Cooperative regulation of p73 promoter by Yin Yang 1 and E2F1

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ABSTRACT

The transcriptional factor Yin Yang 1 (YY1) plays multifunctional role in various biological processes. Here we report that the silencing of YY1 using siRNA expression vectors in U2OS cells led to a significant decrease in transcriptional activity of p73, which is a member of p53 family. Consistently, overexpression of YY1 could enhance the transcriptional activity of p73 in a dose-dependent manner. Moreover, synergistic cooperation between YY1 and E2F1, through a mechanism involving a physical interaction, was observed in the regulation of p73 promoter. In conclusion, our results provide new insights into the function of YY1, and might clarify the complex regulation mechanism by YY1 network in diverse biological processes, such as development, differentiation and cancer biology.

INTRODUCTION

Yin Yang 1 (YY1) is a multifunctional transcription factor, which can act as transcriptional activator, repressor, or initiator element binding protein. YY1 exerts its effects on genes involved in various biological processes via its ability to initiate, activate, or repress transcription depends on the context to which it binds, directly or indirectly via cofactor recruitment (1). YY1-deficient embryos die at the time of implantation, indicating that YY1 plays an indispensable role in embryonic development (2). Nowadays, YY1 has already been known to have a fundamental role in normal biological processes such as embryogenesis, differentiation, DNA replication, and cellular proliferation. Additionally, YY1 has also been shown to be involved in neuronal differentiation as its heterozygote knockout mice displayed significant growth retardation and neurological defects. Recently, YY1 has been reported to possess a potential function in cancer biology. It is important to explain the biological function of YY1 via studying the putative interactions between YY1 and cell cycle regulators, death genes, and furthermore, transcription factors and cofactors in the suppression or progression of various physiological phenomena.

Transcription factor p73 is a structural homologue of tumor suppressor gene p53 and plays an important role in tumorigenesis, cellular differentiation and development. E2F1, a transcription factor known to induce apoptosis via p53-dependent and p53-independent pathways (3), has been identified as a regulation factor for p73 transcription (3). However, transcriptional regulation factors of the p73 promoter, excepting E2F1, remain unknown. Identification of other regulators that might control p73 is needed to understand its functions and roles in tumorigenesis, differentiation and development. Here we identified the transcription factor YY1 as a novel regulator of p73 transcription. More importantly, we showed that YY1 cooperates with E2F1 to induce the transcriptional activity of p73 promoter, indicating the possible role of YY1 and p53 family pathway on the tumorigenesis, development, differentiation.

RESULTS AND DISCUSSION

To elucidate novel functions of YY1, we generated siRNA expression vectors targeted against YY1 for gene knockdown experiments. Compared with the enhancing effect of siYY1 expression vectors on p53-regulated p21 promoter/luciferase reporter by releasing YY1-dependent p53 inhibition, we found that knockdown of YY1 resulted in the 60% reduction of p73 promoter/luciferase reporter activity in both HCT116 and U2OS cells (Figure1). Consistently, overexpression of YY1, as well as E2F1, increased the activity of the p73 luciferase reporter vectors in a plasmid-dose dependent manner. Together, the results from knockdown and overexpression experiments of YY1 had shown the unsuspected role of YY1 in regulating p73 transcription level, specifically, the possibility that YY1 upregulate the p73 promoter activity.

On the other hand, it has been reported that transcription factor E2F1 could be recruited on the p73 promoter and activate its transcription (3). To further reveal the regulatory mechanism of p73 by YY1, we overexpressed YY1 and E2F1 and found out that compared to the overexpression of either YY1 or E2F1 alone, the transcriptional activity of p73 was further enhanced when both of them were overexpressed together, suggesting that YY1 could regulate p73 promoter activity in a synergistic fashion with E2F1.
Next we addressed whether the cooperative effect of YY1 and E2F1 on the p73 promoter is implicated in physiological events. As reported previously, the p73 promoter was activated by doxorubicin (a DNA damaging agent; a topoisomerase II inhibitor) and the event was dependent on E2F1. Under the treatment of doxorubicin, we observed that the activation of p73 promoter could be suppressed by 50% if the cells were transfected with siRNA vectors against YY1 or E2F1, indicating that cooperative action between the constitutive YY1 and the inducible E2F1 contributes to the activation of the p73 promoter on treatment with doxorubicin. Furthermore, immunofluorescence staining and immunoprecipitation experiments revealed that YY1 and E2F1 sublocalized together mainly in the nuclei, and associated physically.

Our data presented here demonstrated two novel roles of YY1 on tumorigenesis. First, YY1 could function as a cooperator of E2F1, which is known to mediate both apoptosis and cellular proliferation. Cooperation E2F1 and YY1 may be at least partially responsible for determining the physiological function (i.e. oncogenic or tumor suppressive function) of E2F1 by modulating specificity and sensitivity of E2F1 on different promoter. Second, YY1 could be involved in the regulation of p73. Recently, it has been reported that p73 could promote cellular growth in a synergistic manner with the proto-oncogene c-Jun (4). Our data that tumor activator gene YY1 upregulates p73 transcription is also consistent with this result. Noteworthy, YY1 has been reported to suppress tumor suppressor gene p53 (5). Therefore, YY1 might contribute to tumorigenesis by modulating p53 family network, i.e., by suppressing p53 pathway and activating p73 pathway.

CONCLUSION

Altogether, our results uncovered a novel function of YY1 on the E2F1-mediated p73 regulation. We summarized a model for the upregulation of the transcriptional activity of p73 by cooperation of YY1 and E2F1 in Figure 2. The comprehensive analysis of the YY1 network will help to clarify, and finally to understand its complex biological function, especially in concern of apoptosis, tumorigenesis, development and differentiation.

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