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IGFBP-3 sensitizes prostate cancer cells to interferon-gamma-induced apoptosis

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Abstract

Objective: Insulin-like growth factor binding protein-3 (IGFBP-3) has been shown to exhibit diverse biological actions, including IGF-independent effects on cell growth and cell death. Here we report that IGFBP-3 sensitizes prostate cancer cells to interferon-gamma (IFN-γ)-induced apoptosis and inhibition of cell proliferation.
Design: The cell growth or cell death of prostate cells in response to the treatments of IGFBPs and/or IFN-γ was measured, and the signaling pathways mediating these actions assessed.
Results: Cell proliferation was minimally affected when M12 prostate cancer cells were treated with exogenous IGFBP-3 (1–5 μg/ml), IGFBP-1 (1–5 μg/ml) or IFN-γ (20 U/ml). However, strong inhibition of cell growth and significant apoptosis were observed when M12 cells were co-treated with IGFBP-3 and IFN-γ, but not with IGFBP-1 and IFN-γ. These effects were IGF-independent and appear not to require intracellular localization of IGFBP-3, as similar results were obtained with mutants of IGFBP-3 that either could not bind IGF or has impaired ability to be internalized. Further analyses revealed that IGFBP-3, but not IGFBP-1, could significantly enhance the weak tyrosine phosphorylation of STAT1 induced by IFN-γ (20 U/ml) alone. The IGFBP-3-promoted apoptosis in the presence of IFN-γ could also be abrogated by blockade of the mTOR pathway with its pharmacological inhibitors, LY294002 or rapamycin.
Conclusions: These results demonstrated that in a cancer cell line not responsive to exogenous IGFBP-3 alone, IGFBP-3 sensitized the cells to the anti-proliferative, proapoptotic actions of IFN-γ through an IGF-independent, STAT1- and mTOR-dependent mechanism.

Keywords: IGFBP-3; Interferon-gamma; STAT1; mTOR; Apoptosis; Prostate cancer

1. Introduction

Insulin-like growth factor binding proteins (IGFBPs), a family of six secreted proteins that share high structural homology, modulate the bioavailability and mitogenic actions of insulin-like growth factors (IGFs) [1,2]. A number of studies have also demonstrated that IGFBPs, especially IGFBP-3, can exert IGF-independent actions, including anti-proliferative

Abbreviations: IGF, insulin-like growth factor; IGFBP-3, insulin-like growth factor binding protein-3; rhIGFBP-3, recombinant human IGFBP-3; IFN-γ, interferon-gamma; IFN-α, interferon-alpha; IFN-β, interferon-beta; JAK, Janus kinase; STAT, signal transducers and activators of transcription; PI-3K, phosphatidylinositol 3-kinase; mTOR, mammalian target of rapamycin.

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and pro-apoptotic effects on various cell types [3,4]. The mechanism(s) underlying the IGF-independent, growth inhibitory actions of IGFBP-3 still remains to be fully understood, yet it has been proposed that such actions may involve specific IGFBP-3 membrane receptors [5,6], inactivation of the IGFIR pathway [7], activation of the Smad 2/Smad 3 pathway [8] or the STAT1 pathway [9], or nuclear localization of IGFBP-3 [10].

In addition to exhibiting direct growth inhibitory effects, IGFBP-3 has also been shown to mediate the anti-proliferative actions of cytokines, such as TGF-β, TNF-α and interferon-gamma [11–13]. Interferon-gamma (IFN-γ), a type II interferon which can inhibit cell proliferation and induce apoptosis [14,15], functions predominantly through activation of the JAK/STAT1 pathway [16]. Other signaling pathways activated by IFN-γ, such as the PI-3K/mTOR pathway and the MAPK/ERK pathways, also significantly contribute to the biological activities exhibited by IFN-γ [15,17]. In this report, we demonstrated that in M12 prostate cancer cells, IGFBP-3 strongly promoted apoptosis with an otherwise non-inhibitory dose of IFN-γ through an IGF-independent mechanism(s), which involved the STAT1 pathway and the mTOR pathway.

