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. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** The Wistar Institute

2. **Reporting Period (start and end date of grant award period):** 01/01/2011 – 6/30/2012

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Russel E. Kaufman, M.D.

4. **Grant Contact Person's Telephone Number:** 215-898-3926

5. **Grant SAP Number:** 4100054879

6. **Project Number and Title of Research Project:** 1 - Isoform Specific p73 Regulatory Networks in Neurogenesis

7. **Start and End Date of Research Project:** 01/01/2011 – 6/30/2012

8. **Name of Principal Investigator for the Research Project:** Ramana V. Davuluri, Ph.D.

9. **Research Project Expenses**.

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

   $ 642,495.80

   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	Position Title	% of Effort on Project	Cost
Davuluri, Ramana,.	Principal Investigator	55.93 %	$ 148,102.21
Gupta, Ravi	Staff Scientist	100 %	$ 47,998.40
Kim, Hyunsoo	Staff Scientist	94 %	$ 36,660.54
Bi, Yingtao	Post-Doctoral Fellow	67 %	$ 45,570.10
Pal, Sharmistha	Post-Doctoral Fellow	100 %	$ 59,762.26

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	Position Title	% of Effort on Project
Showe	Co-Investigator	3%

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___✓_____

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 __√____ 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:
Informatics platform for mammalian gene regulation at isoform-level (Impact/Priority Score - 24 (Three percentile; Pending council review)	☒NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	March, 2012	$ 1,269,750	$ Pending
Molecular sub-typing of gliomas by isoform-level gene network modeling	☒NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	June, 2012	$ 2,880,958	$ Pending
Modeling isoform specific tp73 regulatory networks in neurogenesis	☒NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	November, 2011	$ 2,116,250	$ Not Funded

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:

Based on the promising results on isoform-level expression analysis of genes in melanoma and breast cancer cell lines, and validations in breast cancer tissue samples, we are planning to submit the following grant application in the coming year:

RO1 application to NIH, "Molecular isoform-level transcriptional networks in mammalian cells"

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

We will continue the development of bioinformatics analysis methods for integrating

transcriptome (RNA-seq and exon-array data) and epigenome (DNA methylation) data from public data repositories (e.g. ENCODE, TCGA consortia data) to find expression of TP73 isoforms in different cells, tissues and disease conditions.

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_____ No____X_____

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

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

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

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

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

Yes_____ No____✓_____

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.

CURE funding has increased our capability to conduct large-scale ChIP-seq and RNA-seq studies and integrative computational modeling for genome-wide profiling of the transcriptome and TF-DNA interactions in mammalian genomes, specifically in cancer genomes.

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___X_____ No_____

If yes, please describe the collaborations:

a) New collaboration with Dr. Donald O'Rourk, Director of Penn Brain Tumor Tissue Bank, Neurosurgeon, University of Pennsylvania. This collaboration has led to submission of an R01 application to NIH.
b) New collaboration with Dr. Vivek Mittal, an internationally recognized cancer researcher and the Director of the Lehman Brothers Lung Cancer Laboratory at Weill Cornell Medical College, Cornell University, New York, NY.

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 application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims <u>for the period that the project was funded (i.e., from project start date through end date).</u> 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.

Specific Aims

Aim 1. Conduct ChIP-seq (to derive direct target genes of TP53, TAp73 & DNp73) and mRNA-seq (to find the overall transcriptional outcome, including all the transcript isoforms) experiments in differentiated cells at day 0, 4, and 7 after treating the P19 cells with retinoic acid, in a) wild-type P19 cells, b) TAp73-knock down P19 cells, and c) DNp73-knock down P19 neuronal cells.

Aim 2. Develop novel computational methods to model the combinatorial interaction of p73 isoforms (TAp73 and DNp73) with p53 family members and other TFs and decipher isoform regulatory networks of each isoform in controlling the expression of target genes during neuronal differentiation, under normal and perturbed cellular conditions.

Summary of Research Completed

In vitro neuronal differentiation of P19 cells (embryonal carcinoma) treated with retinoic acid.

