

West Chester University Digital Commons @ West Chester University

Biology Faculty Publications

Biology

2012

Cytoplasmic sequestration of the tumor suppressor p53 by a heat shock protein 70 family member, mortalin, in human colorectal adenocarcinoma cell lines

Erin E. Gestl

West Chester University of Pennsylvania, egestl@wcupa.edu

S. Anne Boettger

West Chester University of Pennsylvania, sboettger@wcupa.edu

Follow this and additional works at: http://digitalcommons.wcupa.edu/bio_facpub

 Part of the [Cancer Biology Commons](#)

Recommended Citation

Gestl, E. E., & Boettger, S. A. (2012). Cytoplasmic sequestration of the tumor suppressor p53 by a heat shock protein 70 family member, mortalin, in human colorectal adenocarcinoma cell lines. *Biochemical and Biophysical Research Communications*, 423(2), 411-416. <http://dx.doi.org/http://dx.doi.org/10.1016/j.bbrc.2012.05.139>

This Article is brought to you for free and open access by the Biology at Digital Commons @ West Chester University. It has been accepted for inclusion in Biology Faculty Publications by an authorized administrator of Digital Commons @ West Chester University. For more information, please contact wcressler@wcupa.edu.

Contents lists available at [SciVerse ScienceDirect](http://www.sciencedirect.com)

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Cytoplasmic sequestration of the tumor suppressor p53 by a heat shock protein 70 family member, mortalin, in human colorectal adenocarcinoma cell lines

Erin E. Gestl¹, S. Anne Böttger*

Department of Biology, West Chester University, 750 S Church Street, West Chester, PA 19383, USA

ARTICLE INFO

Article history:
Received 25 May 2012
Available online xxx

Keywords:
Tumor suppressor
Non-mutational inactivation
Heat shock proteins
Mortalin
Colorectal adenocarcinoma

ABSTRACT

While it is known that cytoplasmic retention of p53 occurs in many solid tumors, the mechanisms responsible for this retention have not been positively identified. Since heatshock proteins like mortalin have been associated with p53 inactivation in other tumors, the current study sought to characterize this potential interaction in never before examined colorectal adenocarcinoma cell lines. Six cell lines, one with 3 different fractions, were examined to determine expression of p53 and mortalin and characterize their cellular localization. Most of these cell lines displayed punctate p53 and mortalin localization in the cell cytoplasm with the exception of HCT-8 and HCT116 379.2 cells, where p53 was not detected. Nuclear p53 was only observed in HCT-116 40–16, LS123, and HT-29 cell lines. Mortalin was only localized in the cytoplasm in all cell lines. Co-immunoprecipitation and immunohistochemistry revealed that p53 and mortalin were bound and co-localized in the cytoplasmic fraction of four cell lines, HCT-116 (40–16 and 386; parental and heterozygous fractions respectively of the same cell line), HT-29, LS123 and LoVo, implying that p53 nuclear function is limited in those cell lines by being restricted to the cytoplasm. Mortalin gene expression levels were higher than gene expression levels of p53 in all cell lines. Cell lines with cytoplasmic sequestration of p53, however, also displayed elevated p53 gene expression levels compared to cell lines without p53 sequestration. Our data reveal the characteristic cytoplasmic sequestration of p53 by the heat shock protein mortalin in human colorectal adenocarcinoma cell lines, as is the case for other cancers, such as glioblastomas and hepatocellular carcinomas.

© 2012 Elsevier Inc. All rights reserved.

1. Introduction

Colorectal carcinomas are the second leading cause of cancer deaths in Western countries directly following lung cancer. Carcinoma patients display the worst prognosis for colorectal adenocarcinomas when the tumor suppressor p53 is retained and thereby inactivated in the cytoplasm [1,2]. The tumor suppressor p53, a transcriptional regulatory protein [3–5], is involved in detection of cellular DNA damage, entry into the cell cycle, apoptosis and maintenance of genomic stability [6]. While p53 protein is present in normal cells in such low quantities that it is virtually undetectable by immunohistochemistry (IHC) [7–10], the wildtype TP53 gene for the p53 protein may become mutated during carcinogenesis in different tumors to display non-sense point mutations and increase protein quantities and subsequently immunoreactivity [2,5]. Though accumulation of mutated tumor suppressors and oncogenes is the leading cause for cancer development [11], functional p53 protein is also inactivated through several other

mechanisms including binding to viral or cellular products [12–14], inefficient nuclear transport [15], and conformation and translational status changes [16,17]. All aberrations can additionally lead to conformational changes of p53 and subsequent stabilization of the protein making it detectable [6], removing its regulatory influence on cell proliferation thus conferring growth advantages [13] and pre-disposing cells to further genetic mutations, including mutations that lead to inactivation of TP53.

