Biochemical and Biophysical Research Communications 445 (2014) 208–213

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Yin Yang 1 regulates the transcriptional repression of survivin

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ARTICLE INFO

Article history:
Received 23 January 2014
Available online 4 February 2014

Keywords:
Survivin
YY1
Transcriptional repressor
HIF-1
IAP

ABSTRACT

The mechanisms for regulation of the Inhibitor of Apoptosis (IAP) Survivin in cells undergoing stress associated with tumor development and the tumor microenvironment are not well understood. The stress response transcription factors HIF-1α and Yin Yang 1 (YY1) were hypothesized to contribute to the upregulation of Survivin in tumor cells. As expected, U2OS cells overexpressing HIF-1α showed a 2- to 3-fold transactivation when transfected. Surprisingly, when YY1 was overexpressed in this survivin promoter reporter system, luciferase expression was repressed 30- to 40-fold. YY1 involvement in survivin promoter repression was confirmed using siRNA directed against YY1. These studies showed that knockdown of YY1 releases the survivin promoter from the observed repression and leads to a 3- to 5-fold increase in promoter activity above basal levels. A U2OS cell line containing a stable YY1 Tet-off system was used to determine whether a temporal increase in YY1 expression affects Survivin protein levels. A low to moderate decrease in Survivin protein was observed 24 h and 48 h after Tet removal. Studies also confirmed that YY1 is capable of directly binding to the survivin promoter. Collectively, these findings identify novel basal transcriptional requirements of survivin gene expression which are likely to play important roles in the development of cancer and resistance to its treatment.

1. Introduction

Survivin an unique mammalian inhibitor of apoptosis (IAP) protein, controls stress from the microenvironment through diverse functions within the cell including surveillance checkpoints, suppression of cell death, regulation of mitosis, and adaptation to unfavorable environments [1–3]. Epigenetic, genetic and post-translational mechanisms for survivin gene regulation have been described in many malignant cell types [4] with various transcription factors including Stat3 [5], HIF-1α [6], Rb-E2F1 [7], Dec1 [8], Sp1 [9], c-myc [10] and KLF5 [11] affecting its transcriptional upregulation. In addition, the tumor suppressor p53 and Rb-E2F2 have been shown to repress survivin transcription by direct binding to the survivin promoter in a lung adenocarcinoma cell line [12] and in normal human melanocytes [4]. An interesting polymorphism has also been described at a CDE/CHR repressor element in the survivin promoter that correlates with increased survivin mRNA and protein in cancer cell lines and not in normal cell line controls [13].

The transcription factor YY1 is known to have a fundamental role in normal biologic processes such as embryogenesis, differentiation, replication, and cellular proliferation [14]. YY1 exerts its effects on genes involved in these processes via its ability to initiate, activate, or repress transcription depending upon the context or recruited cofactors in which it binds [15,16]. One such family of cofactors are the histone deacetylases which have been shown to bind YY1 and repress transcription when targeted to promoters [17]. YY1 has been shown to interact with p300, PCAF and CBP, all which posses the histone acetyltransferase (HAT) activity [17]. YY1 may thus activate transcription by its recruitment of HAT proteins and repress transcription by recruiting HDACs.

Work by Affar et al. [18] previously showed that in a mouse YY1 knockdown model, survivin (BIRC5) levels were decreased. These findings lead to the hypothesis and impetus of our current work which was that YY1 overexpression would lead to survivin promoter control and Survivin protein regulation. The present study, therefore examined the transcriptional affect of YY1 on survivin

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http://dx.doi.org/10.1016/j.bbrc.2014.01.169
0006-291X/

in an osteosarcoma cell line derived from human bone osteosarcoma (U2OS). We found that when YY1 was overexpressed in U2OS cells, survivin mRNA and protein were repressed by YY1. By analyzing the survivin promoter activity, we further found that YY1 works as a transcriptional repressor of the survivin gene and we show for the first time that YY1 is capable of binding directly to the survivin core promoter thus acting as a transcription factor rather than a co-repressor.

2. Materials and methods

2.1. Antibodies and DNA vectors

All antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) unless otherwise indicated. The plasmid expressing YY1 protein, pcDNA3/YY1 as well as the U6/yy1 siRNA and control U6/scrambled were kind gifts of Dr. Sui, Wake Forest and were described previously [19]. U6/yy1 siRNA vector was evaluated in our hands for effective knockdown (Supplemental Fig. 1). Survivin nested deletion constructs were previously described [9] and were a kind gift from Dr. Li, Roswell Park Memorial Institute.

2.2. Cell culture and transfection

The U2OS human osteosarcoma cell line was obtained from ATCC. U2OS cells with stable Tet-off YY1 were a kind gift from Dr. Sui, Wake Forest and were previously described [19]. Cell lines were maintained under an atmosphere of 5% CO_2 at 37 °C in McCoys 5A media supplemented with 10% fetal bovine serum, 2 mmol/L of L-glutamine, and penicillin–streptomycin. The Tet-off cells were additionally maintained in G418, hygromycin B, and the tetracycline analogue doxycycline (50 ng/mL). YY1 expression was induced by transferring the cells to Tet-off medium, which is the same as control (Tet-on) medium except for the lack of doxycycline [19].

