AUTHOR'S VIEW

Complex I and MDM2: hit me baby one more time

Jerry Edward Chipuk^{a,b,c,d,e}

^aDepartment of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA; ^bDepartment of Dermatology, Icahn School of Medicine at Mount Sinai, New York, NY, USA; ^cThe Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA; ^dThe Graduate School of Biomedical Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA; ^eThe Diabetes, Obesity, and Metabolism Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

ABSTRACT

MDM2 (mouse double minute 2) functions as both a tumor suppressor and oncogene, yet little is known if MDM2 regulates cancer cell biology by altering cellular metabolism. We recently found that MDM2 binds NDUFS1 (NADH:ubiquinone oxidoreductase 75 kDa Fe-S protein 1), a key protein involved in Complex I assembly, function, and efficiency. The MDM2-NDUFS1 interaction promotes reactive oxygen species production, DNA damage, and apoptosis.

ARTICLE HISTORY

Received 5 April 2019

Revised 9 April 2019

Accepted 10 April 2019

KEYWORDS

Apoptosis; Complex I;
MDM2; mitochondria;
NDUFS1

Scientific investigations on a specific topic can initiate for many reasons, including: an interesting closely related literature, an upcoming funding opportunity, previous efforts in the laboratory, discussions with your mentor, *etc.* For the project we recently published and discuss here, the investigations began when “control” experiments did not produce the expected results.¹ Let me explain. About 15 y ago, I investigated how the tumor suppressor protein p53 (TP53, best known as p53) engaged the mitochondrial pathway of apoptosis via interactions with the BCL-2 (B-cell lymphoma 2) family of proteins.² Transient expression of p53 in a variety of transformed human and murine cell lines elicited marked apoptotic responses, and according to the literature, the negative regulator of p53, MDM2 (mouse double minute 2), should block these p53-mediated responses. Indeed, that was the case; however, expression of MDM2 alone, the “control” experiment, elicited a cell death response almost as robust as p53. This was observed in many cell lines, and minimal literature pointed to a mechanism explaining this curious result.

Over the years, we investigated the cellular requirements for MDM2-mediated cell death and discovered that a canonical pathway including multiple pro-apoptotic BCL-2 family members (*i.e.*, BCL-2 associated X protein, BAX; BCL-2 homologous antagonist killer, BAK; and BCL-2 interacting mediator of cell death, BIM) were responsible for initiating death; and as expected, anti-apoptotic BCL-2 proteins and the apoptotic caspases influenced cell death kinetics.¹ The source of stress linking MDM2 to the apoptotic machinery remained unknown to us; and we screened several stress signaling cascades (*e.g.*, the endoplasmic reticulum stress signaling pathway) for hints, and observed that MDM2 correlated with increased DNA damage accumulation that was independent of cell cycle arrest. As no pharmacological or radiation was added to the cells, the inducer of DNA damage had to be

cellular, and we turned to reactive oxygen species (ROS) generation as the mediator. Indeed, cellular ROS levels were increased upon MDM2 expression, and we identified that the amino-terminal domain of MDM2 as the culprit. Deeper investigations pointed to mitochondrial ROS generation; but again, what linked MDM2 to mitochondrial ROS generation remained a mystery. Several years past before discovering a paper that identified several MDM2-interacting proteins and a cohort of these proteins was localized within mitochondria.³ One of the proteins, NDUFS1 (NADH:ubiquinone oxidoreductase 75 kDa Fe-S protein 1), piqued our interest because of a role in Complex I assembly, efficiency, and function; so we investigated if MDM2 expression impacted on mitochondrial function.⁴ Our first experiments immediately revealed that MDM2 decreased mitochondrial respiration, and pointed to a loss in Complex I as the explanation.