The mitochondrial heat shock protein mortalin was originally discovered in embryonic mouse fibroblasts, where two forms were isolated, mot-1 and mot-2, differing in two amino acids. Mot-1 was found throughout the cytoplasm, while mot-2 was detected in the perinuclear region of immortal cells. A single human homologue of mortalin (HSPA9) was identified, resulting in a 679 amino acid protein with a molecular weight of 73.9 kD associating it with the Hsp70 proteins. Functions attributed to mortalin include energy generation, stress response, carcinogenesis and involvement in aging-associated diseases [18–21]. In addition, mortalin and its ability to bind cytoplasmic p53 in a number of vertebrate cells suggest that it also operates as a chaperone [22–25]. In normal human cells, cytoplasmic mortalin binds to p53 resulting in the delivery of the tumor suppressor to the nucleus, as a response to stress [26–28]. However, during severe stress, wild-type p53 protein also

* Corresponding author. Fax: +1 610 436 2183.

E-mail addresses: egestl@wcupa.edu (E.E. Gestl), aboettger@wcupa.edu (S. Anne Böttger).¹ Fax: +1 610 436 2183.

interacts with Bcl-2 (B-cell lymphoma 2, an apoptosis regulator protein) in the outer mitochondrial membrane subsequently resulting in non-transcriptionally activated apoptosis [29]. Overexpression of mortalin leads to permanent tethering of p53 protein in the cytoplasm [30,31], a phenomenon referred to as cytoplasmic sequestration in human and mouse cells. As a result, p53/mortalin complexes cannot be translocated to either the nucleus or the mitochondria. These outcomes block not only the transcriptional function of p53, but also its non-transcriptional activity in the mitochondrion thus preventing cell repair or apoptosis. This phenomenon has been observed in an unrelated group of human cancers, including undifferentiated neuroblastomas [12,15,32] but has not been verified for colorectal adenocarcinomas [33]. Increased mortalin levels have been reported in several cancers including leukemias, liver cancer metastasis, and brain tumors. In fact, overexpression of mortalin has been found to be indicative of a patient's poor prognosis as well as a predictor of tumor recurrence.

In this study, mortalin and p53 protein levels were analyzed in eight colorectal adenocarcinoma cell lines. Cytoplasmic sequestration and retention of p53 as well as the interaction and binding of mortalin with p53 was characterized for the first time in four adenocarcinoma cell lines. Targeting this specific mechanism of p53 inactivation, could be a therapeutic approach leading to reactivation of p53 and subsequent apoptosis of tumor cells.

2. Materials and methods

2.1. Cell lines and maintenance

Eight colorectal adenocarcinoma cell lines were selected based on their p53 status and cytogenetic analysis. HCT-8, HCT-15, LS123 and LoVo cells were purchased from ATCC. The HT-29 cells were a gift from Dr. Kristin Eckert (Jake Gittlen Cancer Research Institute, Penn State University, Hershey PA), while the three fractions of the HCT-116 cell line were a gift from Dr. Bert Vogelstein (Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore MD). The HCT-116 cell line fractions differed in their p53 status, 40–16 was wild-type (+/+, parent cell line), 386 was heterozygous (+/-), and 379.2 p53 deficient (-/-).

HCT-8 and HCT-15 were cultured in RPMI media containing 10% FBS with the HCT-15 cells also receiving 1 mM sodium pyruvate. LS123 was cultured in ATCC-formulated Eagles' Minimum Essential media (#30–2003). The remaining cell lines were cultured in DMEM/F12 media with 10% FBS and all cell lines were grown at 37 °C with 5% CO₂.

2.2. Primary antibodies

All antibodies were examined at the start of the experiment for their effectiveness in the HCT-116 parent cell line used in the current studies (results not shown). Primary antibodies for immunohistochemistry, protein localizations through Western blots and co-immunoprecipitation were: A) p-53 (DO-1, mouse anti-human monoclonal, Santa Cruz Biotechnology, catalogue number sc-126), B) mortalin (Grp75, mouse anti-human monoclonal, Thermo Scientific Pierce, catalogue number MA3–028), C) β -actin (mouse anti-human monoclonal, Santa Cruz Biotechnology, catalogue number sc-47778, used as a cytoplasmic loading control) and D) lamin A/C (mouse anti-human monoclonal, Santa Cruz Biotechnology, catalogue number sc-7292, used as a nuclear loading control).

2.3. Immunohistochemistry and protein localization

Cytospins of 60 μ l of freshly collected cells (diluted 1:1 in PBS) from all cell lines were fixed and permeabilized by immersion in

equal amounts of acetone and methanol. Resulting preparations were incubated in primary antibodies (1:50 μ l primary antibody:PBS) and developed with the appropriate peroxidase-labeled secondary antibody (Vectastain ABC Elite IgG kit, Vector Laboratories) followed by treatment with DAB. Control cytopins received identical treatment, however, lacking the primary antibodies.

