Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format.

1. **Grantee Institution:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2010 - 12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA

4. **Grant Contact Person's Telephone Number:** 814 935 1081

5. **Grant SAP Number:** 4100050904

6. **Project Number and Title of Research Project:** 53. Targeting the Oncogenic Mutant p53 Signaling in Colorectal Cancer Therapy

7. **Start and End Date of Research Project:** 3/12/2013 - 12/31/2013

8. **Name of Principal Investigator for the Research Project:** Wafik S. El-Deiry, MD, PhD, FACP

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 107,100

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Hong, Bo	Post Doc	15.7%	$6,682.77
Shang, Shengliang	Research Tech	41.3%	$32,109.39
Van Den Huvel, Peter	Instructor	20.6%	$16,851.20

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
El-Deiry	PI	< 2%

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
NONE		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No_____X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes___X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If

you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Targeting the oncogenic mutant p53 signaling in colorectal cancer therapy	☒ NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	June 2013	$ 1,907,162	$ 316,656

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes___X_____ No_____

If yes, please describe your plans:

The RO1 grant that we applied for will be funded for the period 2014-2019. We will apply for renewal of this grant in the future.

12. Future of Research Project. What are the future plans for this research project?

The project has a number of future directions:

1. The p53 pathway restoring compounds currently being evaluated will be further tested in vitro and in vivo for safety, efficacy and drug targets.
2. The most suitable compounds will be evaluated as lead candidates for translation into clinical trials.
3. Further screening will be performed to identify novel p53 pathway restoration lead candidates for preclinical testing.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes___X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male			1	1
Female				
Unknown				
Total			**1**	**1**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic			1	1
Unknown				
Total			**1**	**1**

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian			1	1
Other				
Unknown				
Total			**1**	**1**

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No_____X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes____X_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

This CURE-funded project has further strengthened and expanded the base of translational research on colorectal cancer therapy and related research training opportunities at the Penn State Hershey Cancer Institute.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No_____X_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No_____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a

performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Our goal is to specifically target mt p53 in colorectal cancer by an indirect mechanism involving the p53 family member p73. We aim to evaluate the safety and efficacy of the highly potent small molecules Prodigiosin, Prodigiosin-related compound and CB-7587351 with *in vitro* and *in vivo* studies. We will optimize and improve the *in vivo* experiments by using various mutant p53 expressing cell lines to demonstrate robust anti-tumor activity with Prodigiosin, its related compound and CB-7587351 in xenograft tumor models. HPLC/ Mass spectrometry will be used to develop and optimize a method for detection of Prodigiosin, its related compound and CB-7587351 that will be utilized for pharmacokinetic analysis of these small molecules in *in vivo* studies. The p53 pathway restoration and apoptotic effects of the compounds will be demonstrated in a dose dependent manner.

Specific aims:
Aim 1: Demonstrate effects in p53 null cells and evaluate the effect of varying doses of compounds on p53 reporter activation and apoptosis
Aim 2: Perform *in vivo* pharmacokinetic analysis of p53 restoring compounds using HPLC detection of small molecules.
Aim 3: Optimize *in vivo* studies to demonstrate robust anti-tumor efficacy with p53-pathway restoring compounds in various xenograft tumor models.

Summary of Research Completed

Aim 1: Demonstrate effects in p53 null cells and evaluate the effect of varying doses of compounds on p53 reporter activation and apoptosis

Methods:
p53 reporter activation: Prodigiosin-mediated p53-dependent transcriptional activity was determined by using noninvasive bioluminescence imaging. Human colon cancer cells stably expressing a p53-responsive luciferase reporter were used. After the treatment, cells were imaged by using an IVIS imaging system (Xenogen, Alameda, CA) to detect luciferase activity.

Establishment of SW480 p53 reporter cell line with stable p73 knock-down (p73 KD): The retroviral vector containing p73 siRNA (pSIREN-RetrolQ-p73 siRNA- blasticidin-retroviral

vector) was used. Phoenix cells were transfected with the retroviral vector containing p73 siRNA. After 48 hr transfection, supernatant (packaged retrovirals) was collected, and then infected into SW480 p53 reporter containing cells. Blasticidin at 5 µg/ml was added for selection. After two weeks of selection, blasticidin-resistant cells were collected for p73 knock-down validation.

Western blot: After treatment, protein lysates were collected for Western blot. Twenty-five micrograms of protein was used for SDS-PAGE. After primary and secondary antibody incubations, the signal was detected by chemiluminescent detection kit, followed by autoradiography.

Flow cytometry: After treatment, cells were harvested, fixed by ethanol and stained by PI, and then flow cytometry was performed.

Results: HCT116 p53-/- cells with p53 reporter were treated with different concentrations of Prodigiosin or DMSO control for 2, 20 and 72 hr. After treatment, p53-responsive luciferase reporter activity was imaged by an IVIS imaging system. As shown in figure 1A, Prodigiosin activated p53-responsive reporter activity at early time points (2 hr and 20 hr) in a dose dependent manner. At the 72 hr time point, all cell lines exhibited the phenotypes of a dose-dependent cell death by the treatment of Prodigiosin. We have observed cell death in HCT116 p53-/- cells (PARP cleavage) with Prodigiosin after incubation for 12 hours using a dose of 1 µM (Fig 2C).We believe that p53 reporter activation is maximal at earlier stages and is reduced later as the cells begin to undergo apoptosis. Thus, the doses used for p53 reporter activation correlate with cell death.

To further validate that Prodigiosin can activate p53 signaling pathway in p53-null cancer cells, we examined whether Prodigiosin can induce the expression of endogenous p53 target genes. Western blot indicated that Prodigiosin induced the expression of p53 target genes (p21, DR5 and PUMA) in a dose dependent manner in HCT116 p53-/- cells (Figure 1B). The data demonstrate that p53 responsive signaling pathway is activated by Prodigiosin in p53-null cancer cells.