We performed *in vitro* neuronal differentiation of P19 cells treated with retinoic acid as summarized in Figure 1A (1). As a proof of principle, to follow the neuronal differentiation, we measured the expression of ES cell marker (Oct3/4), neuronal precursor cell marker (Nestin), and neuronal marker (Map2) during the course of nine days differentiation (Figure 1B). As expected, we observed a drop in Oct3/4 expression and an increase in Nestin expression from days 0 to 4 and a highly induced expression of Map2 by days 6 to 9. When we looked at the expression of the p73 isoforms, we found opposing behavior (Figure 1B). As cells are subjected to the differentiation program, there is a marked decrease in DNp73 expression accompanied by an increased expression of TAp73 from day 0 to 4 followed by a decline by day 9.

Strikingly, the protein expression of TAp73 increases during differentiation from day 0 to 9, although mRNA expression tends to decrease between day 4-9; and for DNp73, despite the rapid loss of its mRNA, no change is observed in its protein levels until day 9 during neuronal differentiation (Figure 2A). These results indicate that p73 gene expression is also regulated through translation and protein stability (2). Furthermore, in agreement with previous studies, we found that TAp73 is present in both nucleus and cytoplasm while DNp73 is a nuclear protein (3).

ChIP-seq & RNA-seq results suggest isoform-specific gene regulation by TAp73 and DNp73.

Recent studies using neural stem cells from WT and p73-/- mice indicate that both TAp73 and DNp73 are expressed in neural stem cells and that they are involved in both embryonic and adult neurogenesis, a process involving differentiation of neural stem cells to mature postmitotic neurons (4). Having established that p73 isoforms are differentially expressed during differentiation of neurons, we hypothesized that TAp73 and DNp73 regulate distinct genes by interacting with different co-regulatory proteins/complexes during neurogenesis. To identify the target genes of each isoform, we decided to conduct ChIP-seq experiments using commercially available isoform-specific antibodies (Imgenex Inc.). We first tested the specificity of the antibodies by Western blotting experiments on over-expressed flag-tagged TAp73 or DNp73 protein in 293T cells and found that the antibodies do not cross-react (Figure 2B). Additionally, we performed immunoprecipitation followed by Western blotting experiments, which confirmed that the antibodies are effective for immunoprecipitating the p73 protein isoforms (Figure 2C).

Next, we performed ChIP-seq experiments with the tested isoform-specific antibodies on cross-linked chromatin from undifferentiated P19 cells (day 0). Following sequencing and bioinformatics data analysis, we identified approximately 151,844 and 122,900 significant ChIP-seq peaks (p-value ≤ 0.0001) for TAp73 and DNp73; and it was found that TAp73 and DNp73 were recruited to the 5' proximal region (-5Kb to + 1Kb around transcription start site (TSS)) of 21,316 and 19,153 gene promoters, respectively (Figure 3).

We found that, for some genes like *Timeless*, the two isoforms are regulating the expression of distinct promoter driven mRNA transcripts of the same gene (Figure 4A), while on the other hand TAp73 and DNp73 recruitment is mutually exclusive on promoters of *Spp1* and *NeuroD2* (Figure 4A). We also observed co-localization of both isoforms on some promoters, such as the downstream promoter of *RTN4* (Figure 4A). It has been suggested that DNp73 can

heterodimerize with TAp73 and function as a dominant negative protein, and we speculate that this contributes to the presence of both isoforms on specific gene promoters (3).

Further analysis on TAp73 and DNp73 binding on -1Kb to +1Kb region of TSS has revealed that the recruitment of both isoforms is mostly mutually exclusive with 7759 and 6750 promoters being TAp73 and DNp73 specific, respectively, while both isoforms co-exist on 1600 promoters, thus identifying distinct pathways that are regulated by the p73 isoforms (Figure 4B). Our results show that, while TAp73 regulates members of mTOR, axonal guidance, synaptic long term potentiation, type II diabetes mellitus, eIF4 and p70S6K signaling pathways, DNp73 regulates FAK, Reelin, Rac, Integrin, and PAK signaling. The identification of p73 isoform-specific target promoters provides a strong basis for our hypothesis that each isoform interacts with different co-regulatory complex to regulate target gene expression.