To determine the distribution of p53 protein between the nucleus and cytoplasm, one 100 mm plate at approximately 80% confluence of each cell line was used to collect cells and isolate nuclear and cytoplasmic proteins using an NE-PER kit (Thermo Scientific Pierce) according to the manufacturer's instructions. Total protein was determined using the colorimetric BioRad D_C Protein Assay. Distribution of p53 and mortalin proteins in the nuclear and cytoplasmic fractions were determined by electrophoresis using 4–15% Tris-HCl precast Mini Protean[®] TGX[™] gels (BioRad), followed by blotting onto PVDF membranes (BioRad), treatment with the monoclonal primary and secondary anti-mouse antibodies and visualization using Western Blue[®] (Promega). All experimental lanes were loaded with 20 μ g total protein, lanes displaying standards were loaded with 10 μ l Precision Plus Protein[™] Dual Color Standard (BioRad). Loading controls (stained with β -actin primary antibody for cytoplasmic and lamin A/C primary antibody for nuclear samples) were conducted for all cytoplasmic and nuclear protein extracts.

2.4. Co-immunoprecipitation of p53 and mortalin

Co-immunoprecipitation (Co-IP) of p53 and mortalin was accomplished using an antibody-coupling gel to precipitate the "bait" protein and co-immunoprecipitate the interacting "prey" protein. Anti-p53 (DO-1) and in a separate reaction anti-mortalin (Grp75) were coupled to amine-reactive resin (ProFound[™] Co-Immunoprecipitation Kit, Pierce) using slow agitation at 22 °C for 3 h. Cytoplasmic lysates for all cell lines (diluted to 100 μ l and containing 50 μ g total protein) were incubated with the different antibody-coupled gels in separate spin-columns. Two negative controls were also prepared, a "control" gel that utilized an inactivated form of the resin and a "quenched" gel, in which a quenching buffer was applied in place of the antibody. Both negative controls were incubated with a cytoplasmic protein from the HCT-116 40–16 (parent cell line) and processed alongside the treatments in an otherwise identical manner. The first elution following antibody conjugation and the first three elutions from each Co-IP were stored for Western Blot analysis, the former to establish proper antibody conjugation (not shown), the latter to evaluate p53-mortalin binding. Elutions were separated by electrophoresis on a 4–15% Tris-HCl precast Mini Protean[®] TGX[™] gel, transferred to a PVDF membrane and probed with the coupling antibody first to ensure presence of "bait" protein followed by probing with the prey antibody. Bands for mortalin and p53 were visualized using anti-mouse alkaline phosphatase antibody and Western Blue[®] (stabilized substrate for alkaline phosphatase, Promega).

2.5. RNA isolation and quantitative RT-PCR

To document expression of HSPA9 and TP53, 100 mm plates of the colorectal cell lines were collected and RNA extracted using Trizol (Life Technologies, Invitrogen[®]) as per the manufacturer's instructions. Synthesis of cDNA was completed using 2 μ g of total RNA with the SuperScript[™] First-Strand Synthesis System (Life Technologies, Invitrogen[®]). All samples were prepared for qRT-PCR using TP53 (#Hs01034249_m1) and HSPA9 (#Hs00269818_m1) Taqman[®] primers and probes (Life Technologies, Applied Biosystems[®]). The GAPDH gene (#Hs0275899_g1) was utilized as a baseline for comparison among the different cell lines. All reactions were run in a Stratagene Mx3005P thermal

cycler (Agilent Technologies) utilizing the following program, 10 min at 95 °C, followed by 40 cycles of 30 s at 95 °C and 60 s at 60 °C.

3. Results

3.1. Protein localization in all cell lines

Localization of p53 was verified in the cell cytoplasm of cell lines LS123, HCT-15, HCT-116 40–16, HCT-116 386, LoVo and HT-29 (Table 1, Fig. 1A). Staining with primary antibody actin was conducted to verify separation of cytoplasmic and nuclear protein fractions (Fig. 1B). In addition, p53 was also located in the nucleus of LS123, HCT-116 40–16 and HT-29 (Table 1, Fig. 1C). Lamin A/C served as a nuclear standard to verify successful protein separation between nucleus and cytoplasm (Fig. 1D).

Mortalin was detected in the cell cytoplasm of all cell lines but never in the nuclear extracts (Fig. 1E). Using colorimetric IHC techniques, both p53 and mortalin displayed a punctate localization within all cell lines (Fig. 2A–D), with the exception of HCT-8 and HCT-116 397.2 (p53 deficient) that lacked p53 localization but displayed regular mortalin localization. Localization of p53 was not consistently present in all cells visualized through immunohistochemistry.