In order to confirm that Prodigiosin can induce cell cycle arrest and/or apoptosis following p53 pathway activation, we performed flow cytometric analysis and PARP cleavage analysis. The cell cycle analysis indicated that 1 µM of Prodigiosin induced G1 and/or G2 arrest at an early time point (12 hr) in SW480 (p53 mutant) and HCT116 p53-/- cells (Figure 2A). When the cells were treated for a longer time (72 hr), 1 µM of Prodigiosin induced a large sub-G1 peak (apoptotic peak) (Figure 2B). Western blot indicated that Prodigiosin induced PARP cleavage (a marker of apoptosis due to caspase activation) in SW480 and HCT116 p53-/- cells (Figure 2C). These results indicate that the activation of the p53 pathway by Prodigiosin is accompanied by the induction of cell cycle arrest and apoptosis in p53-mutant and p53-null cancer cells.

We observed that Prodigiosin induced p53-dependent transcriptional activity and its downstream genes in p53-null cancer cells. p53 expression is not induced by Prodigiosin in SW480 (p53 mutant) and HCT116 (p53 wide type) cells, but p53 target proteins (DR5, p21 and PUMA) are induced by Prodigiosin in HCT116 cells (Figure 3A). These results suggest that Prodigiosin can

restore p53 transcriptional responses in a p53-independent manner. p73, a p53 family protein, can induce cell cycle arrest and/or apoptosis in a p53-like manner and recognizes the same genomic DNA-binding responsive element as p53. Therefore, we hypothesized that Prodigiosin and compound R induce p73 expression to activate p53 pathway signaling leading to cellular apoptosis in p53-deficient cancer cells. Indeed, as shown in figure 3B, Prodigiosin induced p73 expression in mutant p53-expressing SW480 and p53-/- HCT116 cells. p73 expression is also induced by Prodigiosin in p53 wide type HCT116 cells (Figure 3A).

To further confirm that p73 plays a role in p53 pathway activation and apoptosis induction by Prodigiosin, we knocked-down p73 by using p73 shRNA. When p73 is stably knocked-down in SW480 cells (Figure 4A), we found that the induction of p53-responsive transcriptional activity by Prodigiosin is reduced in p73-knockdown SW480 cells, as compared to parental SW480 cells (Figure 4B). The sub-G1 analysis demonstrates that Prodigiosin induced less apoptosis in SW480 cells with stable p73 knockdown than parental SW480 cells (Figure 4C). Together, these findings indicate that p73 up-regulation is involved in the mechanism of p53 pathway activation and apoptosis induction by Prodigiosin.

Aim 2: Perform in vivo pharmacokinetic analysis of p53 restoring compounds using HPLC detection of small molecules.
Method: p53 pathway restoring compound CB-7587351 was provided to the Penn State Hershey Mass Spectrometry core facility for chromatography and mass spectrometry detection.

Results: The compound was detected by HPLC and its identity was confirmed by mass spectrometry (Figure 6). We have established a robust HPLC/Mass spectrometry detection method to obtain the pharmacokinetic profile for the p53 pathway restoring compounds to be tested. The identity of the compounds was confirmed by mass spectrometric detection.

Aim 3: Optimize in vivo studies to demonstrate robust anti-tumor efficacy with p53-pathway restoring compounds in various xenograft tumor models.

Methods: Athymic nude mice were injected subcutaneously in the left and right dorsal flank, each with a 100 µL suspension of 1–4×106 cancer cells in an equal volume of Matrigel. When tumors grew to 4–5 mm in diameter, the mice were treated with the Prodigiosin or DMSO by i.p. injection. The tumor size was monitored by caliper measurements. Tumors were harvested from euthanized mice and fixed in 4% paraformaldehyde in PBS for 48 hours.

All data were analyzed using PRISM4 Software (GraphPad Software, Inc., San Diego, CA, USA). Statistical analysis was performed using t-test. Results were considered as statistically significant when $p < 0.05$.

Results: The anti-tumor efficacy of Prodigiosin was evaluated in vivo by measuring tumor volumes. In SW480 xenografts, tumor volume in Prodigiosin-treated mice appeared to be reduced as compared with the DMSO-treated mice (Figure 5A). Importantly, we found that tumor growth of SW480 p73 knockdown xenografts is enhanced as compared with SW480 xenografts (Figure 5A). Prodigiosin did not reduce tumor growth in SW480 p73 knockdown xenografts (Figure 5A). An additional human colon tumor xenograft, HT29 xenograft (p53

mutant R273H), was also used to test the antitumor efficacy of Prodigiosin. As shown in figures 5B and 5C, Prodigiosin significantly inhibits tumor growth of HT29 xenograft as compared with DMSO-treated control.

The RO1 grant related to this work was resubmitted to the NIH in June 2013 and scored in the top 2 percentile in December 2013. Work related to this grant was published in the journal Cancer Research: Hong B, Prabhu VV, Zhang S, van den Heuvel AP, Dicker DT, Kopelovich L, El-Deiry WS. . Cancer Res. 2014 Feb 15;74(4):1153-65. The Pennsylvania Department of Health was not cited on this publication due to an inadvertent oversight.

