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NF90 regulates PARP1 mRNA stability in hepatocellular carcinoma

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ABSTRACT

Poly (ADP-ribose) polymerase 1 (PARP1) is an ADP- ribosylation enzyme and plays important roles in a variety of cellular processes, including DNA damage response and tumor development. However, the post-transcriptional regulation of PARP1 remains largely unknown. In this study, we identified that the mRNA of PARP1 is associated with nuclear factor 90 (NF90) by RNA immunoprecipitation plus sequencing (RIP-seq) assay. The mRNA and protein levels of PARP1 are dramatically decreased in NF90-depleted cells, and NF90 stabilizes PARP1's mRNA through its 3′UTR. Moreover, the expression levels of PARP1 and NF90 are positively correlated in hepatocellular carcinoma (HCC). Finally, we demonstrated that NF90-depleted cells are sensitive to PARP inhibitor Olaparib (AZD2281) and DNA damage agents. Taken together, these results suggest that NF90 regulates PARP1 mRNA stability in hepatocellular carcinoma cells, and NF90 is a potential target to inhibit PARP1 activity.

1. Introduction

Poly (ADP-ribose) polymerase 1(PARP1) is a DNA-dependent ADP-ribosylation transferase, which transfers ADP-ribose moiety from co-substrate β-NAD^+ to acceptor proteins including itself, leading to protein ADP-ribosylation and regulating the functions of the modified proteins [1]. PARP1 has been found to be involved in a variety of biological processes, including chromatin reorganization, DNA damage repair, transcriptional regulation, apoptosis, mitosis and protein degradation [2]. PARP1 has been found to over express in multitude of human cancers and plays important role in tumorigenesis and tumor development [3,4]. Inhibition of the catalytic activity of PARP1 was found to result in synthetic lethality in BRCA1/2-deficient cancer cells. This cancer therapy strategy was clinically established by the approval of the PARP inhibitor Olaparib for treatment the advanced ovarian cancer patient containing germline BRCA mutation [5,6]. Although the functions of PARP1 are well established, the regulation of PARP1, especially the post-transcriptional regulation, remains largely unclear.

Nuclear factor 90 (NF90) is one of the major products of alternative splicing of the interleukin enhancer binding factor 3(ILF3), also known as Nuclear factor of activated T-cells 90 kDa (NFAR1) or Double-stranded RNA-binding protein 76 (DRBP76) [7]. NF90 is a RNA binding protein, contains two conserved dsRNA binding motifs (dsRBM), which are responsible for its association with AU-rich elements (AREs) of mRNA [8]. NF90 has been found to post-transcriptionally regulate the mRNA stability of many genes, such as IL-2, Tau, VEGF, bcl-2, MKP-1, MyoD, p21, CDC2 and cyclin E1 [9–12], and to be involved in several biological processes, including T cell activation, RNA processing, protein translation, DNA repair, host resistance to viral infections and mitosis [11,13,14]. Recently, we have found that NF90 is upregulated in hepatocellular carcinoma (HCC) and promotes the growth of HCC cells, indicating that NF90 may also play important roles in tumorigenesis and tumor development [15].

To investigate the function of NF90, here we performed RNA immunoprecipitation (RIP) plus sequencing (seq) (RIP-seq) assay to identify the NF90-associated RNAs. We present evidences that NF90 associates with the mRNA of PARP1 and regulates its stability in hepatocellular carcinoma cells, and that NF90 is a potential target to inhibit PARP1 activity.

2. Materials and methods

2.1. Cell lines and hepatocellular carcinoma samples

Human Embryonic Kidney (HEK) 293T cells, human HCC cell lines QGY-7703 and SMMC-7721 were obtained directly from

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Shanghai cell bank of Chinese academy of sciences (Shanghai, China). NF90 stably knock down QGY-7703 (QGY-4 and QGY-6) and SMMC-7721(7721-2 and 7721-3) is generated before [15]. Cells were cultured in Dulbecco's Modified Eagle's Medium-high glucose (Gibico, USA) containing 1% penicillin-streptomycin and 10% fetal bovine serum (FBS) at 37 °C in an atmosphere of 5% CO_2.

Human hepatocellular carcinoma samples were collected from HCC patients in Qidong Liver Cancer Institute (Jiangsu, China) from 2009 to 2013. It was approved for using of human tissues with informed consent by the institutional review board of Fudan University.

2.2. Plasmids, antibodies, and other materials

Full-length cDNA of NF90 was cloned into the SBP vector (SFB,S-FLAG-SBP-tagged) [16]. 3'untranslated region (3′UTR) of PARP1 was cloned into downstream of the renilla luciferase reporter gene of psi-check2 vectors (C8021, Promega, Madison, USA).

Anti-Flag monoclonal antibody was purchased from Sigma. Anti-NF90 polyclonal antibody (ab50832), anti-PARP1 polyclonal antibody (ab194586) and anti-GAPDH monoclonal antibody (ab125247) were purchased from Abcam.

