MOLECULAR & CELLULAR ONCOLOGY
2021, VOL. 8, NO. 1, e1842714 (3 pages)
https://doi.org/10.1080/23723556.2020.1842714

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AUTHOR'S VIEWS

The pan-cancer lncRNA MILIP links c-Myc to p53 repression

Yu Chen Feng[a], Xiao Hong Zhao[a], Liu Teng[b], Rick F. Thorne[b], Lei Jin[b,c], and Xu Dong Zhang[a,b]

[a]School of Biomedical Sciences and Pharmacy, The University of Newcastle, Callaghan, Australia; [b]Translational Research Institute, Henan Provincial People's Hospital and People's Hospital of Zhengzhou University, Academy of Medical Science, Zhengzhou University, Henan, China; [c]School of Medicine and Public Health, The University of Newcastle, Callaghan, Australia

ABSTRACT

We have recently identified the MYC proto-oncogene, bHLH transcription factor (MYC, best known as c-Myc)-responsive pan-cancer lncRNA c-Myc-Inducible Long noncoding RNA Inactivating P53 (MILIP) as an oncogenic driver. Our studies show that MILIP facilitates tumor protein p53 (TP53, best known as p53) turnover by reducing its SUMOylation through suppressing tripartite-motif family-like 2 (TRIML2), thus promoting cell survival, proliferation, and tumorigenicity. MILIP may thus represent an anti-cancer target for counteracting the c-Myc axis.

ARTICLE HISTORY
Received 20 October 2020
Revised 21 October 2020
Accepted 22 October 2020

KEYWORDS
c-Myc; p53; lncRNA; MILIP; pan-cancer

The proto-oncoprotein MYC proto-oncogene, bHLH transcription factor (MYC, best known as c-Myc) and the tumor suppressor tumor protein p53 (TP53, best known as p53) are inextricably linked as "Yin and Yang" partners in normal cells to maintain tissue homeostasis.[1] However, the regulatory interactions are not retained by cancer cells as evidenced by the often-imbalanced expression of c-Myc over wildtype p53.[2] Although p53 repression is frequently associated with the loss of cyclin-dependent kinase inhibitor 2A (CDKN2A, best known as ARF) tumor suppressor ($p14^{ARF}$ in human and $p19^{ARF}$ in mouse),[3] Feng *et al.* have recently reported an alternate mechanism whereby c-Myc inactivates p53 through the c-Myc-Inducible Long noncoding RNA (lncRNA) Inactivating P53 (MILIP) in the pan-cancer context,[4] uncovering an axis inhibiting p53 through a pan-cancer expressed lncRNA accomplice that links c-Myc to repression of p53.

Inspired by the findings that MILIP is one of the most upregulated lncRNA in 18 of 20 cancer types in relation to corresponding normal tissues in the Cancer Genome Atlas (TCGA) and that MILIP is transcriptionally activated by c-Myc, Feng *et al.* set to investigate its potential role in cancer pathogenesis. After confirming the upregulation of MILIP in independent cohorts of non-small cell lung carcinoma and colon cancer tissues, they conducted RNA-sequencing analysis and found that p53 signaling was the most enriched gene pathway resulting from MILIP silencing. In support, the p53 protein itself was upregulated when MILIP was silenced. Conversely, MILIP overexpression caused reduction in p53 expression. These results, along with the finding that c-Myc transcriptionally activates MILIP, suggest that, in contrast to upregulating p53 in normal cells,[5] c-Myc may inactivate p53 through MILIP in cancer cells. Indeed, c-Myc silencing upregulated p53, which was nevertheless abolished by MILIP overexpression. In contrast, c-Myc overexpression caused downregulation of p53 that was diminished by MILIP silencing. Thus, MILIP links c-Myc to inactivating p53.