To identify the transcriptional outcome/consequences of p73 isoform recruitment, we have performed mRNA-seq on undifferentiated P19 (day 0) and specified P19 cells (day 4). We observed that 16,557 and 17,462 genes are expressed in day 0 and retinoic acid treated day4 P19 cells through 21,481 and 22,655 promoters that transcribe 31,938 and 33,272 transcripts respectively. We have observed that some promoters targeted by TAp73 are highly expressed, while others targeted by DNp73 are repressed (e.g. shown in Figure 4A). We also found that the expression of 11,038 and 7,832 transcripts are up and down regulated by day 4 of neurodifferentiation.

Isoform specific knockdown of p73

The disruption of either isoform impacts normal neurogenesis and we speculate that the changes in the p73 networks (altered cell programs) in the neural stem cells are the source of differentiation defects (4). Since the relative levels of TAp73 and ΔNp73 regulate cell fate, we are interested to determine the molecular changes and altered cell programs/networks that follow the perturbation of normal TAp73/ΔNp73 ratio, a phenomenon observed in most human cancers, in undifferentiated P19 cells. To address this issue we have generated lentiviruses carrying shRNA for isoform specific knockdown of TAp73 or ΔNp73. TAp73 shRNA is a kind gift from Dr. Leif W. Ellisen (Dana-Farber/Harvard Cancer Center), while ΔNp73 targeting shRNA has been cloned using siRNA sequences previously used for specific knockdown of ΔNp73. P19 cells were infected with lentivirus containing either TAp73shRNA or ΔNp73 shRNA or vector alone control (Ctrl) for 48 hr before cells were harvested and RNA isolated. We performed RT-PCR analysis to measure the efficacy and specificity of p73 isoform knockdown. Our results indicate that we were able to achieve about 50% knockdown of each isoform and hence are successfully perturbing the system, which is evident from the aberrant expression of neuronal (*Nestin*) and glial cell (*Gfap*) marker in these knockdown cells (Figure 5).

Experimental validation of the transcriptional partners and networks of TAp73, ΔNp73 and p53:

We validated our computational predictions from ChIP-seq and RNA-seq data. We predicted that YY1 and c-Jun (an AP-1 protein) are transcriptional partners of TAp73 (Figures 6 and 7) on a subset of TAp73 target promoters in undifferentiated P19 cells (Day0). Using an integrative approach, that involves analysis of the ChIP-seq data from day0 P19 cells, mRNA-seq data from

day 0 and specified day 4 P19 cells, identification of p73 binding sites and their neighboring TFBS, we predicted several TF partners that included YY1 and AP1 proteins. We validated the in vivo interaction of TAp73 and YY1 or c-Jun by ChIP-re-ChIP (First antibody:anti-TAp73; second antibody:anti-YY1 or c-Jun) experiment on the promoters of fasn, zfp217, etv5, which demonstrate the simultaneous binding of these TFs on the same DNA fragment in day0 and not day4 P19 cells (Figure 6). In contrast, we failed to observe either TAp73/YY1 or TAp73/c-Jun co-occupancy on maml3 promoter. Furthermore, we performed individual ChIP experiments on the above promoters using anti-TAp73, ΔNp73, YY1, c-Jun antibodies, which indicated that there is a loss of TAp73 binding on all the above four promoters while binding of YY1 and c-Jun is retained on the promoters of etv5, maml3 and zfp217 and etv5, respectively in day 4 P19 cells (Figure 7). One of the TF partners, c-Jun has been recently reported by Koeppel etal using an inducible TAp73alpha- Saos2 osteosarcoma cell line. Taken together, our results indicate that on some promoters, YY1 and c-Jun function as TF partners for TAp73 in undifferentiated P19 cells

Alternative transcription and alternative splicing in cancer

We conducted review of literature to understand the mechanism of 'cancer specific' alternative transcription and alternative splicing, which have long been implicated in the development of cancer. We prepared this review in response to an invitation from Pharmacology & Therapeutics journal editor. This article will be published by the end of the year (5). The summary of this review is:

