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The role of MDM2 in DNA damage signaling

by

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Submitted in partial fulfillment of the requirements for the degree of
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Thesis sponsor: Dr. Jill Bargonetti

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Date	Signature of Second Reader

Abstract

The role of MDM2 in DNA damage signaling

By

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Mouse double minute 2 (MDM2) is an E3 ubiquitin ligase that negatively regulates the tumor suppressor p53. MDM2 has tumorigenic capacity because of its ability to inhibit p53-mediated apoptosis and cell cycle arrest. In addition, MDM2 is known to function in tumorigenesis independently of p53. In particular, MDM2 is involved in DNA double-strand break (DSB) repair, due to its interaction with the Nbs1 subunit of the Mre11-Rad50-Nbs1 (MRN) complex. The MRN complex localizes to DSBs and recruits the DNA damage mediator ATM, which can then phosphorylate histone H2AX near the break, forming γH2AX. γH2AX acts as a signal for the localization of DNA repair factors. It has been shown that MDM2 overexpression causes delays in DSB repair, resulting in an increase of DSBs and γH2AX. Therefore, knockdown of MDM2 should lower γH2AX formation after DNA damage induction.

In order to study the role of MDM2 in DNA damage signaling, we applied DNA damaging agents on MDM2-overexpressing A875 melanoma and T47D breast cancer and their respective shMDM2 knockdown lines, and probed for γH2AX. We induced DNA damage by treating cells with the topoisomerase II inhibitor etoposide or the radiomimetic neocarzinostatin and then isolated chromatin-associated proteins. After Western blot analysis we observed that there was a statistically significant increase in γH2AX in the A875 vector control line treated with etoposide but not the shMDM2 knockdown line. Also, MDM2 knockdown did not lower γH2AX formation in T47D cells treated with etoposide. These results suggested that the knockdown of MDM2 may not reverse the effect of MDM2 overexpression on increased γH2AX levels in response to DNA damage. Instead, MDM2 may be affecting the recruitment of other

DNA repair factors, therefore contributing to genome stability. In the future, it will be interesting to study if MDM2 influences DNA repair pathway choice by blocking one pathway while activating another.

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I am also thankful for the support of the Bargonetti Lab members including Alla Polotskaia, Chong Gao, Gu Xiao, Avdar San, Jun Yeob Kim, Iffath Islam and Devon Lundine. They made my experience at the lab more rewarding by offering advice and help when I needed it most.

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Chapter 1: Introduction

1.1 MDM2

1.1.1 The discovery and functions of MDM2

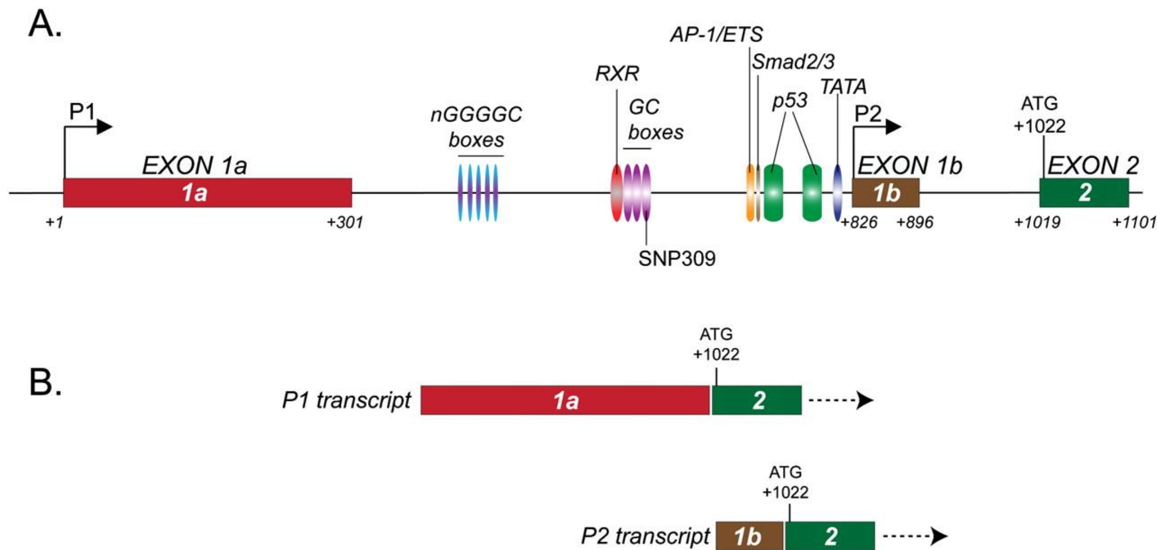
The mouse double minute 2 (*MDM2*) gene was discovered in the 3T3-DM cell, a spontaneously transformed derivative of the mouse 3T3 cell line, which exhibited double minute extrachromosomal DNA fragments (Cahilly-Snyder et al., 1987). MDM2 was shown to have tumorigenic potential by providing a growth advantage when overexpressed in 3T3 cells ((Fakharzadeh, Trusko, & George, 1991). The MDM2 protein was shown to bind and inhibit the transactivation functions of the tumor suppressor p53 (Momand et al., 1992). Later, MDM2 was shown to be an E3 ubiquitin ligase that could degrade p53 via the ubiquitin-proteasome system, further cementing the role of MDM2 as an oncogene (Haupt et al., 1997). Additionally, overexpression of MDM2 in mice with or lacking wild-type p53 leads to spontaneous tumor formation, suggesting that MDM2 has pro-tumorigenic functions independent of p53 (Jones et al., 1998).

