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TITLE:  Regulation of c-Myc mRNA by L11 in Response to UV and Gamma Irradiation

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The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.
In previous funding years, we have discovered a novel regulatory paradigm wherein L11 plays a critical role in controlling c-myc mRNA turnover via recruiting miR-24-loaded miRISC to the c-myc mRNA 3′-UTR in response to ribosomal stress. We also found that c-myc mRNA is down-regulated in response to DNA damage including UV and \( \gamma \)-irradiation in a L11-dependent manner. RNA-IP-RNAseq analysis identified that miR-130a as a L11-associated microRNA. We further showed that miR-130a directly targets c-myc mRNA. Overexpression of miR-130a mimics reduced the levels of c-myc mRNA whereas inhibiting miR-130a drastically induced the levels of c-myc mRNA. Also, overexpression of miR-130a reduced the luciferase activity driven by luciferase reporter containing the c-myc 3′-UTR and increased the association of Ago2/miRISC with c-myc mRNA. Interestingly, UV treatment enhances the association of L11, Ago2 as well as miR-130a with the c-myc mRNA. Together, our current results suggest that L11 may recruit miR-130a-loaded miRISC to mediate c-myc decay in response to DNA damage and implying that miR-130a may possesses a tumor suppressor function through down regulating c-Myc.
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A. INTRODUCTION

The c-Myc oncoprotein is deregulated in many human cancers. Thus, proper control of c-Myc level and activity is essential for normal cell growth and proliferation. We have previously identified that ribosomal protein L11 suppresses c-Myc transactivation activity (1,2) and reduces c-myc mRNA levels (3). Interestingly, c-myc mRNA is markedly reduced in response to ribosomal stress and L11 plays a novel and key role in mediating this ribosomal stress-induced c-myc mRNA turnover (4). Interestingly, c-Myc is also down-regulated in cells following DNA damage, such as those induced by ultraviolet (UV) and γ-irradiation (IR). The purpose of this proposal is to examine whether and how L11 is involved in the regulation of c-Myc in response to DNA damage. Specifically, we will determine whether L11 regulates c-myc mRNA levels and stability by recruiting miRNAs in response to DNA damage as well as the mechanism underlying the L11 regulation of c-myc mRNA. Results from these experiments would demonstrate an important function of L11 in regulating c-myc mRNA in response to DNA damage and offer useful information for developing anti-tumor drugs that target c-myc mRNA in cancers and thus have a significant impact on the understanding of c-Myc-induced tumorigenesis.

B. BODY

During the past funding years, we have found that L11 recruits microRNA (miRNA)-24 (miR-24) loaded RNA interference silencing complex (miRISC) to suppress c-myc mRNA expression. L11 binds to the c-myc mRNA at its 3'-untranslated region (3'-UTR). Overexpression of L11 suppresses the expression of luciferase mRNA and activity, whereas knockdown of L11 increases these levels and activity, in cells transfected with luciferase reporter containing the c-myc 3'-UTR (pGL3-myc 3'UTR), but not the control pGL3 vector. We further confirmed that L11 binds to the miRISC component Ago2 and miR-24. Knockdown of L11 rescued the c-myc mRNA reduction mediated by either overexpression of miR-24 or knockdown of Ago2, suggesting that L11 recruits miR24/miRISC to repress c-Myc. Interestingly, ribosomal stress induced by perturbation of ribosomal biogenesis results in a significant c-myc mRNA reduction in a L11-dependent manner in cells. L11 binding to c-myc mRNA, miR-24, and Ago2 was significantly increased following ribosomal stress. Together, our data identify a novel regulatory paradigm wherein L11 plays a critical role in controlling c-myc mRNA turnover via recruiting miRISC in response to ribosomal stress, thus ensuing a tight coordination between the levels and activity of c-Myc and ribosomal biogenesis. This work has been published in *Mol Cell Biol (2011)* (4).

In addition, we purified L11-associated-miRNAs and mRNAs from 293 cells using deep sequencing. Our initial results identified that L11 associates with a number of novel miRNAs (see below), including miR-130a in addition to miR-24, and mRNAs (including L11 itself, ctBP, Bel-2, etc.) Now we have evidence indicating that miR-130a may regulate c-Myc by directly targeting c-myc mRNA (See below). We are currently working on how L11-miR-130a pathway plays a role in DNA-damage-induced c-myc downregulation.

Specifically relating to the statement of Work (SOW) of this award, following points are either addressed or under planning:

**Aim 1. To determine if L11 regulates c-myc mRNA in response to UV and γ-IR.**

This aim has been **completed** (please see progress report from the previous years). Our results suggest that L11 plays an important role in regulating c-myc mRNA turnover in response to DNA damage.

**Aim 2. To examine if L11 recruits miRNA(s) to the 3'UTR of c-myc mRNA in response to UV and γ-IR.**

We have found that UV damage enhances L11 association with c-myc mRNA (task 2(I)). We also showed that L11 binding to the 3'-UTR of c-myc mRNA was induced by UV treatment, suggesting that L11 regulates c-myc mRNA levels by acting on c-myc 3'-UTR in response to UV-induced DNA damage (please see progress report from the previous years). During this funding year, we have focused on the role of miR-130a in regulating c-Myc levels following DNA damage. Our RNA-IP-RNAseq assays from 293 cells stably expressed Flag-L11 using anti-Flag antibody (task 2(S)) showed that miR-130a is one of the L11-associated miRNAs.
We further confirmed that L11 associates with miR-130a in cells, suggesting that L11 may recruit miR-130a to target c-myc mRNA (please see progress report from the previous years).