3.2. Co-immunoprecipitation of mortalin and p53

Western blot analysis of the wash following antibody coupling revealed absence of the DO-1 and Grp75 antibodies in the wash, indicating that the antibody/resin coupling was fully successful. The “bait” proteins, p53 and mortalin respectively, were localized in all eludes, which served as a control to ensure successful capture of the bait protein. With p53 as “bait” protein mortalin co-localization and binding was observed in the tumorigenic cell lines HCT-116 40–16, HCT-116 386, HT-29 (not shown), LS123 and LoVo (Fig. 3A). Co-localization and binding for the same cell lines was confirmed with mortalin as the “bait” (Fig. 3B).

3.3. Quantitative gene expression

Gene expression of HSPA9 was higher when compared to TP53 gene expression in all cell lines. The cell lines displaying cytoplasmic sequestration of p53 by mortalin (HCT-116 40–16, HCT-116 386, HT-29, LoVo and LS123) displayed higher TP53 gene expression levels than the cell lines without cytoplasmic sequestration of p53. When standardized to levels of GAPDH expression, the resulting HSPA9:TP53 ratios for cell lines with cytoplasmic sequestration of p53 by mortalin were the lowest evaluated (Fig. 2E) with

the exception of the HCT-116 386 (heterozygous) cell line. HT-29, LoVo, LS123 and both HCT-116 fraction displayed the lowest HSPA9:TP53 ratios, significantly lower compared to cell lines lacking p53 sequestration ($p < 0.001$; HCT-8 and HCT-15).

4. Discussion

With nuclear and cytoplasmic protein fractions successfully separated, mortalin was detected exclusively in the cytoplasmic fractions of all the colorectal carcinoma cell lines while p53 was detected in the cytoplasmic fraction of six of the eight cell lines. Exceptions were the cytoplasmic fractions of p53 wild-type cell line (HCT-8) and the p53 deficient cell line (HCT-116 397.2). p53 was also detected in the nuclear fraction of HT-29, LS123 and HCT-116 40–16 (parent cell line). Wild-type p53, has previously been reported in HCT-8 and LoVo in low quantities non-detectable by protein detection methods such as IHC and Western blotting [7–10]. The reason for lack of p53 detection in HCT-8 cells in this study may therefore be a result of levels of p53 protein below detection rather than an inefficient antibody, as DO-1 has been used to detect wild type and mutant p53 in a variety of other tumors [15,34–36]. The LoVo cell line, having wild-type p53, also displayed cytoplasmic sequestration and binding of p53 by mortalin. The reason for evidence of p53 staining in LoVo may be the stabilization and hence overexpression of the p53 protein, not by mutation, but due to retention of p53 in the cytoplasm as a result of tethering by another protein [13,37] such as mortalin.

HCT-116 40–16, HT-29, LS123 and LoVo cell lines displayed an approximate 50% increase in HSPA9 expression compared to other adenocarcinoma cells. Increased levels of HSPA9 expression have been detected in several cancers including brain tumors and hepatocellular carcinomas [31,38,39]. The increased TP53 expression in the cell lines displaying cytoplasmic sequestration may be due to the p53 not reaching its desired target thus not turning off the intracellular signal to increase transcription of TP53 to compensate for the lack of functional activity. Proteomic profiling of matched tumor and non-tumor tissues from patients with hepatocellular carcinoma revealed a 1.8-fold increase in HSPA9 expression. A similar increase in HSPA9 expression was detected, at both the protein and RNA levels, when comparing those patient samples which had an early recurrence to those that were disease free after one year indicating the use of HSPA9 as a potential prognostic biomarker [39]. Therefore, upregulation of HSPA9 expression has been suggested to aid in tumorigenesis [31,40,41], since increases in HSPA9 could lead to increased amounts of mortalin protein which can directly interact with and inappropriately bind the tumor suppressor p53. Cytoplasmic tethering of p53 by mortalin has previously been reported in undifferentiated neuroblastoma cells [33]

Table 1
Colorectal Adenocarcinoma Cell Line Characteristics and p53 Distribution.

Cell Line	Tumorigenicity	p53 Status	Cytogenetic	Base Media	p53 Distribution Cyto/ Nuc
LS123	Yes, Duke's type B	Yes, Arg > His at 175	Modal 63, Range 54–70	Eagle's MEM	+/+
LoVo	Yes, Duke's type C, grade IV	Yes, wild-type	Hyperdiploid Modal number at 49	DMEM/F12	+/-
HCT-8	Yes	Yes, wild-type	Unknown	RPMI 1640	-/-
HCT-116; 40–16	Yes	Yes, Wildtype	Near diploid, modal number at 45, 6.8 % polyploids	DMEM/F12	+/+
HCT-116; 386	Yes	Yes, +/-, p53 heterozygous	Near diploid	DMEM/F12	+/-
HCT-116; 397.2	Yes	No, -/-, p53 deficient	Near diploid	DMEM/F12	-/-
HCT-15	Yes, Duke's type C	Yes, +/-Pro > Ala at 153	Quasidiploid Modal 46 in 76%	RPMI-1640	+/-
HT-29	Yes	Yes, Arg > His at 273	Hypertriploid, modal number 71, range 68–72	DMEM/F12	+/+