1.1.2 Regulation of MDM2 expression.

The expression of MDM2 is in part regulated by p53 in a negative feedback loop in which p53 activates transcription of MDM2 (Juven et al., 1993). MDM2 is transcribed from two distinct promoters, the P1 promoter, which is responsible for basal transcription and the P2 promoter, which is inducible by p53 (Barak et al., 1994). These two promoters produce two distinct mRNA transcripts of different lengths (Figure 1) (Barak et al., 1994). Later, it was shown that a single nucleotide polymorphism (SNP) switch from thymine to guanine at position 309 (SNP309) results in increased expression of MDM2 due to binding of the transcription factor

Sp1 near the P2 promoter (Bond et al., 2004). Individuals with homozygous G/G at SNP309 have an increased likelihood to develop certain cancers due to MDM2 overexpression (Bond et al., 2004).

Figure 1 – Differential MDM2 transcription regulation.



Taken from (Manfredi, 2010). MDM2 is expressed from two distinct promoters. The P1 promoter is responsible for basal transcription of MDM2. The P2 promoter is inducible by transcription factors such as p53. The mRNA transcripts derived from the P1 and P2 promoters have 5' untranslated regions of different length.

1.1.3 MDM2 overexpression in cancer

MDM2 was shown to be highly expressed due to gene amplification in a variety of sarcomas, leading to inhibition of p53-mediated growth regulation (Oliner et al., 1992). Furthermore, experimental overexpression of MDM2 in mouse mammary tissue leads to formation of mammary tumors, suggesting that high levels of MDM2 expression has transformative properties in vivo (Lundgren et al., 1997). MDM2 is also overexpressed in a broad variety of human cancers such as breast and melanoma, and higher expression typically correlates with poorer patient survival (reviewed by Rayburn et al., 2005). In particular, MDM2

is overexpressed in estrogen receptor alpha positive ($ER\alpha_+$) breast cancers (Hori et al., 2002). $ER\alpha_+$ breast cancer cell lines such as T47D have high MDM2 levels due to transcription from the P2 promoter independent of p53 regulation (Phelps et al., 2003).

1.2 DNA damage

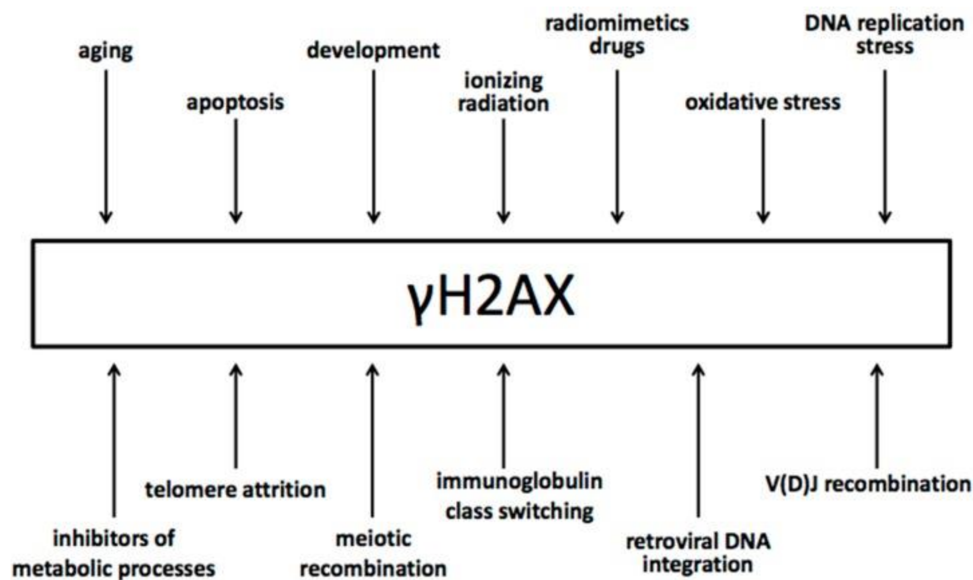
1.2.1 DNA damage and repair

DNA damage is a constant threat to both normal and cancerous cells and poses a risk to their genomic stability. Stress, toxins and radiation either indirectly or directly produce reactive oxygen species that can damage DNA and drive genome instability (reviewed by Levine et al., 2017). In particular, DNA double-strand breaks (DSBs) are especially dangerous due to the risk of major chromosomal damage or loss, and can be generated by radiation and certain chemotherapeutics (reviewed by Jekimovs et al., 2014). In order to deal with DNA DSBs (and DNA damage in general), organisms have evolved responses to detect and manage DNA lesions.

