Award Number:  
W81XWH-10-1-1029

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

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REPORT DATE:  
October 2012

TYPE OF REPORT:  
Annual

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

DISTRIBUTION STATEMENT:  
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**Abstract**

In previous funding year, 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 show that ribosome-free L11 binds to c-myc mRNA in the cytoplasm and this binding is enhanced in response to ribosomal stress. Meanwhile, we found that c-myc mRNA is also down-regulated in response to DNA damage including UV and γ-irradiation in an L11-dependent manner. In current funding year, we have explored the role of miR-130a in DNA damage-mediated c-myc down-regulation. We provided evidence indicating that miR-130a directly targets c-myc mRNA. 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 UV-induced DNA damage and implying that miR-130a may possesses a growth-inhibitory function through down regulating c-Myc.

**Subject Terms**

Ribosomal protein, L11, c-Myc, DNA damage, microRNA
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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 by treatment of ribosomal stress-inducing agents actinomycin D and 5-fluorouracil. During this funding period, we have found that L11 plays a novel and key role in mediating 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 UV and γ-IR 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 two funding years, we have characterized a novel mechanism of L11 regulation of c-myc mRNA stability. 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. We further demonstrated that it is the ribosome-free L11 that binds to the c-myc mRNA in the cytoplasm. Ribosomal stress triggers the release of L11 from the nucleolus to both the nucleoplasm, where it binds to c-Myc protein, and the cytoplasm, where it binds to c-myc mRNA. Importantly, we show that L11 regulation of c-myc mRNA is specific as several other tested ribosomal proteins do not regulates c-myc mRNA levels. Altogether, 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 have 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, Bcl-2, etc.) Now we have evidence indicating that miR-130a may regulate c-Myc by directly targeting c-myc mRNA (See below).

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 was completed during the first funding year (please see progress report for the previous year). We have also expanded the experiments by focusing on UV irradiation and confirmed that UV treatment decreases the levels of c-Myc protein and c-myc mRNA in a dose-dependent manner (data not shown). This reduction requires L11 as it was abolished by L11 knockdown in cells. Thus L11 plays an important role in regulating c-myc levels following DNA damage (please see last years’ progress report).

**Aim 2. To examine if L11 recruits miRNA(s) to the 3'UTR of c-myc mRNA in response to UV and γ-IR.**
During the first funding year, we have found that UV damage enhances L11 association with c-myc mRNA (task 2(1)). We further 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 last years’ progress report for 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 (part of task 2(5)) showed that miR-130a is one of the L11-associated miRNAs (Fig. 1). Bioinformatics prediction using TargetScan (http://www.targetscan.org) identified three putative seedless miR-130a-binding elements at the c-myc 3’-UTR (Fig. 2). We further confirmed the association of L11 with miR-130a in 293 and U2OS cells (Fig. 3), suggesting that L11 may recruit miR-130a to target c-myc mRNA.

To further characterize the role of miR-130a in c-Myc regulation, we have performed experiments covered in part 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. As shown in Fig. 4, overexpression of miR-130a mimics significantly reduced the levels of c-Myc protein and c-myc mRNA. To further test whether miR-130a targets c-myc 3’-UTR, we performed luciferase reporter assays. As shown in Fig. 5, overexpression of miR-130a significantly reduced the luciferase activity in cells transfected with pGL3-myc 3’UTR, but not the control pGL3, reporter plasmid. Also, Overexpression of miR-130a significantly increased the binding of Ago2 to the c-myc, but not GAPDH, mRNA (Fig. 6). Altogether, these data strongly indicate that miR-130a might directly target the c-myc mRNA to regulate the levels of c-Myc.

**Aim3. 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. 7) and miR-130a (not shown) in cells (as proposed in task 3(1)). Interestingly, UV treatment induced the levels of miR-130a in cells (Fig. 8), suggesting that the increased recruitment of miR-130a-loaded
miRISC to \textit{c-myc} mRNA might be due to increased levels of miR-130a expression. It is interesting to test whether L11 is required for this increase of miR-130a expression following UVC treatment, and if yes, whether L11 plays a role in the miR-130a biogenesis--- the processing of pri- or pri-miR-130a to mature miR-130a. Supporting the possible role of L11 in miRNA biogenesis, we have found that L11 interacts with DGCR8 (Fig. 9) and Drosha (Fig. 10), two core components of the microRNA microprocessor complex.

We are currently investigating the role of L11 in regulating miR-130a biogenesis in response to UV-induced DNA damage as well as its implication in miR-130a-mediated down-regulation of \textit{c-myc} upon UV treatment. We will also explore whether other RNA-binding proteins could participate in the L11 regulation of the \textit{c-myc} mRNA stability in response to UV treatment as proposed in aim3. We expect that at the end of the funding period, a manuscript summarizing above finding will be ready for publication.

C. \textbf{KEY RESEARCH ACCOMPLISHMENTS:}

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

D. \textbf{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. \textbf{CONCLUSIONS}

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

G. APPENDICES
The article by Challagundla KB et al. Mol Cell Biol, 31(19): 4007-4021 (2011) was attached in last years’ annual progress report.

H. SUPPORTING DATA
N/A