Feng *et al.* then investigated the functional significance of MILIP. MILIP silencing retarded the tumorigenicity of A549 and MCF-7 cells *in vitro* and in A549 cancer xenograft models, whereas MILIP overexpression promoted clonogenicity of A549 and MCF-7 cells. As anticipated, MILIP silencing triggered apoptosis and cell cycle arrest at G0/G1 phase, functional characteristics of p53 activation.[6] In accord, co-silencing of p53 reversed the inhibitory effect of MILIP silencing on clonogenicity, consolidating that MILIP expression is integral for sustaining cancer cell survival and division through repressing p53. Similar to c-Myc, high MILIP expression was associated with poor overall survival in various cancer types, supporting the notion that MILIP upregulation contributes to c-Myc-driven cancer maintenance and progression. Importantly, although MILIP silencing did not influence the viability of normal human mammary epithelial cells, it decelerated anchorage-independent growth of normal cells caused by c-Myc overexpression in conjunction with knockdown of $p14^{ARF}$. In contrast, MILIP overexpression promoted the anchorage-independent growth of normal cells. Therefore, MILIP upregulation contributes to c-Myc-driven neoplastic transformation. In support, MILIP expression was increased in pre-neoplastic lesions (adenomas) compared with normal epithelia.

How does MILIP repress p53 expression? Feng *et al.* found that the increase in p53 protein expression caused by MILIP silencing was due to its prolonged half-life time. Although this was associated with reduction in p53 polyubiquitination, there were no significant alterations in the association between p53 and MDM2 proto-oncogene (MDM2), the major ubiquitin E3 ligase responsible for p53 ubiquitination.[7] Strikingly, MILIP appeared to be an RNA binding partner of p53 and a MILIP

CONTACT Lei Jin Lei.Jin@newcastle.edu.au; Xu Dong Zhang Xu.Zhang@newcastle.edu.au LS3-49, Life Science Building, University of Newcastle, Callaghan, NSW, Australia.