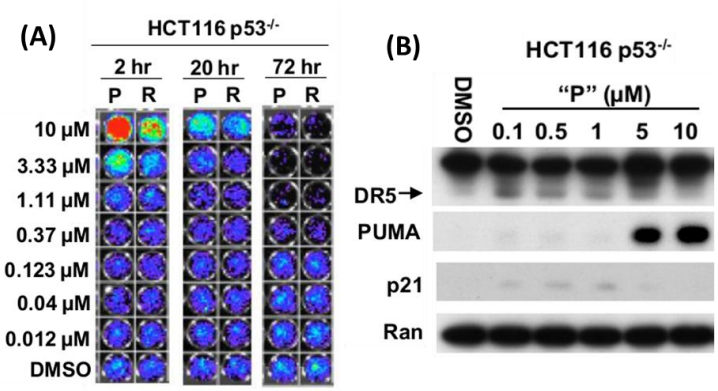


Fig 1. p53 responsive signaling pathway is activated by Prodigiosin in p53-null cancer cells. (A) HCT116 p53-/- cells with p53-responsive reporter were treated with different concentrations of Prodigiosin (P) and its structurally related compound (R) or DMSO control for 2, 20 and 72 hr. p53-responsive reporter activity was imaged by the IVIS imaging system after treatment. (red/green/blue color indicates bioluminescent signal from p53 responsive luciferase reporter, lack of signal (black) indicates absence of cells due to cell death). (B) HCT116 p53-/- cells were treated with the indicated concentrations of P for 12 hr. DR5, PUMA and p21 were detected by Western Blotting. Ran protein expression was used as the loading control.

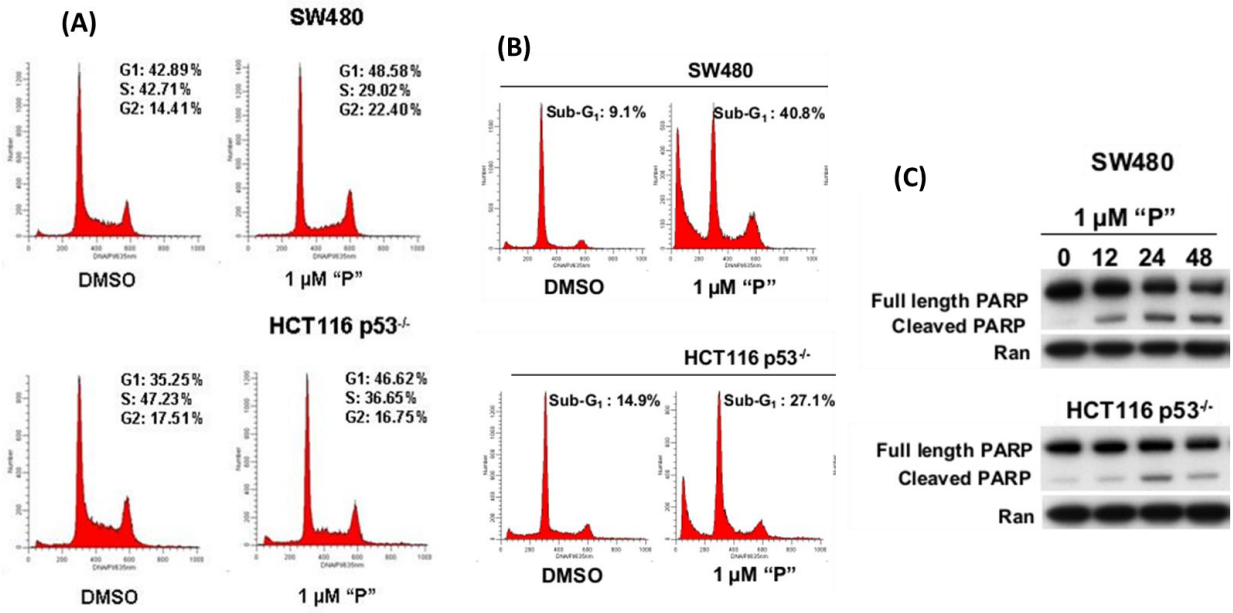


Fig 2. Activation of the p53 pathway by Prodigiosin is accompanied by the induction of cell cycle arrest and apoptosis (A) SW480 and HCT116 p53-/- cells were treated with 1 μM Prodigiosin (P) or DMSO control for 12 hr. After treatment, cells were harvested, fixed by ethanol and stained by PI, then flow cytometry was performed.(B) SW480 and HCT116 p53-/- cells were treated with 1 μM P or DMSO control for 72 hr. After treatment, flow cytometry was performed. (C) SW480 and HCT116 p53-/- cells were treated with 1 μM P for 0, 12, 24 and 48 hr. Full-length, cleaved PARP and loading control Ran were detected by Western Blotting.

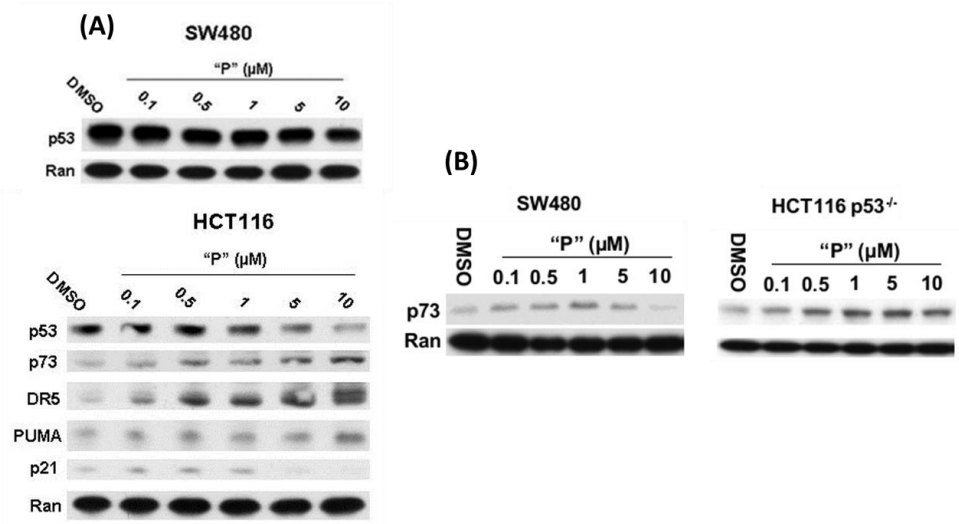


Fig 3. Prodigiosin can restore p53 transcriptional responses in a p53-independent manner by stimulating p73 expression. (A) SW480 and HCT116 cells were treated with the indicated concentrations of Prodigiosin (P) for 12 hr. p53, p73, DR5, PUMA, p21 and loading control Ran were detected by Western Blotting. (B) SW480 and HCT116 p53-/- cells were treated with the indicated concentrations of P for 12 hr. p73 and loading control Ran were detected by Western Blotting.

