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p53 represses the transcription of *snRNA* genes by preventing the formation of little elongation complex

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ABSTRACT

The regulation of transcription by RNA polymerase II (Pol II) is important for a variety of cellular functions. ELL/EAF-containing little elongation complex (LEC) was found to be required for transcription of Pol II-dependent *small nuclear RNA* (*snRNA*) genes. It was shown that the tumor suppressor p53 interacts with ELL and inhibits transcription elongation activity of ELL. Here, we show that p53 inhibits interaction between ELL/EAF and ICE1 in LEC and thereby p53 represses transcription of Pol II-dependent *snRNA* genes through inhibiting LEC function. Furthermore, induction of p53 expression by ultraviolet (UV) irradiation decreases the occupancy of ICE1 at Pol II-dependent *snRNA* genes. Consistent with the results, knockdown of p53 increased both the expression of *snRNA* genes and the occupancy of Pol II and components of LEC at *snRNA* genes. Our results indicate that p53 interferes with the interaction between ELL/EAF and ICE1 and represses transcription of *snRNA* genes by Pol II.

1. Introduction

Several regulatory systems of transcription by RNA polymerase II (Pol II) play important roles in many functions including signal transduction, cell proliferation/differentiation and development. Many genes including protein-coding genes and non-coding RNA (ncRNA) genes are transcribed by Pol II [1,2]. The processes of gene transcription by Pol II basically consist of three steps: transcription initiation, elongation and termination. Many recent studies have shown that the process of transcription elongation has a crucial role in the regulation of gene expression. Transcription elongation factors including Eleven Lysine-rich Leukemia (ELL)/ELL-associated factors (EAF) family members and positive transcription elongation factor (P-TEFb) restart the transcription of Pol II by repressing transient pausing [3]. It has been reported that two ELL/EAF-containing complexes, super elongation complex (SEC) and little elongation complex (LEC), specifically regulate the transcription of distinct genes [4,5]. LEC is composed of multiple subunits including the ELL/EAF component, KIAA0947, NARG2 and ZC3H8 [3,6,7]. KIAA0947 and NARG2 were also known as ICE1 and ICE2 (interacts with C-terminus of ELL subunits 1 and 2), respectively [4]. It has been shown that LEC plays an essential role in transcription of *small nuclear RNA* (*snRNA*) genes. Recently, we showed that human Mediator subunit MED26 contributes to the recruitment of LEC to a subset of *snRNA* genes [6]. ICE1 is one of the central components for the assembly of LEC [5]. It has been shown that LEC plays a role in both the transcription initiation and elongation phases of *snRNA* gene transcription [5]. However, the detailed function of ICE1 and ICE2 in LEC has not been elucidated.

p53 is an important tumor suppressor that is involved in many processes of carcinogenesis in human cancers [8–12]. p53 directly binds to DNA and regulates gene expression to prevent mutations of the genome [13]. p53 is activated in response to alteration of normal cell homeostasis including DNA damage, nutrient starvation, heat shock, virus infection, pH change, hypoxia and oncogene activation [14,15]. In response to DNA damage, p53 is stabilized and acts as a transcription factor that directly regulates several hundred genes and indirectly more than one thousand genes [16]. p53 forms a tetramer on p53-responsive elements of target genes to induce or repress gene expression [17]. Expression of p53-targeted genes induced by exposure to ultraviolet (UV) irradiation includes both proto-oncogenes and tumor suppressor genes. Mutations in the *p53* gene are thought to be involved in the development of precancerous lesions in many types of human cancers. Although p53 directly regulates the transcription of protein-coding genes, recent studies have shown novel roles of p53 in regulation of the transcription of non-coding RNA genes. It has been reported that p53 represses the transcription of *snRNA* genes [18]. In addition, accumulating evidence indicates that snRNA plays a role in the carcinogenesis in lung cancer, germ cell tumors and several types of leukemia [19–21] and in the progression of human diseases including Alzheimer's disease [22–24].

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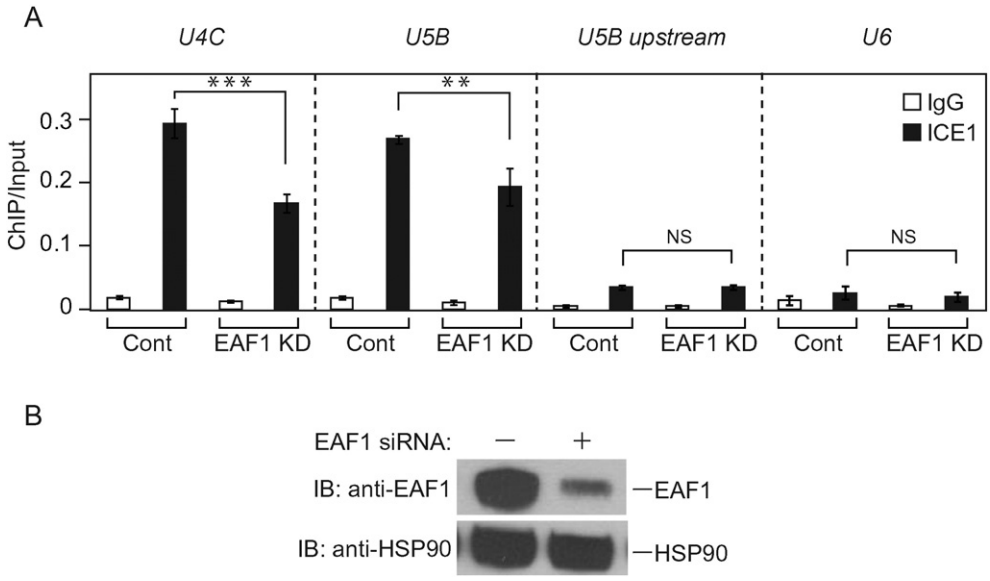