One such measure is the Mre11-Rad50-Xrs2 (MRX) complex, which was discovered in yeast to be important for the repair of DSBs during meiotic recombination (Usui et al., 1998). The MRX complex is homologous to the Mre11-Rad50-Nbs1 (MRN) complex in mammals. The MRN complex is required for activation of the DSB mediator ataxia telangiectasia mutated (ATM) in response to DNA damage (Uziel et al., 2003). ATM is responsible for phosphorylating a variety of proteins to initiate the DNA damage response (reviewed by Shiloh & Ziv, 2013) including CHK2, which prevents the cell from entering mitosis before the damage is repaired ((Matsuoka, Huang, & Elledge, 1998). Central to the DNA damage response is the phosphorylation by ATM of histone H2AX on serine 139 to form γH2AX (Burma et al., 2001). γH2AX acts as signal for the presence of DNA DSBs which need to be repaired (Rogakou et al.,

1998). It has been previously shown that MDM2 affects γH2AX formation in cells lacking p53 (Bouska et al., 2008). As detected by Western blot, γH2AX levels are higher in MDM2 overexpressing mouse embryonic fibroblasts (MEFs) exposed to gamma radiation compared to control MEFs, which shows that MDM2 plays a role in γH2AX signaling (Bouska et al., 2008). Thus, analyzing γH2AX formation in response to DNA damage could elucidate the role of MDM2 in the DNA damage response.

Figure 2 – Various exogenous and endogenous causes lead to formation of γH2AX.



Taken from (Georgoulis et al., 2017). Different factors and processes create DNA double-strand breaks that trigger phosphorylation of histone H2AX into γH2AX.

Chapter 2: Materials and Methods

2.1 Cell culture – A875 empty vector and constitutive shMDM2 cells were generated by Melissa Rosso and were obtained from shared laboratory stocks for use in this thesis (Rosso et al., 2015). T47D empty vector and constitutive shMDM2 cells were generated by Chong Gao from the Bargonetti lab and were a generous gift (unpublished). A875 cells and T47D cells were grown in DMEM (Cellgro) supplemented with 5% fetal bovine serum (Gemini), 50 U/ml penicillin and 50 ug/ml streptomycin (Mediatech) in a 5% CO_2, 37ºC humidified incubator.

2.2 Drug treatments – A875 and T47D cells were treated with either 10 µM etoposide (Sigma) for 3 hours or 100 ng/ml neocarzinostatin (Sigma) for 2 hours in fresh supplemented DMEM. A875 and T47D cells were harvested by scraping after treatment, washed in cold PBS and spun at 1100 rpm for 5 min at 4ºC to pellet the cells. Cell pellets were stored at -80ºC until histone extractions or chromatin fractionations were performed.

2.3 Histone extractions – Cell pellets were lysed in 1 mL of Triton extraction buffer (0.5% Triton X-100 (v/v), 2 mM phenylmethylsulfonyl fluoride, 0.02% sodium azide (w/v) in PBS) for 10 minutes in ice. After centrifugation at 1200 rpm for 10 minutes, the supernatant was discarded and the pellets were rotated overnight in 0.2 N hydrochloric acid with 1:100 phosphatase inhibitor cocktail (Sigma) at 4°C. The next day, the samples were centrifuged at 1200 rpm for 10 minutes, and the supernatant (histone extract) was stored at −20°C.

2.4 Chromatin fractionations – Cell pellets were lysed in Buffer A (10 mM HEPES, 10 mM KCl, 1.5 mM $MgCl_2$, 0.34 M sucrose, 10% glycerol, 1 mM DTT, 0.5 mM PMSF, 2 ug/ml Leupeptin, 8.5 ug/ml Aprotinin, 1:100 phosphatase inhibitor cocktail and 0.1% Triton X-100) on ice for 5 minutes. The solution was spun down at 3600 rpm for 5 minutes, and supernatant was

collected as the S1 cytosolic fraction. The pellet was washed twice in Buffer A, and the resulting pellet was resuspended in Buffer B (3 mM EDTA, 0.2 mM EGTA, 2 ug/ml Leupeptin, 8.5 ug/ml Aprotinin and 1:100 phosphatase inhibitor cocktail), and vortexed every 5 minutes for 30 minutes. The solution was spun down at 3600 rpm for 5 minutes, and supernatant was collected as the S2 nuclear soluble fraction. The pellet was washed twice in Buffer B and the resulting pellet was resuspended in Buffer B, sonicated 3 times for 30 seconds at 97 amplitude and collected as the P3 chromatin fraction. All samples were stored at −80°C.