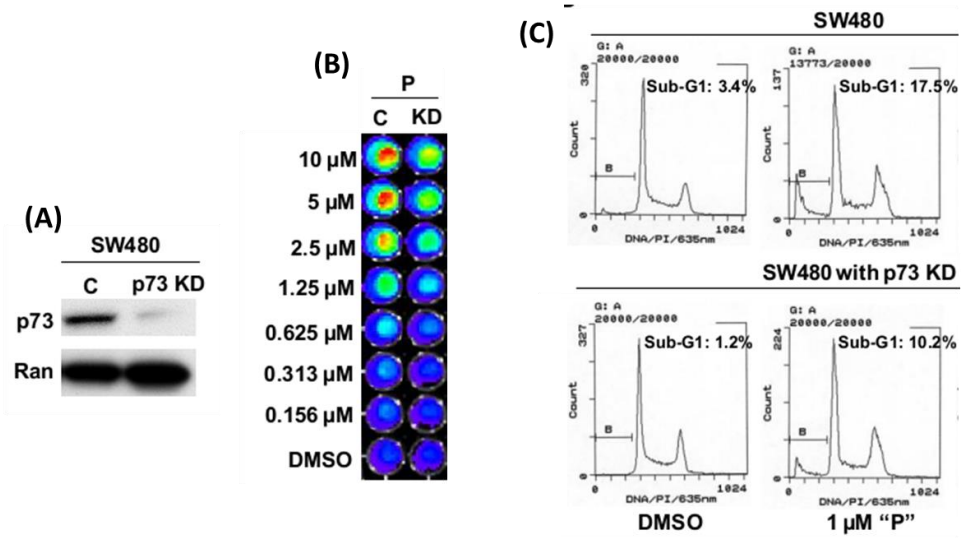


Fig 4. Prodigiosin-mediated p53 pathway activation and apoptosis induction is p73-dependent. (A) p73 knock down was confirmed by Western blot. (B) SW480 p53 reporter cells and SW480 p53 reporter cells with stable p73KD (KD) were treated with different concentrations of Prodigiosin (P) or DMSO control for 2 hr. p53-responsive reporter activity was imaged by the IVIS imaging system after treatment. (C) SW480 cells and SW480 cells with stable p73KD were treated with 1 μM P or DMSO control for 48 hr. After treatment, flow cytometry was performed.

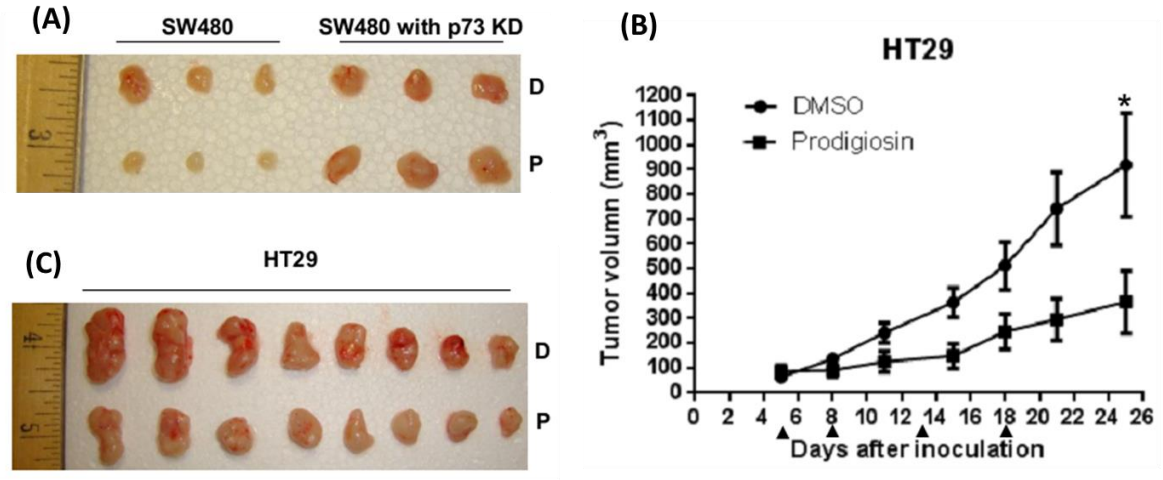
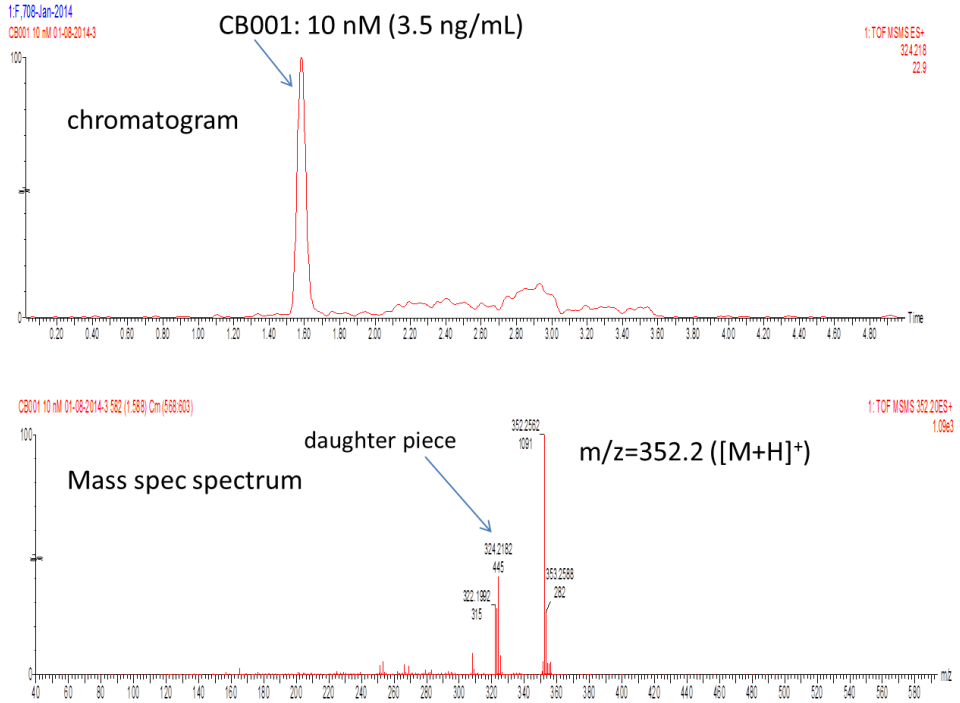