To further characterize the role of miR-130a in c-Myc regulation, we have performed experiments covered in tasks 2(2)-2(4). We focus on miR-130a instead of the proposed miR-145 or let-7, as miR-130a is potentially a tumor suppressive miRNA and therefore the finding of c-Myc targeting by miR-130a would be extremely novel. We first tested whether miR-130a targets c-myc mRNA. We have shown that overexpression of miR-130a mimics significantly reduced the levels of c-Myc protein and c-myc mRNA in U2OS cells (please see progress report from the previous years). We confirmed this finding in human normal fibroblast WI38 cells (Fig. 1). Consistently, inhibition of miR-130a using RNA inhibitor significantly increased the levels of c-Myc protein and c-myc mRNA (Fig. 2). We also showed that overexpression of miR-130a significantly reduced the luciferase activity in cells transfected with pGL3-myc 3’UTR, but not the control pGL3, reporter. Also, overexpression of miR-130a significantly increased the binding of Ago2 to the c-myc, but not GAPDH, mRNA (please see progress report from the previous years). Altogether, these data strongly indicate that miR-130a directly targets the c-myc mRNA to regulate the levels of c-Myc.

**Aim 3. To elucidate the mechanism underlying L11 regulation of c-myc mRNA in response to UV and γ-IR.**

To further examine whether miR-130a targets c-myc following UV treatment, we performed RNA-IP assays. Our initial results indicate that UV treatment significantly increased the binding of Ago2 to both c-myc mRNA (Fig. 3) and miR-130a (Fig. 4) in cells (as proposed in task 3(1)). Furthermore, UV treatment also significantly increased the binding of L11 to both c-myc mRNA in U2OS cells and luciferase mRNA containing c-myc 3’UTR in cells transfected with pGL3-myc 3’UTR reporter (please see progress report from the previous years). Consistently, UV treatment increased the interaction of L11 with Ago2 (Fig. 5). Interestingly, UV treatment induced the levels of miR-130a in cells (please see progress report from the previous years). Altogether, these data strongly suggest that UV treatment increases the recruitment of miR-130a-loaded miRISC to c-myc mRNA to suppress c-Myc expression.
In addition, we have performed miRNA microarray analysis to identify whether knockdown of L11 changes microRNA expression pattern in cells. We have found that 99 miRNAs (e.g. miR-4668-5p) were reduced whereas 79 (e.g. miR-5194) were increased by L11 knockdown (Fig. 6). We will analyze cancer-associated miRNAs (tumor suppressor and oncogenic miRNAs) among these miRNAs. These assays partially address the Task 2(4). We have also performed gene expression profiling using microarray analysis (Affymetrix Human PrimeView Array) by comparing the gene expression profiling of miRNA-130a inhibitor transfected cells with that of control scrambled RNA transfected cells. We are currently analyzing the data, hoping to find that miR-130a-regulated gene network is connected to Myc-regulated network, particularly genes involved in ribosome biogenesis. Regarding the regulation of L11-mediated c-myc mRNA decay by other L11-interacting proteins as proposed in Tasks 3(2)-3(4), we have found that L11 interacts with the microprocessor DGCR8/Drosha complex (please see progress report from the previous years).

For the extended one-year, we will further analyze how L11 is involved in regulation of the c-myc mRNA by the miR-130a-loaded miRISC in response to DNA damage, as proposed in Tasks 2(2) and 2(3). We will further characterize the interaction of L11 with the microprocessor DGCR8/Drosha complex and how this contributes to the L11 regulation of c-myc mRNA stability in response to DNA damage. Meanwhile we will identify additional proteins interacting with L11 and regulating c-myc mRNA stability as proposed in Tasks 3(2)-3(4).

C. KEY RESEARCH ACCOMPLISHMENTS:

1. L11 destabilizes c-myc mRNA via a miRNA-mediated pathway.
2. c-myc mRNA is reduced in response to DNA damage (UV or IR) and ribosomal stress.
3. DNA damage or ribosomal stress-induced c-myc mRNA downregulation requires L11.
4. miR-130a targets c-myc mRNA in cells.
5. UV treatment increased the levels of miR-130a expression
6. UV treatment increased the recruitment of miR-130a-loaded miRISC to the c-myc mRNA.
7. L11 interacts with the miRNA microprocessor complex Drosha-DGCR8.

D. Reportable Outcomes.

1. Manuscript: This award supports the following manuscripts:
2. Employment/training. This award supports one postdoctoral in the lab for his employment and training.

E. CONCLUSIONS

L11 plays an important role in c-myc downregulation in response to DNA damage, suggesting that microRNA-mediated c-myc mRNA decay is an important mechanism that coordinates ribosomal biogenesis and c-Myc activity during stress conditions.

F. REFERENCE.


**G. APPENDICES**


**H. SUPPORTING DATA**

N/A