2.3. Transient transfection and reporter assays

U2OS cells were seeded in 12-well plates and grown to 60–80% confluence. A total 0.4 μg of the various survivin promoter-luciferase reporter plasmids were cotransfected with either 0.6 μg of pcDNA/YY1 or empty vector expression plasmids and 0.01 μg of pRL-tk using FuGENE 6 (Roche, Indianapolis, IN). Twenty-four hours after transfection, cells were lysed and assayed for luciferase activity by luminometer (Turner Design Systems, Sunnyvale, CA). Luciferase activity measurement was accomplished according to manufacturer's instructions. The pLuc230 vector containing the CAT → GGG mutation was purchased from Origene, Rockville, MD.

2.4. Western blots

Cells were solubilized, proteins (20–40 μg) separated using 12 % Bis–Tris polyacrylamide gels, transferred onto polyvinylidene difluoride membranes (Millipore) and probed using the following antibodies: mouse monoclonal anti-YY1 (cat. # sc-7341; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), and rabbit polyclonal anti-Survivin (cat. # NB500-201; Novus, Littleton, CO). Secondary antibodies (IR-Dye-conjugated) were goat anti-rabbit and goat anti-mouse immunoglobulin (LICOR, Lincoln, Nebraska). Immunoreactive bands were detected using the Odyssey imaging system (LICOR) and quantified using ImageQuant software.

2.5. Reverse transcriptase-PCR

Total RNA was extracted from cells at various time intervals using TRI-Reagent (Sigma, St. Louis, MO) and reverse-transcribed with SuperScript™ II RNase H^- Reverse Transcriptase (Invitrogen™, Carlsbad, CA), as described by the manufacturer. One hundred nanograms of the resulting first-strand cDNA was used as template and amplified by PCR. Sequences of the oligonucleotide primer sets used for reverse transcription-PCR analysis are as follows: 5′-GCA TGG CTG CCC CGA CGT TG-3′ (sense) and 5′-GCT CCG GCC AGA GGC CTC AA-3′ (antisense) for survivin, 5′-GCT TCG AGG ATC AGA TTC TCA TCC-3′ (sense) and 5′-GAC TAC ATT GAA CAA ACG CTG GTC-3′ (antisense) for YY1, 5′-GCC AGA TCT CGG CGA AGT AAA-3′ (sense) and 5′-ATA TCC AGG CTG TGT CGA CTG-3′ (antisense) for HIF1, 5′-ATG ACT CGC GAT TTC AAA CCT-3′ (sense) and 5′-CTT TGA AGT CGA GAA TCC ATT-3′ (antisense) for p75/LEDGF, and, 5′-CTCATGACCACAGTCCATGC-3′ (sense) and 5′-TTACTCCTTGGAGGCCATGT-3′ (antisense) for beta actin. Products were visualized on ethidium bromide-stained agarose gels. Amplification of beta actin served as an internal control.

2.6. Electrophoretic mobility shift assay

Nuclear extracts were prepared as previously described [20], with the only modification that N-N-(L-3-trans-carboxyoxirane-2-carbonyl)-L-leucyl-agmatine (E64) and 4-(2-aminoethyl)-benzolsulfonyl fluoride ("Pefabloc SC") were included as protease inhibitors in concentrations suggested by the manufacturer (Boehringer, Mannheim, Germany). Protein concentration in nuclear extracts was determined using the BCA assay (Pierce) according to the manufacturers instructions. Oligos used were as follows: Two YY1 sites (YY1 sites underlined): 5′-GC GCT CCC GAC ATG CCC CGC GGC GCG CCA TTA ACC GCC A-3′; YY1 Site 1 5′-TG CGC TCC CGA CAT GCC CCG CG-3′; YY1 Site 2 CGC GGC GCG CCA TTA ACC GCC A-3′ YY1 Consensus 5′-CGC TCC CCG GCC ATC TTG GCG GCT GGT-3′. All oligos were annealed by incubating at 95 °C for 2 min, then ramp cooling to room temperature. The DNA–protein binding reaction was performed in 20 μl reaction mixtures including 10% glycerol, 12 mM HEPES pH 7.9, 4 mM Tris HCl pH 8.0, 1 mM EDTA, and 3 μg BSA. Binding reactions were incubated at room temperature for 30 min, then for an addition 60 min at 4 °C with anti-YY1 antibody (cat. # sc-281; Santa Cruz Biotechnology, Santa Cruz, CA) added to the appropriate reactions. The DNA–protein complexes were resolved on 5.5% non-denaturing polyacrylamide gel (29:1 cross-linking ratio) run in Tris–Borate buffer, dried and exposed using the Storm 860 Phosphoimager (Amersham Biosciences). Competitions in EMSA were performed as above, except that 25 ng of poly (dI–dC)·(dI–dC) per reaction were used.

2.7. Statistical analysis

All data in reporter assays and semiquantitative PCR are presented as means ± standard deviation.

3. Results

3.1. HIF-1α and YY1 transcriptionally regulate surviving

HIF-1α has previously been shown to be a transcriptional regulator of survivin [6,21,22]. However, it is currently unknown if YY1 plays a role in survivin transcription. To determine possible bindings sites for YY1, using a computer-based approach, the survivin promoter was scanned for putative HIF-1α, and YY1 binding sites using the online tools TFSearch (http://www.cbrc.jp/research/db/TFSEARCH.html), Promo Program (http://alggen.lsi.upc.es/cgi-bin/