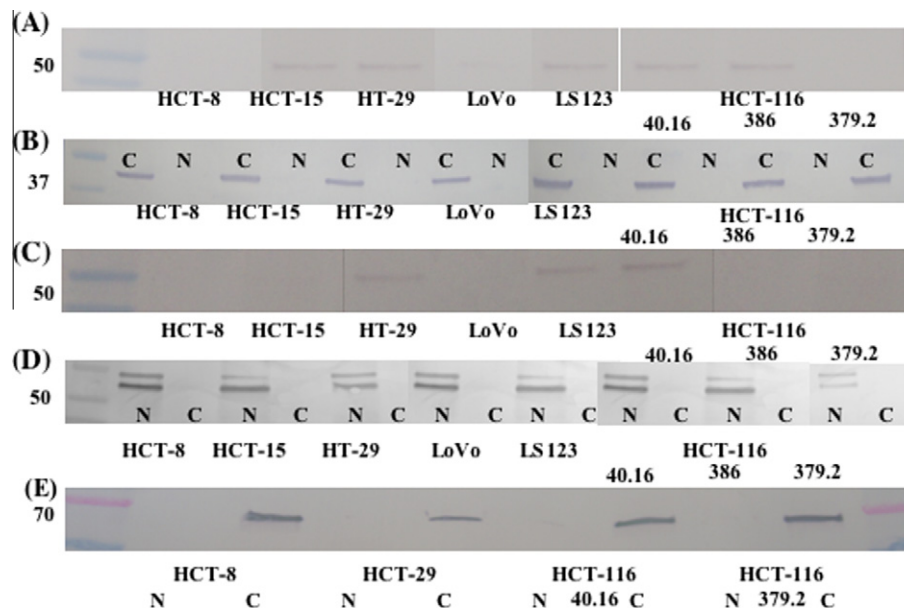


Fig. 1. p53 and mortalin cellular distribution. Protein distribution is displayed separately for p53 (A) cytoplasmic distribution (positive for cell lines HCT-15, HT-29, LoVo, LS123 and HCT-116 40-16 and 386), (B) cytoplasmic loading control (actin), (C) nuclear distribution (positive for cell lines HT-29, LS123 and HCT-116 40-16), (D) nuclear loading controls (lamin A/C) and (E) concurrently for nuclear and cytoplasmic distribution of mortalin (cell line examples, all cell lines displayed only cytoplasmic distribution of mortalin).

