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Award Number: DAMD17-96-1-6076

TITLE: Stimulation of p53-Dependent Transcription by the Growth Suppressor, c-Abl

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REPORT DATE: July 2001

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 2001	Final (01 Jul 96 - 31 May 01)

4. TITLE AND SUBTITLE
Stimulation of p53-Dependent Transcription by the Growth Suppressor, c-Abl

5. FUNDING NUMBERS
DAMD17-96-1-6076

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

Although it is known that c-Abl stimulates p53-dependent transcription, a function required for c-Abl growth suppressor activity, the molecular mechanism by which this occurs remain elusive. The results obtained with this grant award show that c-Abl interacts with the C-terminal regulatory domain of tetrameric form of p53 and functions to activate the p53 DNA-binding. In an effort to assess the mechanism of c-Abl activation, we also show that c-Abl activates p53 DNA-binding by stabilizing the p53-DNA complex. Collectively, these results suggest a model for c-Abl activation. In this model c-Abl activates latent p53 by relieving the C-terminal inhibitory domain of p53 and enhances p53 DNA-binding by forming a stable p53-DNA complex (see text).

14. SUBJECT TERMS	15. NUMBER OF PAGES
Breast Cancer	17
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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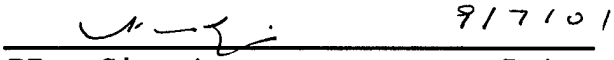

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INTRODUCTION

Loss of cell growth regulation is a characteristic of cancer cells. To achieve our goal of designing therapies for cancer, we must understand how cancer proteins affect cell growth. The aim of this proposal is to address this question for the cancer related proteins, p53 and c-Abl. Specifically, we have proposed:

To define the domains on p53 required for c-Abl binding

To examine the effect of c-Abl on the DNA-binding activity of p53

To characterize the effect of c-Abl on p53-dependent transcription *in vitro*

To determine the effect of Gal4-Abl on transcription from a promoter containing Gal4 sites

To examine whether general transcription factors are phosphorylated by c-Abl

PREVIOUS WORK AND ACCOMPLISHMENTS

When this grant was submitted in 1995, we had only a limited understanding of the regulation of p53-mediated transcription by cell cycle proteins. We proposed to study molecular mechanisms by which c-Abl activates p53-mediated transcription as I have observed, while I was a postdoctoral fellow in Arnold Berk's laboratory at UCLA, that c-Abl exhibits an p53-dependent growth suppression and enhances the transcriptional activity of p53 *in vivo*.

To address Specific Aim 1, we performed an extensive deletion and site-specific mutational analysis and showed two regions on p53 that are required for c-Abl interaction, an extreme C-terminal region (aa 363 to 393) and a tetramerization domain (aa 325 to 356). We also constructed a tetramerization impair mutant, 341K344E348E355K and showed that this mutant fails to bind to c-Abl, which demonstrates the requirement of the tetrameric conformation of p53 for c-Abl interaction (Nie et al). The C-terminal region was previously identified as a regulatory domain to inhibit the DNA binding activity of p53. Thus, we proposed a model in which c-Abl interacts with the negative regulatory domain to enhance the DNA binding activity of p53.

To address Specific Aim 2, we obtained highly purified c-Abl protein via a baculovirus expression system and showed that c-Abl stimulates the DNA-binding activity of full-length p53 but not a carboxyl terminal mutant p53, Δ363. This result suggested that interaction of c-Abl abolished the inhibitory effect of the C-terminal domain. In addition, we studied mechanisms for c-Abl activation and showed that c-Abl stimulates p53's DNA binding by decreasing the dissociation rate of the p53-DNA complex (Nie et al, 2000). The C-terminal regulatory domain was proposed by others to interact with a motif in the core DNA binding domain of the p53 tetramer, thereby forming a conformationally inactive complex. Despite compelling evidence for such a model, the motif on the core domain that interacts with the C-terminus remains to be identified. In addition, the increased association rate of p53 and DNA after disrupting the C-terminal inhibition has never been observed. An alternative explanation, therefore, is that the C-terminal domain may interfere with the tetramerization of p53, resulting in a less stable p53-DNA complex. To support this alternative model (Fig. 1), we first performed analytical ultracentrifugation and showed that interaction with c-Abl stabilized p53 tetrameric conformation. Second, we have shown that c-Abl stimulates p53 DNA binding only when all four quarter-sites on a p53 promoter are present. This was a very significant result to us, raising the possibility that in response to DNA damage, c-Abl may selectively stimulate p53 DNA binding on the promoters that contain four perfect quarter-sites (Wei and Liu, manuscript in preparation).