2.5 Western blot analysis – To twenty microgram histone aliquots, 4X NuPAGE LDS buffer (Invitrogen) and DTT (Sigma) were added such that the working concentrations were 1X NuPAGE and 50 mM DTT. The samples were boiled at 70°C for 10 mins and iodoacetamide was added to reach 100 mM. The samples separated by either 10% or 4-12% (Invitrogen) SDS-PAGE, wet transferred to PVDF membranes, blocked in 5% nonfat milk in TBS with 0.1% Tween-20. The membranes were probed with primary antibodies overnight at 4°C. The next day, the membranes were washed in TBS with Tween-20, and probed with secondary antibodies at room temperature for 1 hour. The membranes were dried in a 37°C incubator and scanned using the Typhoon™ FLA 7000 (GE Healthcare). Images were analyzed using ImageQuant TL v8.1 software (GE Healthcare), ImageJ 1.50i (National Institutes of Health) and the data was graphed using Prism v7.0c (Graphpad Software).

2.6 Antibodies – Primary antibodies used for Western Blots were α-γH2AX (EMD Millipore 05-636), α-H2B (EMD Millipore 07-371), α-Lamin (Sigma L1293) and a mixture of α-MDM2 monoclonal antibodies 4B2, 2A9 and 4B11. Secondary antibodies used were α-mouse IgG-Cy3 (GE Healthcare) and α-rabbit IgG-Cy5 (GE Healthcare). Primary antibodies were of different animal origins and thus were probed together on the same membrane.

Chapter 3: The role of MDM2 in DNA damage signaling and repair.

3.1 Introduction

Recent studies are starting to uncover the role of MDM2 in increasing genome instability, which may be beneficial for the progression of certain cancers (reviewed by Saadatzadeh et al., 2017). MDM2 has been shown to act as E3 ubiquitin ligase to degrade proteins related to the DNA damage response and cell cycle control including the p53 family (p53, p63, p73), pRB and CHK2 (reviewed by Riley & Lozano, 2011). In addition to degrading proteins that control genome integrity, MDM2 was shown to bind the Nbs1 subunit of the MRN complex (Alt et al., 2005). Nbs1 is required for efficient recruitment of ATM to DSBs, which is important for the subsequent localization of DNA repair factors by ATM (Falck et al., 2005).

MDM2 overexpression was shown to delay the repair of DSBs induced by gamma irradiation in vitro (Bouska et al., 2008). The effects of MDM2 overexpression were abrogated by mutating the MDM2 residues that interact with Nbs1 (Bouska et al., 2008). After gamma irradiation, MDM2 overexpressing MEFs had higher γH2AX levels compared to controls (Bouska et al., 2008) which suggests that MDM2 may be affecting DNA damage signaling through Nbs1. However, it has not been shown if the knockdown of MDM2 reduces γH2AX formation after DNA damage induction. We tested the role of MDM2 in γH2AX formation by causing DNA damage in MDM2 overexpressing cells using the topoisomerase II inhibitor etoposide or the radiomimetic neocarzinostatin (NCS). A875 melanoma cells and T47D ERα+ breast cancer cells both have homozygous G/G SNP309 overexpression of MDM2 (Table 1). The extent of γH2AX formation in these cells was compared to A875 and T47D with MDM2 knockdown via constitutively expressed shRNA.

Table 1 – Cell lines used in this study.

Cell line	*p53* status	*MDM2* SNP309 status	Cell type
A875	Wild-type	G/G homozygous	Melanoma
T47D	Mutant	G/G homozygous	Breast cancer

3.2 Results

3.2.1 MDM2 knockdown lowers γH2AX formation in response to DNA damage in A875 cells but not T47D cells.

A875 and T47D cells and their respective MDM2 knockdown lines were used to compare the extent of γH2AX formation after DNA damage induction. In A875 vector control cells, etoposide treatment significantly increased γH2AX levels, however in the MDM2 knockdown line there was no significant increase (Figure 3A and B). This suggests that high levels of MDM2 were contributing to greater DNA damage and thus γH2AX formation in A875 cells. However, in T47D cells the initial one time data suggested that MDM2 overexpression before knockdown did not result in increased DNA damage as assessed by γH2AX levels. Both T47D vector control and MDM2 knockdown cells had similar levels of γH2AX with either etoposide or NCS treatment (Figure 3C and D). This suggested that MDM2 did not play a role in γH2AX signaling in T47D cells. Further testing of γH2AX levels in chromatin fractions showed that MDM2 knockdown did not lower γH2AX in T47D cells treated with etoposide, although there was a statistically significant increase in γH2AX in the untreated controls (Figure 3E and F). The level of MDM2 protein in untreated T47D cells was decreased by about 65% between the vector control and shMDM2 lines (Figure 3G).

Figure 3 – MDM2 knockdown lowers γH2AX formation in response to DNA damage in A875 cells but not T47D cells.