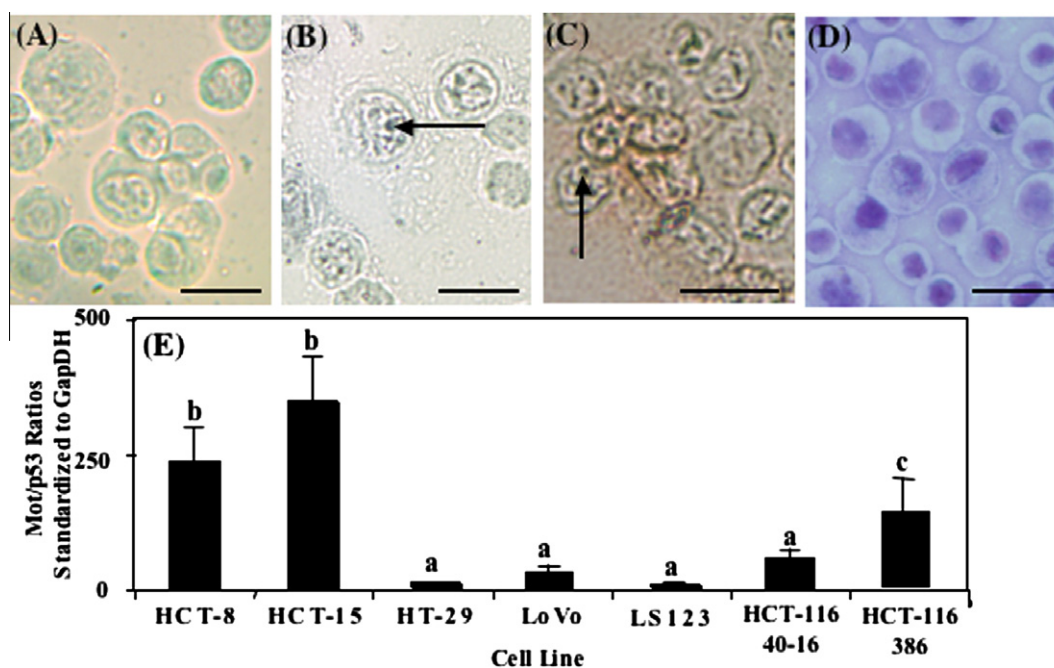


Fig. 2. p53 and mortalin localization, distribution and expression. (A) negative (untreated by DO-1 primary antibody) control, (B) p53 distribution (arrow), (C) mortalin distribution (arrow), (D) nuclear to cytoplasmic ratio and overall cellular outline (nuclear methylene blue stain) and (E) HSPA9 and TP53 gene expression ratios standardized using the housekeeping gene GAPDH. Scale bars = 10 μ m; a, b and c referring to significant difference between groups.

and in primary and secondary glioblastomas (with possible involvement of other tethering molecules e.g., cullin 7 or PARC) [42,43] when mortalin is over-expressed. In addition to HSPA9, hsp27 has been shown to be upregulated in head and neck squamous cancer cells as well as some melanomas [44,45]. Mortalin overexpression in the cell lines examined could enhance tumorigenesis through direct interaction with p53. Sequestration of tumor suppressors in the cytoplasm would prevent the apoptotic pathway as observed in four of the eight examined cell lines

(HCT-116 parent and heterozygous fractions, HT-29, LS123 and LoVo).

The involvement of mortalin and other heatshock proteins in both cancer and aging-related diseases is an important area of research that may result in a therapy for these conditions. The findings in this study confirm for the first time the interaction between mortalin and p53 in four out of eight colorectal carcinoma cell lines. Our results also suggest that therapies that disrupt the p53/mortalin interaction in cancers should be explored further

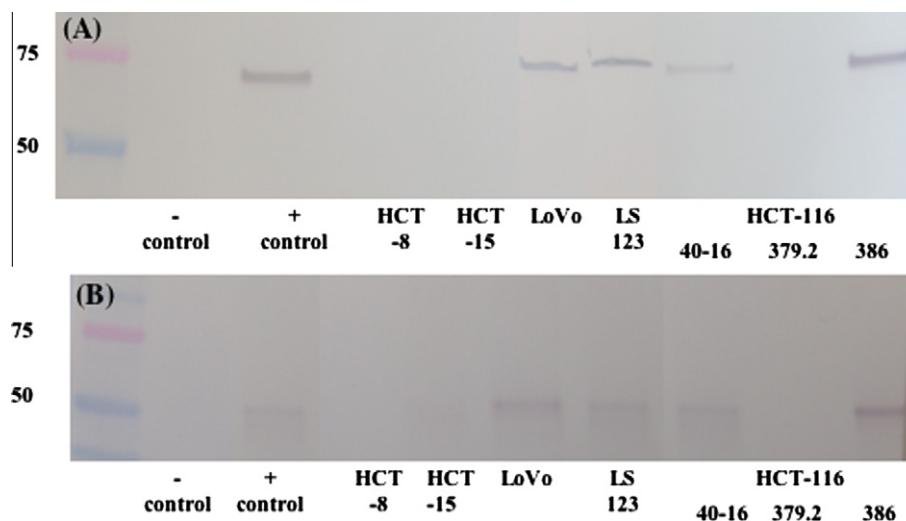


Fig. 3. Co-immunoprecipitation of p53 and mortalin. Cytoplasmic proteins were pulled down (A) using DO-1 (p53) antibody for pull down and staining for mortalin. p53 protein pull down was verified for all cell lines (B) using Grp-75 (mortalin) antibody for pull down and staining for p53. The negative control utilized quenched and thereby inactivated antibodies, the positive control consisted of 10 μ g cytoplasmic protein extract from cell line HCT-116 40–16. (Loading controls for actin not provided).

for colorectal adenocarcinomas as they may allow normal localization and function of p53 to be re-established, resulting in activation of downstream genes allowing apoptosis to occur and reduce cancer burden.

Acknowledgments

We would like to thank Endo Pharmaceuticals, West Chester, PA (<http://www.endo.com/>), for their generous financial support and to Nicholas Laping, Sanjeeva Reddy and Alan Butcher (Endo Pharmaceuticals) for their editorial contribution to this manuscript. Our sincere gratitude to the Vogelstein lab (Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University) for providing the HCT-116 cell lines and the Eckert lab (Jake Gittlen Cancer Research Institute, Penn State University) for the HT-29 cell line. Our gratitude also extends to the Vice President for Advancement, Mark Pavlovich, and the Office of Sponsored Research at West Chester University for their support. Lastly we would like to acknowledge the WCU undergraduate researchers who helped with cell maintenance, protein and RNA techniques, Norah Taraska, Rebecca Giovenella and Samantha Terkowski.