These observations focused our investigations to interrogate and connect how MDM2 impacted upon Complex I to promote mitochondrial ROS generation, DNA damage, and apoptosis. We confirmed that MDM2 interacted with NDUFS1, and that this interaction was necessary for Complex I loss and mitochondrial ROS production. Furthermore, we identified a mutant of MDM2 (MDM2^{G581}) that exhibited reduced binding to NDUFS1, and subsequently, did not induce mitochondrial ROS production.¹ We honed into the mechanism by carefully examining a recent paper on NDUFS1 and Complex I assembly and function in neurons,⁵ and demonstrated that MDM2 could promote NDUFS1 accumulation in the cytosol. This result was key to our study because it allowed us to formulate the hypothesis that MDM2-mediated NDUFS1 cytosolic accumulation led to decreased super-complex assembly (*i.e.*, interactions between Complex I and Complex III that promote efficiency), ROS production, and apoptosis (Figure 1). Over the next year or so, we

Figure 1. MDM2 (mouse double minute 2) integrates cellular respiration and apoptotic signaling through NDUFS1 (NADH:ubiquinone oxidoreductase 75 kDa Fe-S protein 1) and the mitochondrial network. In the absence of MDM2, the nuclear-encoded protein NDUFS1 is imported into the mitochondrial network, integrates within Complex I (CI), and allows for super-complex assembly between Complexes I and III (CIII). This promotes efficient electron (e^-) transfer between CI and CIII, and maintains reactive oxygen species (ROS) production at basal levels – all of which do not perturb cell survival (*top panels*). When MDM2 is exogenously expressed, amplified, or present due to transgenes, NDUFS1 is sequestered in the cytoplasm by MDM2, which causes nascent CI to not integrate appropriately with CIII, leading to decreased e^- transfer efficiency, ROS production, and the accumulation of DNA damage. In numerous model systems, this can commit cells to the mitochondrial pathway of apoptosis (*bottom panels*). IMS: inner membrane space.

evaluated several gain-of-function and loss-of-function approaches to test the hypothesis, and the data continued to support our hypothesis.¹

Throughout the years of investigation on this project, I kept an open mind to model systems that allowed for us to demonstrate impact and broad relevance. As such, we corroborated our results in multiple model systems ranging from yeast, fruit flies, and transgenic mice. As yeast do not have a Complex I assembly machinery like metazoa, we speculated that MDM2 would have minimal impact on their mitochondrial functions – and indeed, MDM2 did not influence yeast growth or survival irrespective of mitochondrial requirements in either aspect.¹ On the contrary, fruit flies do have a NDUFS1 homolog (named ND75 for NADH dehydrogenase ubiquinone 75 kDa subunit), and MDM2 expression in the fly causes marked cellular stress and tissue ablation that is mediated by the apoptosis machinery. In the transgenic *Mdm2* mouse, we detected elevated mitochondrial ROS, decreased Complex I activity, and DNA damage accumulation compared to wild-type littermates.^{1,6} In our opinion, the transgenic *Mdm2* mouse somewhat reveals what may occur in human cancers that harbor amplified *HDM2* (human MDM2). Indeed, we compared many of the above phenotypes in a human sarcoma cell line with amplified *HDM2* and observed alterations in Complex I activity compared to non-*HDM2*-amplified sarcoma lines; and noted a curious observation that a small molecule antagonist (*i.e.*, Nutlin-3A) to *HDM2* that binds to the same region responsible for NDUFS1.⁷ Nutlin-3A treatment promoted Complex I dysfunction in a *HDM2* allele-dependent manner, and we discovered that Nutlin-3A promoted the MDM2–NDUFS1 interaction.

A few months before submitting our work, a manuscript was published that demonstrated MDM2 repressed the expression of *MT-ND6* (mitochondrially encoded NADH: Ubiquinone oxidoreductase core subunit 6), a critical component of Complex I.⁸ This study also showed that MDM2 entered mitochondria to repress mitochondrial gene expression, and this may be relevant to muscle endurance, hypoxia, and cellular migration/invasion. While mechanistically distinct, yet complementary in phenotype, these studies established an unexpected new literature on how the MDM2

protein can take several hits at Complex I with consequences reaching beyond the expected.⁹ In the context of normal physiology, gene amplification, metastasis, and quite possibly, pharmacological interventions aimed at the MDM2 pathway, we need further investigations into both the inducers and regulators of MDM2-mediated mitochondrial dysfunction, and given the number of mitochondrial proteins identified to interact with MDM2, and no mechanistic insights into these interactions, we are far from a complete understanding of the impact and value of this biology in human health and disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Funding