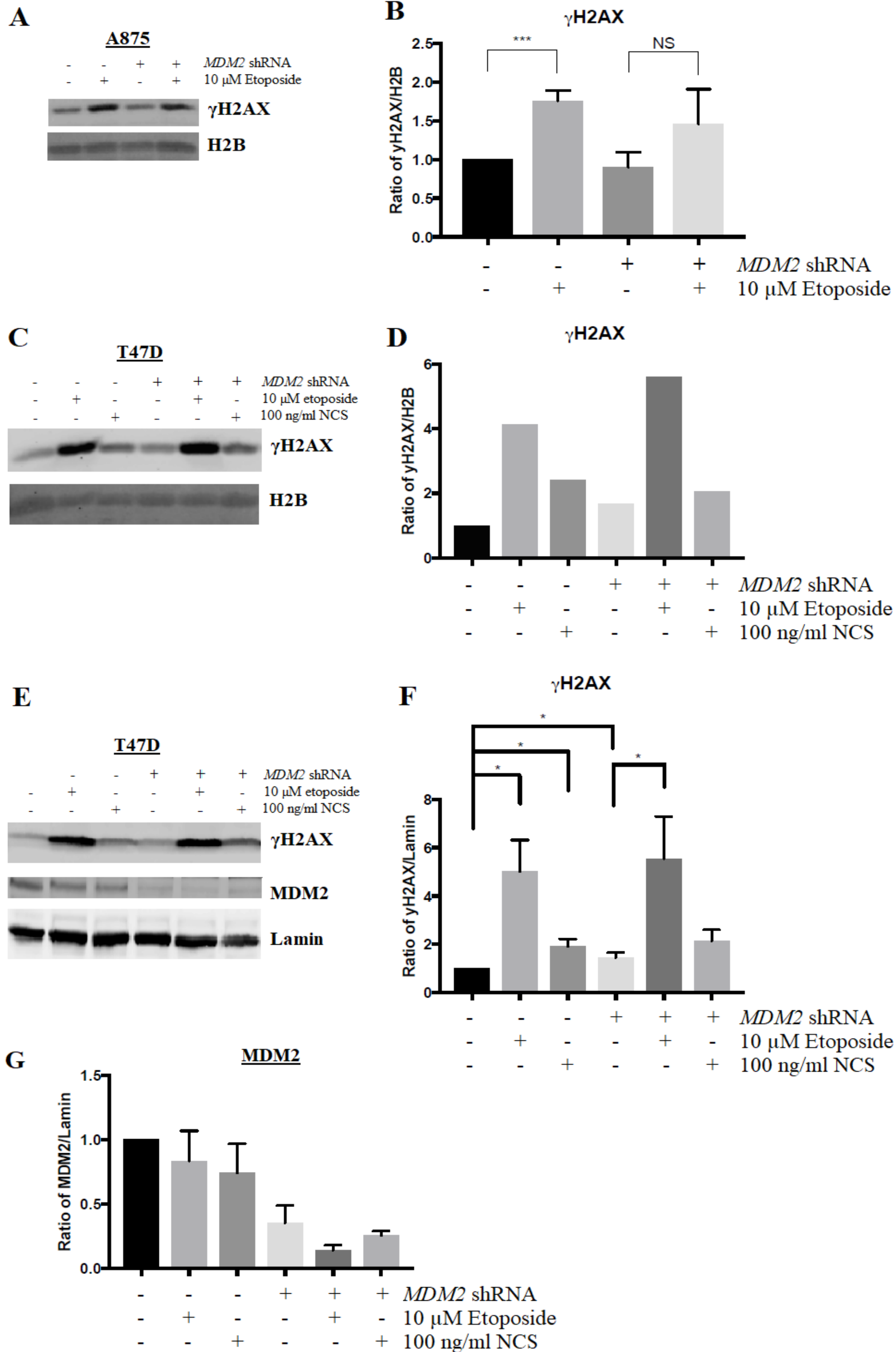


Figure 3 – A875 and T47D MLP vector and *MDM2* shRNA constitutive knockdown cells were treated with 10 μM etoposide for 3 hours or 100 ng/ml neocarzinostatin (NCS) for 2 hours. Cells were harvested and subjected to either histone extractions or chromatin fractionations. A, C) Histone extracts were run using SDS-PAGE and probed for γH2AX and H2B as a loading control. A representative image is shown. B) Relative intensity of γH2AX was normalized against H2B and averaged over 3 independent experiments. The non-significant p value was 0.118. D) Relative intensity of γH2AX was normalized against H2B in one experiment. E) T47D chromatin extracts were run using SDS-PAGE and probed for γH2AX, MDM2 (mix of 4B2, 2A9 and 4B11) and Lamin as a loading control. A representative image is shown. F) Relative intensity of γH2AX was normalized against Lamin and averaged over 3 independent experiments. G) Relative intensity of MDM2 in T47D cells was normalized against Lamin and averaged over 2 independent experiments. * represents a p value < 0.05. *** represents a p value < 0.0005. NS represents a non-significant p value > 0.05. Student's t test was used for statistical analysis.