References

- [1] G. Flamini, G. Curigliano, C. Ratto, et al., Prognostic significance of cytoplasmic p53 overexpression in colorectal cancer: An immunohistochemical analysis, *Eur. J. Cancer* 32 (1996) 802–806.
- [2] S. Bosari, G. Viale, P. Bossi, et al., An independent prognostic indicator in colorectal adenocarcinomas, *J. Natl. Cancer Inst.* 86 (1994) 681–687.
- [3] C. Miller, T. Mohandas, D. Wolf, et al., Human p53 gene localized to short arm of chromosome 17, *Nature* 319 (1986) 783–784.
- [4] N. Lassam, L. From, H. Kahn, Overexpression of p53 is a late event in the development of malignant melanoma, *Cancer Res.* 53 (1993) 2235–2238.
- [5] R. Hamelin, P. Laurent-Puig, S. Alschwang, et al., Association of p53 mutations with short survival in colorectal cancer, *Gastroenterology* 106 (1994) 42–48.
- [6] R. Soong, P. Robbins, B. Dix, et al., Concordance between p53 protein overexpression and gene mutation in a large series of common human carcinomas, *Hum. Pathol.* 27 (1996) 1050–1055.
- [7] J. Marks, A. Davidoff, B. Kerns, et al., Overexpression and mutation of p53 in epithelial ovarian cancer, *Cancer Res.* 51 (1991) 2979–2984.
- [8] J. Kupryjanczyk, A. Thor, R. Beauchamp, et al., P53 and K-ras gene mutations in epithelial ovarian neoplasms, *Cancer Res.* 53 (1993) 1–6.
- [9] M. Ebina, S. Steinberg, J. Muishine, et al., Relationship of p53 overexpression and up-regulation of proliferating cell nuclear antigen with the clinical course of non-small cell lung cancer, *Cancer Res.* 54 (1994) 2496–2503.
- [10] T. Starzynska, M. Bromley, A. Ghosh, et al., Prognostic significance of p53 overexpression in gastric and colorectal carcinoma, *Br. J. Cancer* 66 (1992) 558–562.
- [11] V. van Houten, M. Tabor, M. van den Brekel, et al., Mutated p53 as a molecular marker for the diagnosis of head and neck cancer, *J. Pathol.* 198 (2002) 476–486.
- [12] U. Moll, G. Riou, A. Levine, Two distinct mechanisms alter p53 in breast cancer: Mutation and nuclear exclusion, *Proc. Natl. Acad. Sci. USA* 89 (1992) 7262–7266.
- [13] B. Dix, P. Robbins, S. Carello, et al., Comparison of p53 gene mutation and protein overexpression in colorectal carcinomas, *Br. J. Cancer* 70 (1994) 585–590.
- [14] S. van den Heuvel, T. van Laar, I. The, et al., Large E1B proteins of adenovirus types 5 and 12 have different effects on p53 and distinct roles in cell transformation, *J. Virol.* 67 (1993) 5226–5234.
- [15] U. Moll, M. LaQuaglia, J. Benard, et al., Wild-type p53 protein undergoes cytoplasmic sequestration in undifferentiated neuroblastomas but not in differentiated tumors, *Proc. Natl. Acad. Sci. USA* 92 (1995) 4407–4411.
- [16] K. Suzuki, T. Ono, K. Takahashi, Inhibition of DNA synthesis by TGF-beta1 coincides with inhibition of phosphorylation and cytoplasmic translocation of p53 protein, *Biochem. Biophys. Res. Commun.* 183 (1992) 1175–1183.
- [17] T. Takahashi, N. Nau, I. Chiba, et al., P53: A frequent target for genetic abnormalities in lung cancer, *Science* 246 (1989) 491–494.
- [18] J. Hendrick, F. Hartl, Molecular chaperone functions of heat-shock proteins, *Annu. Rev. Biochem.* 62 (1993) 349–384.
- [19] H. Kampinga, E. Craig, The HSP70 chaperone machinery: J proteins as drivers of functional specificity, *Nat. Rev. Mol. Cell Biol.* 11 (2010) 579–592.
- [20] M. Akerfelt, R. Morimoto, L. Sistonen, Heat shock factors: Integrators of cell stress, development and lifespan, *Nat. Rev. Mol. Cell Biol.* 11 (2010) 545–555.
- [21] S. Kaul, C. Deocaris, R. Wadhwa, Three faces of mortalin: A housekeeper, guardian and killer, *Exp. Gerontol.* 42 (2007) 263–274.
- [22] C. Deocaris, K. Yamasaki, S. Kaul, et al., Structural and functional differences between mouse Mot-1 and Mot-2 proteins that differ in two amino acids, *Ann. NY Acad. Sci.* 1067 (2006) 220–223.
- [23] R. Gupta, N. Ramachandra, T. Bowes, et al., Unusual cellular disposition of the mitochondrial molecular chaperones Hsp60, Hsp70, and Hsp10, *Novart. Fdn. Symp.* 291 (2008) 59–68.
- [24] S. Kaul, K. Taira, O. Pereira-Smith, et al., Mortalin: Present and prospective, *Exp. Gerontol.* 37 (2002) 1157–1164.
- [25] R. Wadhwa, S. Kaul, Y. Mitsui, et al., Differential subcellular distribution of mortalin in mortal and immortal mouse and human fibroblasts, *Exp. Cell Res.* 207 (1993) 442–448.
- [26] M. Galigniana, J. Harrell, H. O'Hagen, et al., Hsp90-binding immunophilins link p53 to dynein during p53 transport to the nucleus, *J. Biol. Chem.* 279 (2004) 22483–22489.
- [27] P. Giannakakou, D. Sackett, Y. Ward, et al., P53 is associated with cellular microtubules and is transported to the nucleus by dynein, *Nat. Cell Biol.* 2 (2000) 709–717.
- [28] P. Giannakakou, M. Nakano, K. Nicolaou, et al., Enhance microtubule-dependent trafficking and p53 nuclear accumulation by suppression of microtubule dynamics, *Proc. Natl. Acad. Sci. USA* 99 (2002) 10855–10860.
- [29] K. Yee, K. Vousden, Complicating the complexity of p53, *Carcinogenesis* 26 (2005) 1317–1322.
- [30] R. Wadhwa, S. Kaul, Y. Ikawa, et al., Identification of a novel member of mouse hsp70 family. Its association with cellular mortal phenotype, *J. Biol. Chem.* 268 (1993) 6615–6621.