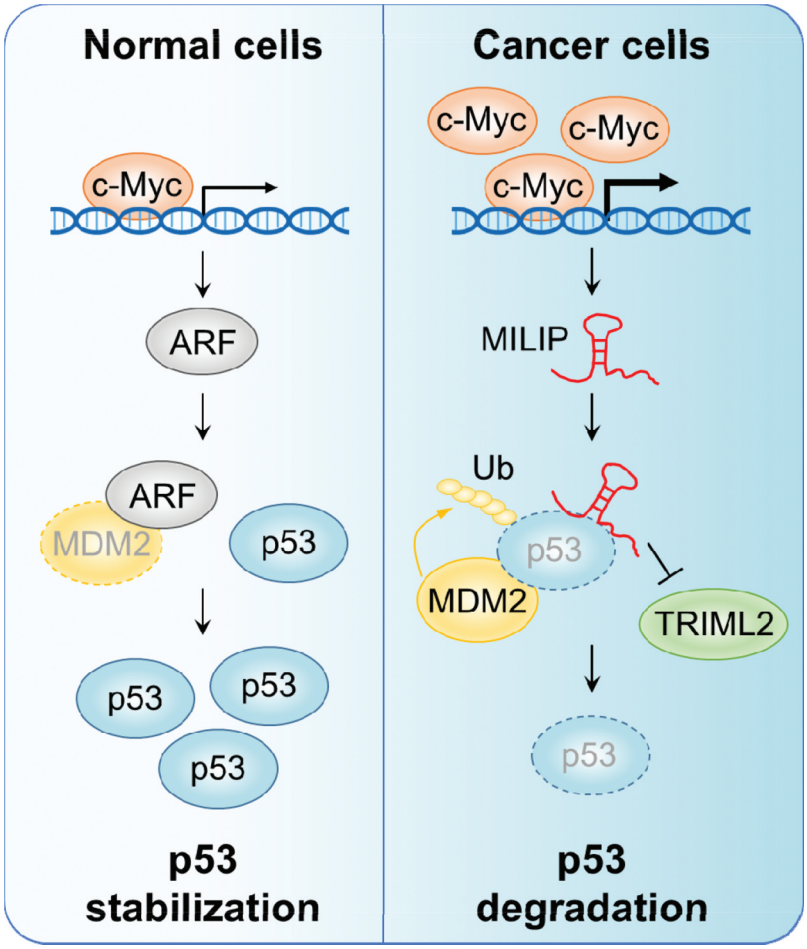

Figure 1. Molecular mechanisms underlying the differential impact of MYC proto-oncogene, bHLH transcription factor (MYC, best known as c-Myc) signaling on tumor protein p53 (TP53, best known as p53) expression in normal and cancer cells. In normal cells, physiological (relatively low) levels of c-Myc is sufficient for transcriptional activation of cyclin-dependent kinase inhibitor 2A (CDKN2A, best known as ARF) tumor suppressor that binds to and inhibits MDM2 proto-oncogene (MDM2), the major ubiquitin E3 ligase responsible for p53 polyubiquitination and degradation, leading to p53 activation. In contrast, oncogenic (relatively high) levels of c-Myc in cancer cells transcriptionally activates c-Myc-Inducible Long noncoding RNA (lncRNA) Inactivating P53 (MILIP), which compete with tripartite motif family like 2 (TRIML2) for binding to p53 resulting in decreases in p53 SUMOylation and occurrent increases in its polyubiquitination and subsequent proteasomal degradation.

mutant with the segment required for its binding to p53 deleted did not regulate p53 expression, suggesting that the direct interaction between MILIP and p53 is required for MILIP-mediated p53 repression.

To further understand the mechanism responsible for MILIP-mediated repression of p53, Feng *et al.* identified tripartite motif family like 2 (TRIML2) was one of the most upregulated genes encoding proteins that interact with p53.[8] Silencing of TRIML2 reduced p53 protein levels, proposing that TRIML2 may be involved in the regulation of p53 by MILIP. Consistent with its role as a SUMO E3 ligase,[8] TRIML2 bound to and modified p53 with SUMO-2/3. Nevertheless, TRIML2-mediated p53 SUMOylation was diminished by MILIP overexpression, whereas MILIP silencing increased modification of p53 by SUMO-2/3, which was counteracted by co-silencing of TRIML2. Therefore, MILIP negatively regulates p53 SUMOylation through suppressing TRIML2. Mechanistically, MILIP competes with TRIML2 for binding to p53. This was demonstrated by the findings that MILIP overexpression reduced the association between TRIML2 and p53, and in contrast, MILIP silencing increased the amount of TRIML2 co-immunoprecipitated with p53. On the other hand, TRIML2 overexpression reduced the amount of MILIP associating with p53, whereas its silencing increased binding of MILIP to p53.

In summary, Feng *et al.* have demonstrated that the pan-cancer lncRNA MILIP links c-Myc to repression of p53 (Figure 1). As a proto-oncoprotein, c-Myc is cast as an enigmatic actor, playing dualistic roles as both villain and hero. For the latter, c-Myc triggers activation of p53 through ARF, which serves as a key checkpoint to curb malignant transformation through induction of apoptosis.[5] Feng *et al.* establish that c-Myc inactivates p53 through MILIP,[4] providing an explanation as to how wild-type p53 can be repressed by c-Myc independently of the loss of ARF. Noticeably, $p14^{ARF}$ mRNA but not MILIP levels correlate with *MYC* gene expression in normal human tissues,[9] whereas Feng *et al.* found that MILIP is upregulated and its expression is positively associated with the levels of *MYC* expression in human cancers. Thus, it is conceivable that transcriptional activation of *MILIP* requires

oncogenic (relatively high) levels of c-Myc, whereas physiological (relatively low) levels of c-Myc are sufficient for transcriptional activation of $p14^{ARF}$ (Figure 1).[10]

Abbreviations

c-Myc	MYC proto-oncogene, bHLH transcription factor (MYC, best known as c-Myc)
p53	Tumor protein p53 (TP53, best known as p53)
ARF	Cyclin dependent kinase inhibitor 2A (CDKN2A, best known as ARF)
lncRNA	Long noncoding RNA
MILIP	c-Myc-Inducible Long noncoding RNA Inactivating P53
TCGA	The Cancer Genome Atlas
MDM2	MDM2 proto-oncogene
TRIML2	Tripartite motif family like 2

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

.This work was supported by the National Health and Medical Research Council (NHMRC) under Grants APP1147271, APP1162753, APP1177087; and Cancer Council NSW under Grant RG20-01

ORCID

Yu Chen Feng http://orcid.org/0000-0001-5507-8129
Rick F. Thorne http://orcid.org/0000-0001-7882-7081

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