IGFBP-3 and Nur77 are dramatically suppressed by androgens and are up-regulated during apoptosis induced by castration in the ventral rat prostate affords another unique in vivo model to study IGFBP-3-induced Nur77 translocation (60, 61). This phenomenon also suggests that uncontrolled androgen receptor signaling implicated in androgen-independent prostate cancer involves the loss of the IGFBP-3/Nur77 apoptotic pathway.

It is now well recognized that Nur77 mediates apoptosis (62) and has roles as both a transcription factor and a co-activator-binding site (68). Nur77 is often overexpressed in androgen-independent prostate cancer involves the loss of the IGFBP-3/Nur77 apoptotic pathway. Finally, we have described a novel interface between the nuclear receptor superfamily and the growth and survival-regulating IGFBP-3 axis. Beyond its initial description as a serum carrier for the growth-promoting IGFs, IGFBP-3 has emerged as a multifunctional, intrinsic, and IGF-independent signaling protein that mediates important autocrine and paracrine regulation of growth and homeostasis in a variety of tissues (1). Although we have described previously IGFBP-3 binding to the nuclear receptor RXRα and supershifting RXR-RXRα complexes in electrophoretic mobility shift assays, modulating traditional nuclear receptor roles as transcription factors via modulation of signaling via the RXRE and presumably taking on a co-activator/co-repressor role in the nucleus (5–7), we currently describe IGFBP-3 as a modulator of novel nuclear receptor roles as extra-nuclear mediators of cellular processes. Furthermore, both IGFBP-3 and Nur77 are dramatically suppressed by androgens and are up-regulated during apoptosis induced by castration in the ventral rat prostate affords another unique in vivo model to study IGFBP-3-induced Nur77 translocation (60, 61). This phenomenon also suggests that uncontrolled androgen receptor signaling implicated in androgen-independent prostate cancer involves the loss of the IGFBP-3/Nur77 apoptotic pathway.

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