- [31] R. Wadhwa, S. Takano, K. Kamaljit, et al., Upregulation of mortalin/mthsp70/Grp75 contributes to human carcinogenesis, *Int. J. Cancer* 118 (2006) 2973–2980.
- [32] U. Moll, A. Ostermeyer, R. Haladay, et al., Cytoplasmic sequestration of wild-type p53 protein impairs the G1 checkpoint after DNA damage, *Mol. Cell Biol.* 16 (1996) 1126–1137.
- [33] S. Dundas, L. Lawrie, P. Rooney, et al., Mortalin is over-expressed by colorectal adenocarcinomas and correlates with poor survival, *J. Pathol.* 205 (2005) 74–81.
- [34] M. Kubbutat, S. Jones, K. Vousden, Regulation of p53 stability by Mdm2, *Nature* 387 (1997) 299–303.
- [35] J. Ford, P. Hanawalt, Expression of wild-type p53 is required for efficient global genomic nucleotide excision repair in UV-irradiated human fibroblasts, *J. Biol. Chem.* 272 (1997) 28073–28080.
- [36] W. Hoffman, S. Biade, J. Zilfou, et al., Transcriptional repression of the anti-apoptotic surviving gene by wild type p53, *J. Biol. Chem.* 277 (2002) 3247–3257.
- [37] H. Mineta, A. Borg, M. Dictor, et al., P53 mutation, but not p53 overexpression, correlates with survival in head and neck squamous cell carcinoma, *Br. J. Cancer* 78 (1998) 1084–1090.
- [38] S. Takano, R. Wadhwa, Y. Yoshii, et al., Elevated levels of mortalin expression in human brain tumors, *Exp. Cell Res.* 237 (1997) 38–45.
- [39] X. Yi, J. Luk, N. Lee, et al., Association of mortalin (HSP9) with liver cancer metastasis and prediction for early tumor recurrence, *Mol. Cell. Proteomics* 7 (2008) 315–325.
- [40] D. Mosser, R. Morimoto, Molecular chaperones and the stress of oncogenesis, *Oncogene* 23 (2004) 2907–2918.
- [41] A. Czarnecka, C. Campanella, et al. G. Zummo, Mitochondrial Chaperones in Cancer, *Cancer Biol. Ther.* 5 (2006) 714–720.
- [42] J. Nagpal, A. Jamoona, N. Gulati, et al., Revisiting the role of p53 in primary and secondary glioblastomas, *Anticancer Res.* 26 (2006) 4633–4639.
- [43] A. Nikolaev, M. Li, N. Puskas, et al., Parc: A cytoplasmic anchor for p53, *Cell* 112 (2003) 29–40.
- [44] Z. Zhu, X. Xu, M. Graham, et al., Silencing heat shock protein 27 decreases metastatic behavior of human head and neck squamous cell cancer cells in vitro, *Mol. Pharm.* 7 (2010) 1283–1290.
- [45] F. Jmor, H. Kalirai, A. Taktak, et al., HSP-27 protein expression in uveal melanomas: Correlation with predicted survival, *Acta Ophthalmol* 88 (2010) 1–7.