10 -OH camptothecin (10-OH CPT, S1288) and Olaparib (AZD2281, S1060) were purchased from Selleckchem, Houston, USA. High capacity streptavidin agarose resin beads (RE231329) were purchased from Thermo Fisher Scientific, Massachusetts, USA.

2.3. RNA immunoprecipitation (RIP)

RIP was performed as reported by Jing Zhao et al. with some modifications [17,18]. In brief, 5×10^7 293T cells stably expressing SFB or SFB-NF90 was harvested and washed with phosphate-buffered saline. Cells were lysed in NETN buffer (0.5% Nonidet P-40, 50 mM Tris-HCl, pH 8.0, 2 mM EDTA, and 100 mM NaCl plus RNase and proteinase inhibitors) at 4 °C and centrifuged at 12000 rpm for 20 min at 4 °C. The supernatants were incubated with high capacity streptavidin agarose resin beads (streptavidin beads, Thermo) for 4 h at 4 °C. Then the beads were washed three times with NETN buffer and RNA was isolated by using RNA Purification Kit (RNAeasy Mini Elute kit, QIAGEN) according to the manufacturer's protocol.

2.4. Quantitative real-time PCR (qRT-PCR)

Total RNA was purified using RNA Purification Kit (RNAeasy Mini Elute kit, QIAGEN) according to the manufacturer's protocol. The total RNA concentration and purity of samples was assessed using Nanodrop spectrophotometer (Thermo Fisher). Next, 1ug of RNA was subjected to DNase (NEB) treatment and retro-transcribed using the ReverTra Ace PCR RT Kit (Toyobo, Osaka, Japan) in 20ul reaction, according to manufacturer's instructions. Then, the quantification of transcripts in the samples was performed by qRT-PCR analysis using SYBR Green Supermix kit (Toyobo) and it was carry out in CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, USA). The related primers are listed in Supplementary Table 1.

2.5. Cell lysis and western blot

The cells were lysed with buffer containing 0.5% Nonidet P-40, 50 mM Tris-HCl, pH 8.0, 2 mM EDTA, and 300 mM NaCl for further Western bolt analysis using indicated antibodies following standard protocol as described previously [19].

2.6. Cell survival assay

NF90 stably knocked down QGY-7703 and SMMC-7721 cells or control cells were plated in 96-well flat-bottomed plates (5×10^3 cells per well) and treated with different concentration of 10-OH CPT or AZD2281.48 h later, the cells were harvested and cell viability measured by Cell Counting Kit-8 (Beyotime, Shanghai, China) according to the manufacturer's protocol.

2.7. Dual-luciferase reporter assay

Indicated plasmids were transfected into cells cultured in 24-well plates using Lipofectamine 3000(Invitrogen, Carlsbad, USA). 36 h later, renllia and firefly luciferase activities were measured by Multiscan Spectrum Luciferase assays Kit (Transgene, Beijing, China) according to the manufacture's protocol as described before [20].

2.8. Statistical analysis

GraphPad Prism 5.0 statistical software (San Diego, CA, USA) was used to analyze the data. These data were expressed as the mean ± S.D., three independent experiments. Partial data analysis was performed with the student *t*-test(two-tailed, $p < 0.05$ or 0.01 was considered significant). For Quantitative real-time PCR, gene mRNA levels were transformed by formula $2^{-\Delta Ct}$, normalized by β2-microglobulin (β2-MG).

3. Results

3.1. Isolation of NF90 associated RNAs by RIP-seq

NF90 is a RNA binding protein, which has two dsRNA-binding motifs. To explore the functions of NF90, we developed a method to isolate NF90-associated RNAs by combining native RIP and RNA sequencing (RIP-seq). SBP-vector or SBP-NF90 were transfected into 293T cells, and SBP or SBP-NF90 complex were pulled down by streptavidin beads, then associated RNAs were reverse transcribed into cDNA and subjected to sequence by HiSeq 2500 (Supplementary Fig. 1). The expression level of SBP or SBP-NF90 was examined in the RIP complex by western blot using anti-Flag antibody (Fig. 1A). RNA sequencing was performed to compare mRNAs present in SBP-NF90 complex with those present in SBP complex, in order to identify the mRNAs preferentially associating with NF90. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of the transcripts enriched in SBP-NF90 complex suggested that the most significantly enriched mRNAs encoded proteins involved in cell cycle, ribosome, RNA transports and DNA damage repair, including base excision repair (BER) and nucleotide excision repair (NER) (Fig. 1B). Interestingly, out of hundreds of NF90 associated mRNAs related to DNA damage repair, several were found to encode important enzymes and mediators in DNA damage response, including PARP1, ATM, ATR, ERCC1/2/5/6 and XRCC1 and so on. qRT-PCR analysis verified the enrichment of transcripts encoding these enzymes and mediators (Fig. 1C). These findings indicated that NF90 associated with many mRNAs related to DNA damage repair gene.

3.2. NF90 regulates the protein and RNA levels of PARP1

Among these DNA damage repair gene, we are very interested in PARP1, which plays important roles in a variety of cellular processes, including tumorigenesis and tumor development, and it is an important potential target in cancer therapy. To further confirm the interaction between NF90 and PARP1 mRNA, control IgG or

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