Fig 5. Prodigiosin exhibits potent anti-tumor efficacy *in vivo* (A) SW480 xenografts and SW480 p73KD xenografts were treated with 5mg/kg Prodigiosin or DMSO every 4 days for a total of three treatments. The imaging from three representative tumors of each group is shown (SW480 xenografts and SW480 p73KD xenografts). (B) HT29 xenografts were treated with 5mg/kg prodigiosin and DMSO control for four times. The arrows show the day of the treatments. The mean tumor volume ± SEM is shown (* $P < 0.05$). (C), The imaging of tumors from each group is shown (HT29 xenografts). The tumors were excised at the end of the experiment (day 25 after inoculation).

Fig 6: Determination of CB758731 by UPLC/MS/MS



18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
__X__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one

publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
None				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____X_____ No_____

If yes, please describe your plans:

Based on this project we will continue to evaluate the mechanism of action of lead candidates and continue further screening for p53 pathway restoring compounds. The results from these studies will be submitted for publication.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project.
Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

Colorectal cancer accounts for 9% of all cancer-related deaths. Despite the approval of new agents, the 5-year survival remains very poor for patients with CRC that are not diagnosed at an early stage. There is a major unmet need for new tumor-specific therapeutic regimens to overcome resistance and toxicity associated with current therapy.

Tumor suppressor p53 is mutated in more than 50% of all human cancers. p53 mutations are known to contribute to disease progression and resistance to chemotherapy and radiation. Targeting mutant (mt) p53 is advantageous since it is a tumor-specific target. Currently, there are no FDA approved drugs in the clinic targeting mt p53.

We have developed a novel functional cell-based p53 luciferase reporter-based screening approach to screen small molecules that can reactivate a p53 pathway-dependent transcriptional response and induce cell death in mt p53-expressing -/+ p73 knockdown cancer cells. We have identified CB-758731, Prodigiosin and its related compound as potent p53 pathway-restoring compounds using this screening approach and will continue further screening for other small molecules.

Our project aims to validate CB-758731, Prodigiosin and its related compound as safe and effective p73-activating small molecules for CRC therapy. Our proposal evaluates three lead candidate compounds due to mechanistic convergence. This approach is more advantageous than relying on the efficacy of a single candidate. The most efficacious compound will be selected for potential further development and translation to the clinic in the future. These compounds will be evaluated for their efficacy as single agents for CRC therapy. The lead candidates may also be combined with current chemotherapy and radiation regimens in future studies.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

Our proposal includes work related to the novel p53 pathway restoring compound CB-758731. Preclinical data indicates that the compound is safe and effective for anti-cancer therapy.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes___X_____ No_____

If yes, please describe your plans:

The compound CB-758731 is a novel previously unpublished compound with p53 pathway restoring and anti-cancer activities. We plan to file a disclosure related to the work on this compound.

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
El-Deiry, Wafik S.	Professor of Medicine Chief, Division of Hematology/Oncology, Penn State Associate Director, Translational Research, PSHCI
eRA COMMONS USER NAME (credential, e.g., agency login) WEL-DEIRY	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Miami College of Arts & Sciences	B.S.	06/81	Chemistry/Math
University of Miami School of Medicine	M.D.	06/87	Medicine
University of Miami School of Medicine (So Lab)	Ph.D.	06/87	Biochemistry
The Johns Hopkins University (Vogelstein Lab)	post-doc	06/94	Mol. Genetics of Cancer

A. Personal Statement

Dr. El-Deiry's research is focused on unraveling cell death pathways involved in tumor suppression and mechanisms of cancer therapeutic responses. Dr. El-Deiry discovered the p21(WAF1) cell cycle inhibitor as a p53 target gene and mediator of p53-dependent tumor suppression. This is the most highly cited original research paper published in Cell. His group discovered TRAIL death receptor DR5 and elucidated regulation of TRAIL signaling and DR5 by p53. He is developing strategies to reverse therapy resistance by targeting restoration of the p53 pathway in tumors with mutated or dysfunctional p53 with small molecules. Dr. El-Deiry has significant expertise in cancer biology, in vivo imaging, drug screening, and combinatorial targeting of cell cycle, cell death and signal transduction pathways for cancer therapy. He has mentored over 60 post-doctoral fellows and PhD students and has played an active role in the career development of clinical fellows and faculty for over a decade. He serves as Associate Director for Translational Research at his cancer center and PI of several translational clinical protocols. Dr. El-Deiry along with his laboratory research group and clinical colleagues is well-positioned to pursue translational research to develop new cancer therapeutics.

