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Activation of NFAT signal by p53-K120R mutant

Natsuko Shinmen [a,b], Toshifumi Koshida [a], Takeshi Kumazawa [b], Keizo Sato [b], Hideaki Shimada [c], Tomoo Matsutani [d], Yasuo Iwadate [d], Masaki Takiguchi [a], Takaki Hiwasa [a,*]

[a] Department of Biochemistry and Genetics, Chiba University, Graduate School of Medicine, Inohana 1-8-1, Chuo-ku, Chiba 260-8670, Japan
[b] Department of Legal Medicine, Showa University School of Medicine, Hatanodai 1-5-8, Shinagawa-ku, Tokyo 142-8555, Japan
[c] Department of Gastroenterological Surgery, Chiba Cancer Center, Nitona-cho 666-2, Chuo-ku, Chiba 260-8717, Japan
[d] Department of Neurological Surgery, Chiba University, Graduate School of Medicine, Inohana 1-8-1, Chuo-ku, Chiba 260-8670, Japan

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ABSTRACT

The tumor suppressor p53 is activated by phosphorylation and/or acetylation. We constructed 14 non-phosphorylated, 11 phospho-mimetic, and 1 non-acetylated point p53 mutations and compared their transactivation ability in U-87 human glioblastoma cells by the luciferase reporter assay. Despite mutations at the phosphorylation sites, only the p53-K120R and p53-S9E mutants had marginally reduced activities. On the other hand, the Nuclear factor of activated T-cells (NFAT)-luciferase reporter was more potently activated by p53-K120R than by wild-type p53 and other mutants in glioblastoma, hepatoma and esophageal carcinoma cells. This suggests that acetylation at Lys-120 of p53 negatively regulates a signaling pathway leading to NFAT activation.

1. Introduction

The tumor suppressor protein p53 possesses various biological activities such as cell cycle arrest, induction of apoptosis and inhibition of angiogenesis by transactivating p21 [1], Bax [2], PUMA [3], Noxa [4], Bad [5], etc. The transactivation ability and the selectivity of the target genes of p53 are assumed to be regulated mainly by post-translational modifications including phosphorylation, acetylation, ubiquitination, methylation, neddylation and sumoylation [6]. Despite numerous reports on p53, the precise mechanism of selection of the target genes of activated p53 remains to be proven.

Nuclear factor of activated T-cells (NFAT) was first identified as a transcription factor in activated, but not in resting, T cells [7,8]. NFAT is dephosphorylated by calcineurin and translocated into the nucleus [9,10]. p53 also induces proline dehydrogenase 1 (PRODH1, proline oxidase, PIG6) [11,12] and proline dehydrogenase 2 (PRODH2, hydroxyproline oxidase) [13], both of which then activate NFAT pathway through reactive oxygen and Ca^{2+}/calcineurin [14]. Although the NFAT pathway was suggested to be involved in induction of apoptosis [15], NFAT has an oncogenic role in pancreatic carcinoma through transcriptional activation of c-myc [16].

In the present study, we show that a non-acetylated mutant, p53-K120R, markedly activated NFAT.

2. Materials and methods

2.1. Plasmids

pCMV-p53WT, pCMV-p53V143A [17], reporter plasmids and pBV-PUMA-Luc [3] were provided by Dr. Bert Vogelstein (Howard Hughes Medical Institute). pGL3-Bax-Luc, pGL3-p21-Luc and pGL3-Bad-Luc [5] were provided by Dr. Mian Wu (University of Science and Technology of China). pGV-B2 Noxa-Luc [4] was provided by Dr. Nobuyuki Tanaka (Nippon Medical School). NFAT-Luc and SV40-Rluc were purchased from Promega (Madison, WI).

2.2. Cell culture

Human glioblastoma U-87, hepatocarcinoma HepG2, esophageal carcinoma YES-4 and bladder carcinoma T-24 cells were cultured in Dulbecco's modified Eagle's minimum essential medium supplemented with 10% fetal bovine serum.

2.3. Construction of p53 point mutants

p53 mutants were constructed from pCMV-p53 using KOD-Plus-Mutagenesis kit (Toyobo, Osaka, Japan). The primers used are listed in Table 1 (Supplementary data). All constructs were confirmed by base sequencing.

* Corresponding author. Fax: +81 43 226 2037.
E-mail address: hiwasa_takaki@faculty.chiba-u.jp (T. Hiwasa).