3.3 Discussion

One of the ways MDM2 induces genome instability is by binding to Nbs1 and delaying DSB repair, leading to accumulation of DNA damage (reviewed by Eischen, 2016). Although γH2AX formation appears significantly different in A875 cells overexpressing MDM2 compared to the shRNA knockdown, it is not yet determined if and how it is biologically significant. While there was a statistically significant increase in γH2AX levels in A875 vector controls cells treated with etoposide, the same was not observed in A875 MDM2 knockdown cells (Figure 3B). However, the p value (0.118) for the A875 shMDM2 cells treated with etoposide approached statistical significance, suggesting that γH2AX formation is not affected by a decrease of MDM2 (Figure 3B). Therefore, it is possible that DNA damage signaling and repair mechanisms may still function normally even after MDM2 knockdown. Moreover, in T47D cells treated with etoposide, there were statistically significant increases of γH2AX in both vector control and shMDM2 lines (Figure 3F). This suggests that MDM2 knockdown does not lower γH2AX formation after DNA damage induction in cells overexpressing MDM2. Also, there was a statistically significant increase in γH2AX in the untreated T47D cells with MDM2 knockdown

compared to the vector control (Figure 3F), but it is not yet clear if this difference affects DNA repair. Although the MDM2 knockdown in T47D untreated cells was efficient (Figure 3G), it is possible that the decrease in MDM2 is not significant enough to lower γH2AX formation. γH2AX levels may not be meaningfully altered because other DNA repair mediators also phosphorylate H2AX, such as DNA-PK (An et al., 2010). The lack of a decrease of γH2AX levels in T47D cells with MDM2 knockdown suggests that MDM2 may weaken genome stability through targets other than Nbs1. It is possible that γH2AX formation is not particularly important for proper DNA repair in MDM2 overexpressing cancers and that other pathways decrease genome stability. As an alternative, the increase of γH2AX formation due to MDM2 overexpression (Bouska et al., 2008) could instead be acting as a more robust signal to recruit a different variety of DNA repair factors. In order to clarify the role of MDM2 overexpression in DNA repair, it would be necessary to identify which DNA repair factors associate at DSBs and test if these factors are responsible for modifying DNA repair pathway choice.

Chapter 4: Future directions

MDM2 overexpression appears to increase γH2AX in response to DNA damage in A875 cells but not T47D cells. However, it is still not known if MDM2 alters DNA repair pathway choice. Cells use two major repair pathways to resolve DSBs, nonhomologous end-joining (NHEJ) and homologous recombination (HR), with the former being more error-prone than the latter (reviewed by Jekimovs et al., 2014). It would be interesting to see if MDM2 overexpression creates bias towards one of these DNA repair mechanisms. Since the pro-HR factor BRCA1 and the pro-NHEJ factor 53BP1 antagonize each other (Bunting et al., 2010), it is

possible to determine if MDM2 affects the choice between HR and NHEJ. In order to test this, knocking down either BRCA1 or 53BP1 with shRNA and quantifying the extent of DNA damage should show if MDM2 overexpression supports one pathway over another. Another DSB repair pathway, microhomology-mediated end joining (MMEJ), is an alternative repair pathway that cells can use but is highly mutagenic (Sinha et al., 2017). It was shown that ATM represses MMEJ, suggesting that ATM may protect genome integrity by inhibiting this type of DNA repair (Rahal et al., 2010). Interestingly, MMEJ requires the nuclease function of Mre11 of the MRN complex, which MDM2 may alter due to its interaction with Nbs1 (Truong et al., 2013). If MDM2 overexpression increases the use of MMEJ, then inhibiting the nuclease activity of Mre11 by mutation and testing if MMEJ is still active should show if MDM2 is involved in this pathway. In conclusion, the role of MDM2 in genome instability still has not been explored thoroughly but could be improved by studying predispositions in DNA repair pathway choice.

Chapter 5: Bibliography

Alt, J. R., Bouska, A., Fernandez, M. R., Cerny, R. L., Xiao, H., & Eischen, C. M. (2005). Mdm2 binds to Nbs1 at sites of DNA damage and regulates double strand break repair. *The Journal of Biological Chemistry*, *280*(19), 18771–18781. https://doi.org/10.1074/jbc.M413387200

An, J., Huang, Y.-C., Xu, Q.-Z., Zhou, L.-J., Shang, Z.-F., Huang, B., … Zhou, P.-K. (2010). DNA-PKcs plays a dominant role in the regulation of H2AX phosphorylation in response to DNA damage and cell cycle progression. *BMC Molecular Biology*, *11*, 18. https://doi.org/10.1186/1471-2199-11-18

Barak, Y., Gottlieb, E., Juven-Gershon, T., & Oren, M. (1994). Regulation of mdm2 expression by p53: alternative promoters produce transcripts with nonidentical translation potential. *Genes & Development*, *8*(15), 1739–1749.