B. Positions and Honors

Positions and Employment

07/87-06/88 Osler Intern in Medicine, The Johns Hopkins Hospital, Baltimore, MD
07/88-06/90 Junior and Senior Assistant Resident, Osler Medical Service, Johns Hopkins Hosp., Baltimore
07/90-06/94 Senior Clinical Fellow in Medical Oncology, The Johns Hopkins Oncology Center, Baltimore
07/94-06/99 Assistant Professor of Medicine, Department of Medicine, Univ. of Penn. School of Medicine
01/95-08/04 Assistant Investigator, Howard Hughes Medical Institute
06/95-06/99 Assistant Professor of Genetics, University of Pennsylvania School of Medicine
12/95-02/10 Active Medical Staff, Hospital of the University of Pennsylvania
06/96-02/10 Attending Staff (Solid Tumor Oncology), Hospital of the University of Pennsylvania
02/99-06/99 Assistant Professor of Pharmacology, University of Pennsylvania School of Medicine
07/99-06/05 Associate Professor of Medicine (Tenured), Genetics, and Pharmacology, Univ. of Pennsylvania
12/04-02/10 Co-Director, Radiation Biology & Imaging Program, Abramson Comprehensive Cancer Center
07/05-02/10 Professor of Medicine (Hem/Onc), Genetics, and Pharmacology, University of Pennsylvania
02/07-02/10 Associate Director for Physician-Scientist Training (Hematology/Oncology)

01/09- American Cancer Society Research Professor
03/10- Rose Dunlap Professor of Medicine and Chief, Hem/Onc, Penn State Hershey Medical Center
03/10- Associate Director for Translational Research, Penn State Hershey Cancer Institute
02/11- Program Leader, Experimental Therapeutics Program, Penn State Hershey Cancer Institute

Selected Peer-reviewed Publications (Selected from 209 original manuscripts & 114 reviews)

1. El-Deiry, W.S., Kern, S.E., Pietenpol, J.A., Kinzler, K.W., and Vogelstein, B. Definition of a consensus binding site for p53. **Nature Genetics** 1:45, 1992.
2. Kastan, M.B., Zhan, Q., El-Deiry, W.S., Carrier, F., Jacks, T., Walsh, W.V., Plunkett, B.S., Vogelstein, B., and Fornace, Jr., A. J. A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in Ataxia Telangiectasia. **Cell** 71:587, 1992.
3. El-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW, and Vogelstein B. WAF1, a potential mediator of p53 tumor suppression. **Cell,** 75:817-825, 1993.
4. Wales, M.M., Biel, M.A., El-Deiry, W., Nelkin, B.D., Issa, J.-P., Cavenee, W.K., Kuerbitz, S.J., and Baylin, S.B. p53 activates expression of HIC-1, a new candidate tumor suppressor gene on 17p13.3. **Nature Medicine**, 1:570-577, 1995.
5. da Costa, L.T., Liu, B., El-Deiry, W.S., Hamilton, S.R., Kinzler, K.W., Vogelstein, B., Markowitz, S., Willson, J.K.V., de la Chapelle, A., Downey, K.M., and So, A.G. Polymerase delta variants in RER colorectal tumors. **Nature Genetics**, 9:10-11, 1995.
6. Zeng, Y.-X., Somasundaram, K., and El-Deiry, W.S. AP-2 inhibits cancer cell growth and activates $p21^{WAF1/CIP1}$ expression. **Nature Genetics**, 15:78-82, 1997.
7. Somasundaram, K., Zhang, H., Zeng, Y.-X., Houvras, H., Peng, Y., Zhang, H., Wu, G.S., Licht, J.D., Weber, B.L., and El-Deiry, W.S. Arrest of the cell cycle by the tumour suppressor BRCA1 requires the CDK-inhibitor $p21^{WAF1/CIP1}$. **Nature**, 389:187-190, 1997.
8. Wu GS, Burns TF, McDonald III ER, Jiang W, Meng R, Krantz ID, Kao G, Gan D-D, Zhou J-Y, Muschel R, Hamilton SR, Spinner NB, Markowitz S, Wu G, and El-Deiry, WS. KILLER/DR5, a DNA damage-inducible p53-regulated cell death receptor gene. **Nature Genetics,** 17:141-143, 1997.
9. Yeh, W.-C., de la Pompa, J.L., McCurrach, M.E., Shu, H.-B., Elia, A.J., Shahinian, A., Ng, M., Wakeham, A., Mitchell, K., El-Deiry, W.S., Lowe, S.W., Goeddel, D.V., and Mak, T.W. FADD: Essential for embryo development and signaling from some, but not all, inducers of apoptosis. **Science**, 279:1954-1958, 1998.
10. Sax JK, Fei P, Murphy ME, Bernhard EJ, Korsmeyer SJ, and El-Deiry, WS. BID transcriptional regulation by p53 contributes to chemosensitivity. **Nature Cell Biology,** 4:842-849, 2002. PMID: 12402042
11. Ricci MS, Kim S-H, Ogi K, Plastaras JP, Ling J, Wang W, Jin Z, Liu YY, Dicker DT, Chiao PJ, Flaherty, KT, Smith, CD, and El-Deiry WS. Reduction of TRAIL-induced Mcl-1 and CIAP2 expression by c-Myc or Bay 43-9006 (Sorafenib) sensitizes resistant human cancer cells to TRAIL-induced death. **Cancer Cell,** 12:66-80, 2007.