2. Materials and methods

2.1. Cell culture

P69 cells is a SV40-T antigen transformed, low tumorigenic human prostate cancer cell line, and M12 cells are a highly metastatic derivative of P69 cells [18]. P69 cells or M12 cells were cultured in defined RPMI 1640 medium as described previously [15]. For experiments detecting changes in signaling pathways in response to various treatments, M12 cells were grown to 60–70% confluence on 6-well plate and starved in RPMI 1640 overnight before being incubated in RPMI 1640 supplemented with 50 μg/ml bovine serum albumin (BSA) plus human IFN-αA/D (Sigma–Aldrich, St. Louis, MO), human IFN-β (Sigma–Aldrich, St. Louis, MO), human IFN-γ (Roche, Mannheim, Germany), recombinant human IGFBP-3 (rhIGFBP-3), LY294002 (Calbiochem, La Jolla, CA), or rapamycin (Cell Signaling Technology, Beverly, MA) as indicated.

2.2. Generation of recombinant human IGFBP-3

The KpnI/Not1 digested fragment containing cDNA encoding c-terminal FLAG-tagged wild-type rhIGFBP-3, or the mutant rhIGFBP-3($G^{56}G^{80}G^{81}$) was subcloned from pBSSK plasmids [19] into pShuttle-CMV plasmids (AdenoVator™ Vector System, QBiogen, Carlsbad, CA), and the adenovirus carrying cDNA encoding the wild-type or the mutant rhIGFBP-3 was generated following manufacture's instruction. The rhIGFBP-3 was then purified with M2 anti-FLAG antibody (Sigma–Aldrich, St. Louis, MO) from the conditioned medium of M12 cells infected with the adenovirus for 28 h. The concentration of the rhIGFBP-3 in the eluted fractions was quantitated as described previously [19]. It was estimated that at least 90% of the total protein purified from alpha-M2 affinity column was the 42–45KD IGFBP-3 species.

2.3. Cell proliferation assay

M12 cells were plated on 96-well plates (2900 cells/well) and grown for 16 h. The cells were then synchronized in RPMI for 4 h before treatment with defined RPMI culture medium supplemented with 50 μg/ml BSA plus either IFN-α, IFN-β, IFN-γ, rhIGFBP-3 (Protigen, Inc., Sunnyvale, CA; GroPep, Adelaide, Australia; or self-generated, see above), IGFBP-3 NLS mutant (K228E, R230G) (Protigen, Inc., Sunnyvale, CA), LY294002, or rapamycin at the concentrations indicated. Cell proliferation was quantitated with CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay Kit (MTS/PMS assay) (Promega, Madison, WI) according to the manufacturer's instructions.

2.4. Apoptosis assay

M12 cells were plated, cultured and treated on 96-well plates as described above. Apoptosis-induced DNA fragmentation was quantitated with a Cell Death Detection $ELISA^{PLUS}$ assay system (Roche Diagnostics, Indianapolis, IN) according to the manufacturer's protocol. The assay quantitatively measures cytoplasmic histone-associated DNA fragments (mono- and oligonucleosomes) generated in the early phase of apoptosis.

2.5. Western immunoblot

Preparation of the cell lysates and subsequent Western immunoblot analysis were performed as described previously [15]. The density of the bands on X-ray film was determined by using IMAGEQUANT 5.1 (Molecular Dynamics, CA). The antibodies used for Western immunoblot analyses in this study were: rabbit polyclonal IgG against human IGFBP-1 (GroPep, Adelaide, Australia), phospho-Tyr701-STAT1, phospho-Thr389 p70 S6K, and p70 S6K from Cell Signaling Technology (Beverly, MA); rabbit polyclonal anti-phospho-Ser727-STAT1 from Biosource International (Camarillo, CA); mouse monoclonal IgG against IGFBP-3 from Diagnostic Systems Laboratories, Inc. (Webster, TX), and STAT1 (C-136) from Santa Cruz Biotechnology (Santa Cruz, CA). Secondary antibodies (anti-mouse IgG and anti-rabbit IgG) were obtained from Amersham