Blackford, A. N., & Jackson, S. P. (2017). ATM, ATR, and DNA-PK: The Trinity at the Heart of the DNA Damage Response. *Molecular Cell*, *66*(6), 801–817. https://doi.org/10.1016/j.molcel.2017.05.015

Bond, G. L., Hu, W., Bond, E. E., Robins, H., Lutzker, S. G., Arva, N. C., … Levine, A. J. (2004). A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. *Cell*, *119*(5), 591–602. https://doi.org/10.1016/j.cell.2004.11.022

Bouska, A., Lushnikova, T., Plaza, S., & Eischen, C. M. (2008). Mdm2 promotes genetic instability and transformation independent of p53. *Molecular and Cellular Biology*, *28*(15), 4862–4874. https://doi.org/10.1128/MCB.01584-07

Bunting, S. F., Callén, E., Wong, N., Chen, H.-T., Polato, F., Gunn, A., … Nussenzweig, A. (2010). 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. *Cell*, *141*(2), 243–254. https://doi.org/10.1016/j.cell.2010.03.012

Burma, S., Chen, B. P., Murphy, M., Kurimasa, A., & Chen, D. J. (2001). ATM phosphorylates histone H2AX in response to DNA double-strand breaks. *The Journal of Biological Chemistry*, *276*(45), 42462–42467. https://doi.org/10.1074/jbc.C100466200

Cahilly-Snyder, L., Yang-Feng, T., Francke, U., & George, D. L. (1987). Molecular analysis and chromosomal mapping of amplified genes isolated from a transformed mouse 3T3 cell line. *Somatic Cell and Molecular Genetics*, *13*(3), 235–244.

Eischen, C. M. (2017). Role of Mdm2 and Mdmx in DNA repair. *Journal of Molecular Cell Biology*, *9*(1), 69–73. https://doi.org/10.1093/jmcb/mjw052

Fakharzadeh, S. S., Trusko, S. P., & George, D. L. (1991). Tumorigenic potential associated with enhanced expression of a gene that is amplified in a mouse tumor cell line. *The EMBO Journal*, *10*(6), 1565–1569.

Falck, J., Coates, J., & Jackson, S. P. (2005). Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. *Nature*, *434*(7033), 605–611. https://doi.org/10.1038/nature03442

Georgoulis, A., Vorgias, C. E., Chrousos, G. P., & Rogakou, E. P. (2017). Genome Instability and γH2AX. *International Journal of Molecular Sciences*, *18*(9). https://doi.org/10.3390/ijms18091979

Haupt, Y., Maya, R., Kazaz, A., & Oren, M. (1997). Mdm2 promotes the rapid degradation of p53. *Nature*, *387*(6630), 296–299. https://doi.org/10.1038/387296a0

Hori, M., Shimazaki, J., Inagawa, S., Itabashi, M., & Hori, M. (2002). Overexpression of MDM2 oncoprotein correlates with possession of estrogen receptor alpha and lack of MDM2 mRNA splice variants in human breast cancer. *Breast Cancer Research and Treatment*, *71*(1), 77–83.

Jekimovs, C., Bolderson, E., Suraweera, A., Adams, M., O’Byrne, K. J., & Richard, D. J. (2014). Chemotherapeutic Compounds Targeting the DNA Double-Strand Break Repair Pathways: The Good, the Bad, and the Promising. *Frontiers in Oncology*, *4*. https://doi.org/10.3389/fonc.2014.00086

Jones, S. N., Hancock, A. R., Vogel, H., Donehower, L. A., & Bradley, A. (1998). Overexpression of Mdm2 in mice reveals a p53-independent role for Mdm2 in tumorigenesis. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(26), 15608–15612.

Juven, T., Barak, Y., Zauberman, A., George, D. L., & Oren, M. (1993). Wild type p53 can mediate sequence-specific transactivation of an internal promoter within the mdm2 gene. *Oncogene*, *8*(12), 3411–3416.

Kleiner, R. E., Verma, P., Molloy, K. R., Chait, B. T., & Kapoor, T. M. (2015). Chemical proteomics reveals a γH2AX-53BP1 interaction in the DNA damage response. *Nature Chemical Biology*, *11*(10), 807–814. https://doi.org/10.1038/nchembio.1908

Levine, A. S., Sun, L., Tan, R., Gao, Y., Yang, L., Chen, H., … Lan, L. (2017). The oxidative DNA damage response: A review of research undertaken with Tsinghua and Xiangya students at the University of Pittsburgh. *Science China. Life Sciences*, *60*(10), 1077–1080. https://doi.org/10.1007/s11427-017-9184-6

Lundgren, K., Montes de Oca Luna, R., McNeill, Y. B., Emerick, E. P., Spencer, B., Barfield, C. R., … Finlay, C. A. (1997). Targeted expression of MDM2 uncouples S phase from mitosis and inhibits mammary gland development independent of p53. *Genes & Development*, *11*(6), 714–725.