D. Research Support

Ongoing Research

NIH R01 CA 173453-01A1 (PI: El-Deiry) 09/30/13-08/31/18
NIH/National Cancer Institute
Proposal Title: TIC10 ANTI-TUMOR EFFECT THROUGH REGULATION OF FOXO3A AND TRAIL.
Major goals of the project: Specific Aim #1: Identify TIC10-induced effects on Foxo3a expression, phosphorylation, and subcellular localization; Specific Aim #2: Elucidate the role of Akt and ERK kinases in the mechanism of action of TIC10; Specific Aim #3: Determine the differential regulation of TRAIL gene transcription by FOXO family members.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Zhang, Shengliang	POSITION TITLE Director of Research Project
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Dalian Medical University, Dalian CHINA	Bachelor of Medicine	07/93	Laboratory Medicine
Dalian Medical University, Dalian CHINA	MSc	07/99	Biochemistry and Molecular Biology
Tokyo Medical and Dental University, Tokyo JAPAN	Ph.D.	10/04	Molecular Cellular Oncology
University of Pennsylvania, Philadelphia, PA	Postdoctoral	09/09	Dermatology and Orthopaedic Surgery

A. Personal Statement

Dr. Zhang's research has focused on signal transduction in regulating cancer cell growth and death. He is conducting drug discovery and development targeting p53 pathway for cancer therapy by use of cell-based assay, in vivo and in vitro imaging and preclinical translational studies. As a postdoctoral fellow at the University of Pennsylvania, he discovered that tissue specific transcription factor regulates one subset of ribosomal RNA gene transcription. He further extended his research into stem cell differentiation and demonstrated that dynamic G protein-cAMP signal pathway regulates stem cell fate decisions and differentiation. In addition, he discovered new factors related to ERK2 signal pathway, and demonstrated that Naf1 is not only a new factors related to ERK2 signal, but also related to cell cycle and regulates cancer cell apoptosis.

B. Positions and Honors

Positions and Employment

1993-2000 Assistant Professor, Dept. of Clinical Biochemistry, Dalian Medical University, Dalian, CHINA
2002-2004 Research Assistant, Dept. of Molecular Cellular Oncology, Tokyo Medical and Dental University, Tokyo, JAPAN
2009-2010 Research Associate, Dept. of Orthopaedic Surgery, University of Pennsylvania, Philadelphia, PA
2010-present Director of research project. Penn state Hershey Cancer Institute, Hershey,PA

Other Experience and Professional Memberships

1998-2000 member, Chinese Association of Medical Sciences,

2002-2006 member, Japanese Cancer Association
2005-present member, America Association for Cancer Research

Honors

1991, 1992 Dalian City's Best Student Award, Dalian, CHINA
1998 China Medical Board (CMB) Fellowship, Peking Union Medical college and Chinese Academy of Medical Sciences, Beijing, CHINA
1998 The third prize of Liaoning Province Government Award for Advanced Science and Technology Progress, Liaoning province, CHINA
1999 The third prize of Best Thesis Award at Dalian Medical University, CHINA
2000-2001 Takase International Scholarship, Takase international scholarship foundation, Japan.
2002-2004 Honjo International Scholarship, Honjo international scholarship foundation, JAPAN.

C. Selected Peer-reviewed Publications (Selected from 19 peer-reviewed publications)

1. **Zhang S,** Zhang Y, Yan P, Gao H. Mutation and abnormal expression of $P16^{INK4a}$ in hepatocelluar carcinoma. *Chinese Journal of Cancer Research* 11(4): 260-263, 1999.
2. **Zhang S,** Fukushi M, Hashimoto S, Gao C, Huang L, Fukuyo Y, Nakajima T, Tamfasa T, Enomoto S, Koike K, Miura O, Yamamoto N, Tsuchida N. A new ERK2 binding protein, Naf1, attenuates the EGF/ERK2 nuclear signaling. *Biochem Biophys Res Commun* 279: 17-23, 2002.
3. Gao C, Ren S, Wang J, Zhang S, Jin F, Nakajima T, Ikeda M, Tsuchida N. P130 and its truncated form mediate p53-induced cell cycle arrest in $Rb^{-/-}$Saos2 cells. *Oncogene* 21: 7569-7579, 2002.
4. Huang L, Gotoh N, **Zhang S,** Shibuya M, Yamamoto T, Tsuchida N. SNT-2 interacts with ERK2 and negatively regulates ERK2 signaling in response to EGF stimulation. *Biochem Biophys Res Commun* 324:1011-1017, 2004
5. Wang J, * **Zhang S *,** Tseng H. Search for Basonuclin Target Gene. *Biochem Biophys Res Commun* 348:1261-1271, 2006. (*equal contribution)
6. **Zhang S,** Wang J, Tseng H. Basonuclin regulates a subset of ribosomal RNA genes in HaCaT cells. *Plos ONE* 2(9): e902, 2007
7. Tseng H, Chuo W, Wang J, Zhang X, Zhang S, Schultz RM. Mouse ribosomal RNA gene contain multiple differentially regulated variants. *Plos ONE* 3(3):e1843, 2008
8. **Zhang S,** Marthandan M, Tsuchida N. Naf1α is phosphorylated in mitotic phase and required to prevent cells against apoptosis. Biochem Biophys Res Commun 367(2):304-369, 2008.
9. **Zhang S,** Kaplan FS, Shore EM. Different roles of Gnas and cAMP signaling during early and late stages of osteogenic differentiation. *Horm Metab Res***.** 44(10);724-31, 2012

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Bo Hong	Postdoctoral fellow
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Anhui University, China	B.Sc.	06/2003	Biology
University of Science and Technology of China, China	M.Sc.	06/2006	Genetics
The Chinese University of Hong Kong, Hong Kong	Ph.D.	09/2009	Medical Sciences
Penn State Hershey Medical Center, Hershey, PA	Postdoctoral	03/2010	Experimental molecular therapeutics