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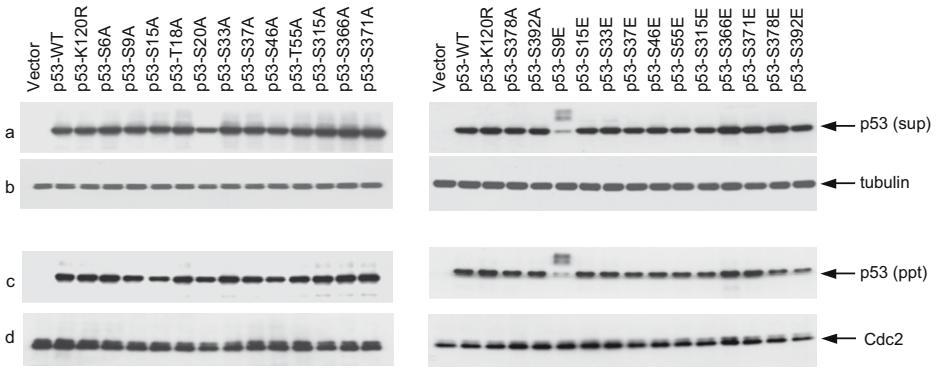

Fig. 1. Expression of p53 in p53-transfected cells. U-87 human glioblastoma cells were transfected with expression plasmids of pCMV-Neo-Bam (Vector), p53-WT or p53 mutants. Expression levels of p53 proteins in the cytoplasmic (A) and nuclear fractions (C) were examined by Western blotting analysis using anti-p53 (DO-1) with loading controls, anti-α/β-tubulin for the cytoplasmic fractions (B) and anti-Cdc2 antibodies for the nuclear fractions (D).

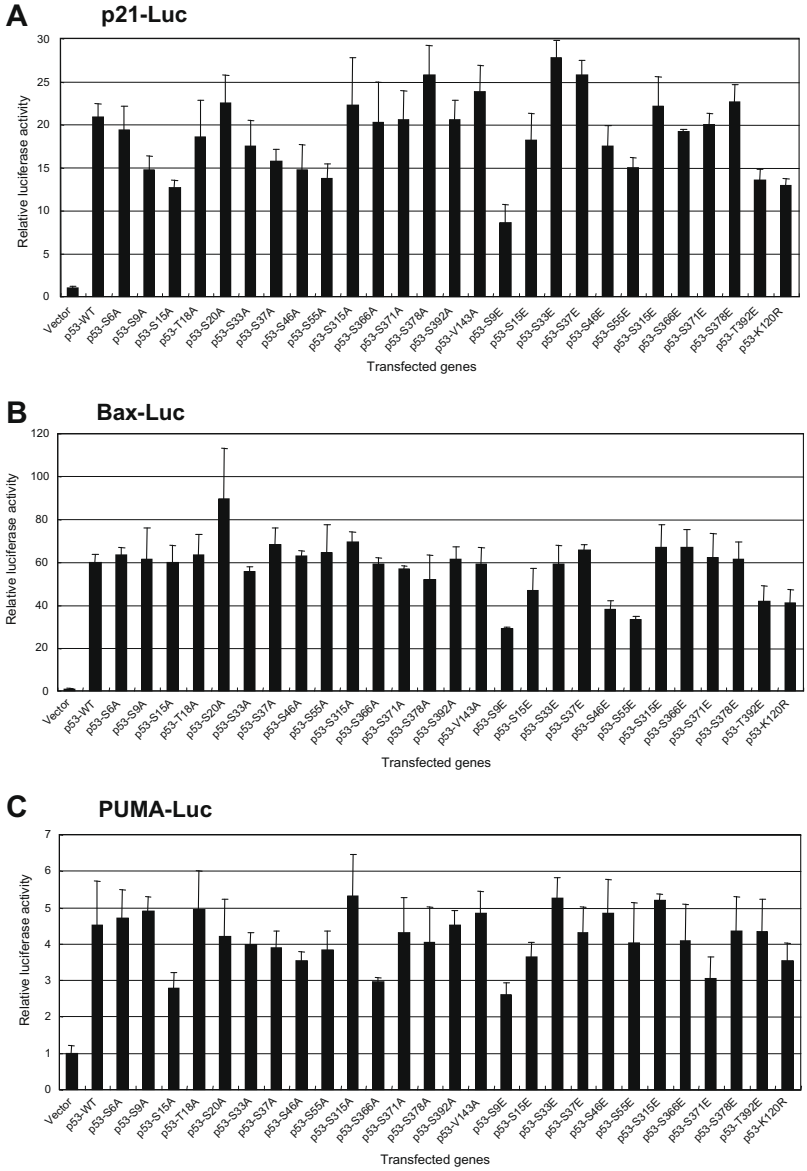

Fig. 2. Activation of p53 reporter plasmids by p53 mutants. U-87 glioblastoma cells (5×10^4 cells) were co-transfected with p53-responsive reporter plasmids (p21-Luc, Bax-Luc, PUMA-Luc, Noxa-Luc, Bad-Luc or NFAT-Luc; 100 ng), transfection standard SV40-Rluc (10 ng), and the expression plasmid (0.5 μg) of p53-WT or p53 mutants, or control empty vector pCMV-Neo-Bam. Cells were harvested 48 h after transfection and luciferase activities in the cell extracts were measured. The error bars represent S.D. ($n = 3$).