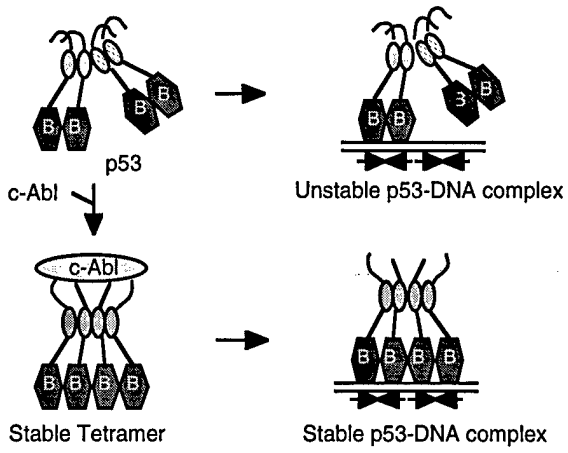

Figure 1. Proposed model of p53 activation by c-Abl. Green: the tetramer domain of p53; Red: the DNA binding domain of p53. Each arrow represents a quarter-site on a p53 promoter.

The goal of Specific Aim 3 was to study the effect of c-Abl on p53-dependent transcription and on the interaction of p53 with its functional transcription targets. We were hoping to identify p53's functional targets (such as general transcription factors or coactivators) that are responsible for c-Abl activation using a reconstitute transcription system. Through our experiments, we identified TFIID and IIA as p53 functional targets (results will be discussed later), but the effect of c-Abl on the interaction of p53 with TFIID or IIA was not observed. These results, together with our results described in Specific Aim 2, suggest that c-Abl affects DNA binding activity but not p53's ability to interact with transcription machinery. During the course of these studies, we did observe that c-Abl inhibits the ability of p300 to acetylate p53 in vitro (Xing and Liu, unpublished results). The significance of this finding is currently unclear.

In the face of the disappointment in identification of the c-Abl targets, we turned our efforts to the study of SV40 T antigen. Recently, we have shown that T antigen, under certain conditions, can repress p53-dependent transcription by a mechanism in which the transactivation domain of p53 is abrogated while DNA binding is unaffected (Sheppard et al 1999). Specifically, p53 purified as a complex with T antigen from mouse cells was found to bind DNA as a transcriptionally inactive intact complex, while that purified from human cells was found to bind DNA independently of T antigen and could activate p53-dependent transcription. Thus, we proposed a model for T antigen transformation (Fig. 2, Sheppard et al, 1999). In human cells, latent p53 and T antigen form a complex that is unable to bind to DNA. Upon activation of p53, a possible conformational change in p53 allows p53 to dissociate from the p53/T complex and bind to DNA. Therefore, p53-activated transcription that is required for growth arrest and apoptosis would not be lost. In mouse cells,

again latent p53 and T antigen form a complex that does not bind DNA. Upon activation, however, the p53-T complex binds DNA as a transcriptionally inactive complex. p53 responsive promoters therefore would be completely blocked. Because SV40 T antigen is known to cause tumors in rodents but has not been shown to be a complete carcinogen in humans, we speculate that our model may provide an explanation at the molecular level. Therefore, we decided to characterize the effect of T antigen on p53-dependent transcription *in vitro* and on the interaction of p53 with its functional targets, like we proposed with c-Abl.