This work was supported by: NIH grants R01 CA157740 and R01 CA206005; the JJR Foundation, the William A. Spivak Fund, the Fridolin Charitable Trust, an American Cancer Society Research Scholar Award, a Leukemia & Lymphoma Society Career Development Award, and an Irma T. Hirsch/Monique Weill Caulier Trust Research Award. This work was also supported in part by two research grants (5FY1174 and 1FY13416) from the March of Dimes Foundation, the Developmental Research Pilot Project Program within the Department of Oncological Sciences at the Icahn School of Medicine at Mount Sinai, a Collaborative Pilot Award from the Melanoma Research Alliance, and the Tisch Cancer Institute Cancer Center Support Grant (P30 CA196521).

References

- Elkholi R, Abraham-Enachescu I, Trotta AP, Rubio-Patiño C, Mohammed JN, Luna-Vargas MPA, Gelles JD, Kaminetsky JR, Searinghe MN, Zou C, et al. MDM2 integrates cellular respiration and apoptotic signaling through NDUFS1 and the mitochondrial network. *Mol Cell*. 2019 Mar 9. pii: S1097-2765(19)30102-9. doi:10.1016/j.molcel.2019.02.012.
- Chipuk JE, Maurer U, Green DR, Schuler M. Pharmacologic activation of p53 elicits Bax-dependent apoptosis in the absence of transcription. *Cancer Cell*. 2003 Nov;4(5):371–381.
- Nicholson J, Scherl A, Way L, Blackburn EA, Walkinshaw MD, Ball KL, Hupp TR. A systems wide mass spectrometric based linear motif screen to identify dominant in-vivo interacting

- proteins for the ubiquitin ligase MDM2. *Cell Signal.* **2014** Jun;26(6):1243–1257. doi:[10.1016/j.cellsig.2014.02.011](https://doi.org/10.1016/j.cellsig.2014.02.011).
4. Janssen RJ, Nijtmans LG, van Den Heuvel LP, Smeitink JA. Mitochondrial complex I: structure, function and pathology. *J Inherit Metab Dis.* **2006** Aug;29(4):499–515. doi:[10.1007/s10545-006-0362-4](https://doi.org/10.1007/s10545-006-0362-4).
 5. Lopez-Fabuel I, Le Douce J, Logan A, James AM, Bonvento G, Murphy MP, Almeida A, Bolaños JP. Complex I assembly into supercomplexes determines differential mitochondrial ROS production in neurons and astrocytes. *Proc Natl Acad Sci U S A.* **2016** Nov 15;113(46):13063–13068. doi:[10.1073/pnas.1613701113](https://doi.org/10.1073/pnas.1613701113).
 6. Jones SN, Hancock AR, Vogel H, Donehower LA, Bradley A. Overexpression of Mdm2 in mice reveals a p53-independent role for Mdm2 in tumorigenesis. *Proc Natl Acad Sci U S A.* **1998** Dec 22;95(26):15608–15612.
 7. Vassilev LT, Vu BT, Graves B, Carvajal D, Podlaski F, Filipovic Z, Kong N, Kammlott U, Lukacs C, Klein C, et al. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. *Science.* **2004** Feb 6;303(5659):844–848. doi:[10.1126/science.1092472](https://doi.org/10.1126/science.1092472).
 8. Arena G, Cissé MY, Pyrdziak S, Chatre L, Riscal R, Fuentes M, Arnold JJ, Kastner M, Gayte L, Bertrand-Gaday C, et al. Mitochondrial MDM2 regulates respiratory complex I activity independently of p53. *Mol Cell.* **2018** Feb 15;69(4):594–609. doi:[10.1016/j.molcel.2018.01.023](https://doi.org/10.1016/j.molcel.2018.01.023).
 9. Rubio-Patiño C, Trotta AP, Chipuk JE. MDM2 and mitochondrial function: one complex intersection. *Biochem Pharmacol.* **2019** Apr;162:14–20. doi:[10.1016/j.bcp.2018.10.032](https://doi.org/10.1016/j.bcp.2018.10.032).