Fig. 1. EAF1 is required for the occupancy of ICE1 at *snRNA* genes. (A) Occupancy of ICE1 at regions of *U4C*, *U5B*, upstream of *U5B*, and *U6 snRNA* genes by EAF1 knockdown (KD). Standard deviations of each result were calculated from three independent experiments. *P* values were determined by Student's *t* test (* $P < 0.05$). Error bars show s.d. NS, not specific. (B) Western blot analysis of EAF1 after EAF1 knockdown. Hsp90 was used as a control.

It has been reported that p53 is one of the specific ELL interactors and that the C-terminal 47 amino acids of p53 are responsible for the interaction [25]. Intriguingly, p53 inhibits the transcription elongation activity of ELL [25]. These results raises the possibility that p53 regulates transcription elongation by Pol II through controlling the activity of ELL or LEC. Here, we provide evidence that p53 is involved in regulation of the function of LEC. Through biochemical analysis and chromatin immunoprecipitation (ChIP) analysis, we showed that p53 inhibits the interaction between ELL/EAF and ICE1 in LEC *in vitro*. Expression of p53 induced by UV irradiation decreased the occupancy of ICE1 at Pol II-dependent *snRNA* genes. p53 knockdown increases both the expression of *snRNA* genes and the occupancy of Pol II and the components of LEC at *snRNA* genes. These findings suggest that p53 interferes with the interaction between ELL/EAF and ICE1 and represses the transcription of *snRNA* genes by Pol II.

2. Materials and methods

2.1. Cell culture

HCT116 cells (ATCC CCL-247) and their derivatives were seeded and cultured in 10-cm dishes (2×10^6 cells per dish) under an atmosphere of 5% CO_2 at 37 °C in McCoy's 5a Medium (GIBCO, Grand Island, NY) supplemented with 10% (v/v) fetal bovine serum (Invitrogen, Carlsbad, CA), 100 units/ml penicillin, and 100 μg/ml streptomycin (GIBCO).

2.2. Production of recombinant protein

Epitope-tagged ELL, EAF1 and p53 were subcloned into pBacPAK8 and were expressed using the BacPAK system (Clontech Laboratories, Mountain View, CA). Full-length ICE1 was subcloned into pFastBac HTb with HA tags and expressed with the BAC-to-BAC system (Clontech). Baculovirus infections, Sf9 culture, and affinity purifications were performed as described previously [6].

2.3. Antibodies and reagents

Anti-FLAG (M2) antibody (F3165), anti-FLAG (M2)-agarose (A2220) and anti-HA-agarose (A2095) were purchased from Sigma-Aldrich (St. Louis, MO). Anti-ELL antibody (A301-645A) and anti-ICE1 antibody (A304-276A) were purchased from Bethyl Laboratories (Montgomery, TX). Anti-Pol II antibody (F-12, sc-55492), anti-p53 antibody (DO-1, ab1101), normal rabbit IgG (sc-2027) and normal mouse IgG (sc-2025) were purchased from Santa Cruz Biotechnology. Anti-Myc antibody (9E10, MMS-164P) and anti-HA antibody (HA11, 901502) were purchased from Covance Research Products (Princeton, NJ). Anti-TBP

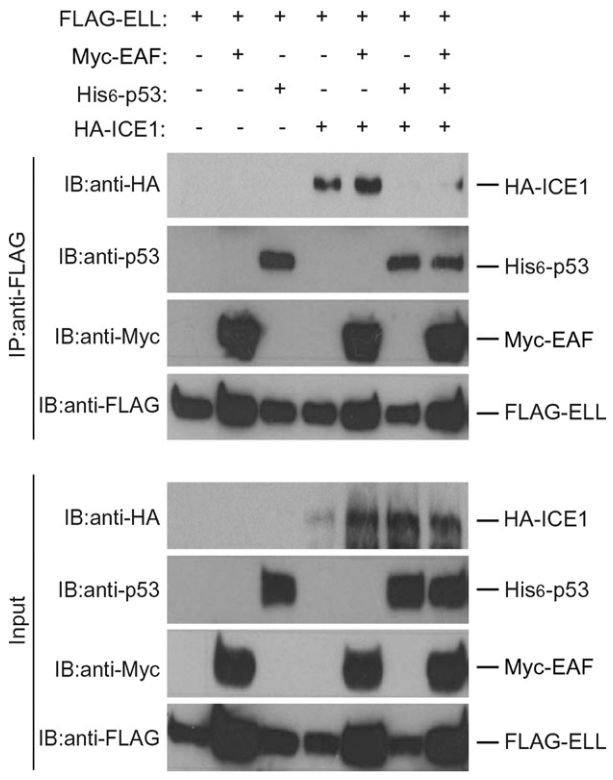

Fig. 2. p53 interferes with the interaction between ELL/EAF and ICE1. Anti-FLAG-immunoprecipitates using lysates from Sf9 cells expressing the indicated combinations of Myc-tagged EAF, FLAG-tagged ELL, HA-tagged ICE1 and His_6-tagged p53 were subjected to immunoblot analysis. IP, immunprecipitation.