A. Personal Statement

I am a third year postdoctoral fellow in Penn State Hershey Cancer Institute. I received my bachelor and master degree in China. As an undergraduate, I majored in biology and got a basic biological education and training including molecular biology, microbiology, biochemistry, cell biology and genetics. During my master degree, I obtained broad laboratory skill training and I identified several novel transcriptional factors which can bind to the promoter of a gene and regulate the expression of the gene. The study has been published in an international journal, Biochimica et Biophysica Acta (BBA). After Master graduation, I decided to study cancer, a most critical challenge facing us. I chose the field of experimental molecular therapeutics, which is most likely to be applied in cancer patient therapy in the future. So I came to the Chinese university of Hong Kong, Department of Clinical Oncology to pursue my PhD degree in Medical Sciences. My PhD project is that hypoxia-targeting in Nasopharyngeal Cancer (NPC). I studied the anti-growth and anti-invasion efficacy of two hypoxia-targeting small molecules and indentified their antitumor mechanisms. The study has been published in two international journals, Biochemical Pharmacology and Investigational New Drugs. Followed my PhD study, I joined Dr. El-Deiry lab, a very famous p53-studying lab, to pursue my postdoctoral training. My project is that the development of the small molecules targeting mutant p53 in cancer. By high-throughput drug screening, we have indentified some promising small molecules targeting mutant p53. I have obtained very promising data, which showed that a p53-targeting compound restored p53 pathway and induced cancer cell death in mutant p53 cancer cells. The proposed research will study these mutant p53 targeting small molecules in detail, focus on the mechanism of p53 rescue in mutant p53 cancers, and study the possibility of clinical application. My academic training and research experience have provided me with an excellent background to perform this study.

B. Positions and Honors

Positions and Employment

2009-2010 Research Assistant, Department of Clinical Oncology, The Chinese University of Hong Kong, Hong Kong

2010-present Postdoctoral fellow, Penn State Hershey Medical Center, Hershey, PA

Other Experience and Professional Memberships

2007-present Associate member, American Association for Cancer Research

Honors

2008 CUHK Postgraduate Student Grants for Overseas Academic Activities (4th Batch of Awards, 2007-08), The Chinese University of Hong Kong, Hong Kong

2011 Highly Rated Poster Presentation in AACR 102nd Annual Meeting 2011, Orlando, FL

C. Selected Peer-reviewed Publications

1. Prabhu VV, Allen JE, **Hong B**, Zhang S, Cheng H, El-Deiry WS. Therapeutic targeting of the p53 pathway in cancer stem cells. **Expert Opin Ther Targets**. 2012;16(12):1161-1174.
2. Cheng H, **Hong B**, Zhou L, Allen JE, Tai G, Humphreys R, Dicker DT, Liu YY, El-Deiry WS. Mitomycin C potentiates TRAIL-induced apoptosis through p53-independent upregulation of death receptors: evidence for the role of c-Jun N-terminal kinase activation. **Cell Cycle**. 2012;11(17):3312-3323.
3. **Hong B**, Lui VW, Hashiguchi M, Hui EP, Chan AT. Targeting tumor hypoxia in nasopharyngeal carcinoma. **Head & Neck**. 2013 Jan;35(1):133-145.
4. Wang W, Gallant JN, Katz SI, Dolloff NG, Smith CD, Abdulghani J, Allen JE, Dicker DT, **Hong B**, Navaraj A, El-Deiry WS. Quinacrine sensitizes hepatocellular carcinoma cells to TRAIL and chemotherapeutic agents. **Cancer Biology & Therapy**. 2011;12(3):229-238.
5. **Hong B***, Lui VW*, Hui EP, Ng MH, Cheng SH, Sung FL, Tsang CM, Tsao SW, Chan AT. Hypoxia-targeting by Tirapazamine (TPZ) Induces Preferential Growth Inhibition of Nasopharyngeal Carcinoma Cells with Chk1/2 Activation. **Investigational new drugs**. 2011;29(3):401-410, (*denotes co-first authorship).
6. Lui VW, Yau DM, Cheung CS, Wong SC, Chan AK, Zhou Q, Wong EY, Lau CP, Lam EK, Hui EP, **Hong B**, Hui CW, Chan AS, Ng PK, Ng YK, Lo KW, Tsang CM, Tsui SK, Tsao SW, Chan AT. FGF8b oncogene mediates proliferation and invasion of Epstein-Barr virus-associated nasopharyngeal carcinoma cells: implication for viral-mediated FGF8b upregulation. **Oncogene**. 2011;30(13):1518-1530.
7. Lui VW, Wong EY, Ho K, Ng PK, Lau CP, Tsui SK, Tsang CM, Tsao SW, Cheng SH, Ng MH, Ng YK, Lam EK, **Hong B**, Lo KW, Mok TS, Chan AT, Mills GB. Inhibition of c-Met downregulates TIGAR expression and reduces NADPH production leading to cell death. **Oncogene**. 2011;30(9):1127-1134.
8. **Hong B***, Lui VW*, Hui EP, Lu Y, Leung HS, Wong EY, Cheng SH, Ng MH, Mills GB, Chan AT. Reverse Phase Protein Array Identifies Novel Anti-invasion Mechanisms of YC-1. **Biochemical Pharmacology**. 2010; 79(6):842-852, (*denotes co-first authorship).
9. Lui VW, Lau CP, Cheung CS, Ho K, Ng MH, Cheng SH, **Hong B**, Tsao SW, Tsang CM, Lei KI, Yamasaki Y, Mita A, Chan AT. An RNA-directed nucleoside anti-metabolite, 1-(3-C-ethynyl-beta-d-ribo-pentofuranosyl)cytosine (ECyd), elicits antitumor effect via TP53-induced Glycolysis and Apoptosis Regulator (TIGAR) downregulation. **Biochemical Pharmacology**. 2010;79(12):1772-1780.