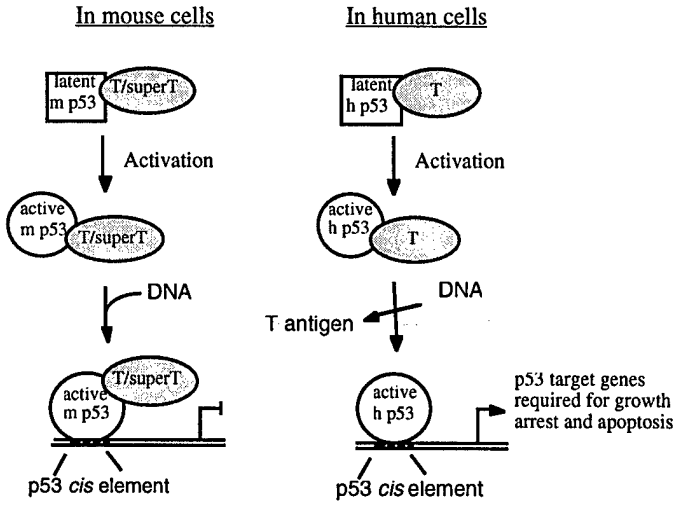

Figure 2. Proposed model of p53 inhibition by T antigen in mouse (m) and human (h) cells.

In Xing et al, 2001, we report that p53 stimulates transcription by enhancing the recruitment of the basal transcription factors TFIIA and TFIID on the promoter (the DA complex) and by inducing a conformational change in the DA complex. Significantly, we have demonstrated that T antigen inhibits p53-mediated transcription by blocking this ability of p53. We investigated the mechanism for this inhibition and found that the DA complex formation was resistant to T antigen repression when the TFIID-DNA complex was formed prior to the addition of the p53-T antigen complex, which indicates that the T antigen, once tethered to the promoter by p53, targets TFIID. Further, we have shown that the p53-T antigen complex prevents TBP from binding to the TATA box. Thus, these data suggest a detailed mechanism by which p53 activates transcription and by which T antigen inhibits p53-mediated transcription.

In addressing Specific Aim 4, we constructed Gal4-Abl construct and found that it is incapable of stimulating transcription from a promoter containing five Gal4 binding sites in a transcient transfection assay. The results suggest that c-Abl does not function as a transcription factor and again support the notion that c-Abl affects p53-mediated transcription via enhancing its DNA binding.

In addressing Specific Aim 5, we performed in vitro phosphorylation assay using purified p53 and c-Abl and found that c-Abl is incapable of phosphorylating p53, suggesting that c-Abl does not function as a kinase to activate p53-mediated transcription. These results are in agreement with data obtained in several other labs, supporting the notion that c-Abl affects p53-mediated transcription via enhancing its DNA binding.

KEY RESEARCH ACCOMPLISHMENTS

Although it is known that c-Abl stimulates p53-dependent transcription, a function required for c-Abl growth suppressor activity, the molecular mechanism by which this occurs remain elusive. The results obtained this year show that c-Abl interacts with the C-terminal regulatory domain of tetrameric form of p53 and functions to activate the p53 DNA-binding. In an effort to assess the mechanism of c-Abl activation, we also show that c-Abl activates p53 DNA-binding by stabilizing the p53-DNA complex. Collectively, these results suggest a model for c-Abl activation. In this model c-Abl activates latent p53 by relieving the C-terminal inhibitory domain of p53 and enhances p53 DNA-binding by forming a stable p53-DNA complex.

REPORTABLE OUTCOMES

Publications:

1. Song, XD, H.M. Sheppard, A.W. Norman and X. Liu. 1998. Mitogen-activated protein kinase is involved in the degradation of p53 protein in the bryostatin-1 induced differentiation of the acute promyelocytic leukemia cell line NB4. J. Biol. Chem. 274:1677-1682.
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CONCLUSIONS

The finding that c-Abl stimulates p53's DNA-binding via the negative regulatory domain may provide important clues about the regulation of p53. It has been suggested by other investigators that peptides designed to interact with this region can be used to reactivate p53 pathway in tumor cells to cause cell cycle arrest. In spite of the significance of this region, however, no growth regulatory protein has been shown to function directly via this region. In this study, we present results that clearly indicate a link between the growth suppressor c-Abl and p53 transcription regulation, and importantly, provide an example of activation of p53 DNA-binding activity via the carboxyl terminal regulatory domain by a cell-cycle protein.

APPENDICES

None

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domain and histone deacetylase activity. Presented at Presented at Keystone Symposium on Mechanism of Eukaryotic Transcription, Santa Fe, NM.

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