Manfredi, J. J. (2010). The Mdm2-p53 relationship evolves: Mdm2 swings both ways as an oncogene and a tumor suppressor. *Genes & Development*, *24*(15), 1580–1589. https://doi.org/10.1101/gad.1941710

Matsuoka, S., Huang, M., & Elledge, S. J. (1998). Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. *Science (New York, N.Y.)*, *282*(5395), 1893–1897.

Momand, J., Zambetti, G. P., Olson, D. C., George, D., & Levine, A. J. (1992). The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. *Cell*, *69*(7), 1237–1245.

Nayak, M. S., Yang, J.-M., & Hait, W. N. (2007). Effect of a single nucleotide polymorphism in the murine double minute 2 promoter (SNP309) on the sensitivity to topoisomerase II-targeting drugs. *Cancer Research*, *67*(12), 5831–5839. https://doi.org/10.1158/0008-5472.CAN-06-4533

Oliner, J. D., Kinzler, K. W., Meltzer, P. S., George, D. L., & Vogelstein, B. (1992). Amplification of a gene encoding a p53-associated protein in human sarcomas. *Nature*, *358*(6381), 80–83. https://doi.org/10.1038/358080a0

Phelps, M., Darley, M., Primrose, J. N., & Blaydes, J. P. (2003). p53-independent activation of the hdm2-P2 promoter through multiple transcription factor response elements results in elevated hdm2 expression in estrogen receptor alpha-positive breast cancer cells. *Cancer Research*, *63*(10), 2616–2623.

Rahal, E. A., Henricksen, L. A., Li, Y., Williams, R. S., Tainer, J. A., & Dixon, K. (2010). ATM regulates Mre11-dependent DNA end-degradation and microhomology-mediated end joining. *Cell Cycle (Georgetown, Tex.)*, *9*(14), 2866–2877. https://doi.org/10.4161/cc.9.14.12363

Rayburn, E., Zhang, R., He, J., & Wang, H. (2005). MDM2 and human malignancies: expression, clinical pathology, prognostic markers, and implications for chemotherapy. *Current Cancer Drug Targets*, *5*(1), 27–41.

Riley, M. F., & Lozano, G. (2012). The Many Faces of MDM2 Binding Partners. *Genes & Cancer*, *3*(3–4), 226–239. https://doi.org/10.1177/1947601912455322

Rogakou, E. P., Pilch, D. R., Orr, A. H., Ivanova, V. S., & Bonner, W. M. (1998). DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *The Journal of Biological Chemistry*, *273*(10), 5858–5868.

Rosso, M. (2015). Exploring Chromatin-bound MDM2 Functions in Compromised Transcriptional Regulation of p53 Target Genes. *All Graduate Works by Year: Dissertations, Theses, and Capstone Projects*. Retrieved from https://academicworks.cuny.edu/gc_etds/1112

Rosso, M., Polotskaia, A., & Bargonetti, J. (2015). Homozygous mdm2 SNP309 cancer cells with compromised transcriptional elongation at p53 target genes are sensitive to induction of p53-independent cell death. *Oncotarget*, *6*(33), 34573–34591.

Saadatzadeh, M. R., Elmi, A. N., Pandya, P. H., Bijangi-Vishehsaraei, K., Ding, J., Stamatkin, C. W., … Pollok, K. E. (2017). The Role of MDM2 in Promoting Genome Stability versus Instability. *International Journal of Molecular Sciences*, *18*(10). https://doi.org/10.3390/ijms18102216

Shiloh, Y., & Ziv, Y. (2013). The ATM protein kinase: regulating the cellular response to genotoxic stress, and more. *Nature Reviews. Molecular Cell Biology*, *14*(4), 197–210.

Sinha, S., Li, F., Villarreal, D., Shim, J. H., Yoon, S., Myung, K., … Lee, S. E. (2017). Microhomology-mediated end joining induces hypermutagenesis at breakpoint junctions. *PLoS Genetics*, *13*(4), e1006714. https://doi.org/10.1371/journal.pgen.1006714

Truong, L. N., Li, Y., Shi, L. Z., Hwang, P. Y.-H., He, J., Wang, H., … Wu, X. (2013). Microhomology-mediated End Joining and Homologous Recombination share the initial end resection step to repair DNA double-strand breaks in mammalian cells. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(19), 7720–7725. https://doi.org/10.1073/pnas.1213431110

Usui, T., Ohta, T., Oshiumi, H., Tomizawa, J., Ogawa, H., & Ogawa, T. (1998). Complex formation and functional versatility of Mre11 of budding yeast in recombination. *Cell*, *95*(5), 705–716.

Uziel, T., Lerenthal, Y., Moyal, L., Andegeko, Y., Mittelman, L., & Shiloh, Y. (2003). Requirement of the MRN complex for ATM activation by DNA damage. *The EMBO Journal*, *22*(20), 5612–5621. https://doi.org/10.1093/emboj/cdg541