In recent years, the notion of "one gene makes one protein that functions in one signaling pathway" in mammalian cells has been shown to be overly simplistic. Recent genome-wide studies suggest that at least half of the human genes, including many therapeutic target genes, produce multiple protein isoforms through alternative splicing and alternative usage of transcription initiation and/or termination. For example, alternative splicing of the vascular endothelial growth factor gene (VEGFA) produces multiple protein isoforms, which display either pro-angiogenic or anti-angiogenic activities. Similarly, for the majority of human genes, the inclusion or exclusion of exonic sequences enhances the generation of transcript variants and/or protein isoforms that can vary in structure and functional properties. Many of the isoforms produced in this manner are tightly regulated during normal development but are misregulated in cancer cells. Altered expression of transcript variants and protein isoforms for numerous genes is linked with disease and its prognosis, and cancer cells manipulate regulatory mechanisms to express specific isoforms that confer drug resistance and survival advantages. Emerging insights indicate that modulating the expression of transcript and protein isoforms of a gene may hold the key to impeding tumor growth and act as a model for efficient targeting of disease-associated genes at the isoform level. This review highlights the role and regulation of alternative transcription and splicing mechanisms in generating the transcriptome, and the misuse and diagnostic/prognostic potential of alternative transcription and splicing in cancer.

References:

1. Endo M, Antonyak MA, Cerione RA. Cdc42-mTOR signaling pathway controls Hes5 and Pax6 expression in retinoic acid-dependent neural differentiation. J Biol Chem. 2009;284(8):5107-18. Epub 2008/12/23. doi: M807745200 [pii] 10.1074/jbc.M807745200. PubMed PMID: 19097998.

2. Tomkova K, Tomka M, Zajac V. Contribution of p53, p63, and p73 to the developmental diseases and cancer. Neoplasma. 2008;55(3):177-81. Epub 2008/03/20. PubMed PMID: 18348649.
3. Grob TJ, Novak U, Maisse C, Barcaroli D, Luthi AU, Pirnia F, et al. Human delta Np73 regulates a dominant negative feedback loop for TAp73 and p53. Cell Death Differ. 2001;8(12):1213-23. Epub 2001/12/26. doi: 10.1038/sj.cdd.4400962. PubMed PMID: 11753569.
4. Talos F, Abraham A, Vaseva AV, Holembowski L, Tsirka SE, Scheel A, et al. p73 is an essential regulator of neural stem cell maintenance in embryonal and adult CNS neurogenesis. Cell Death Differ. 2011;17(12):1816-29. Epub 2010/11/16. doi: cdd2010131 [pii] 10.1038/cdd.2010.131. PubMed PMID: 21076477.
5. Pal S, Gupta R, Davuluri RV. Alternative transcription and alternative splicing in cancer. Pharmacology & Therapeutics. 2012:(In Press).

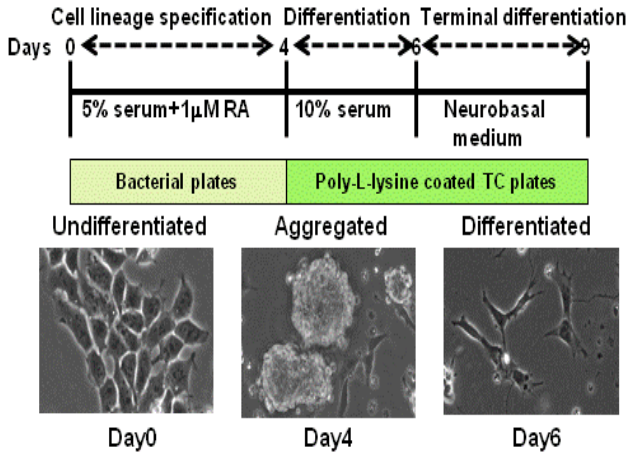

Figure 1A: In vitro differentiation of P19 cells to neurons.

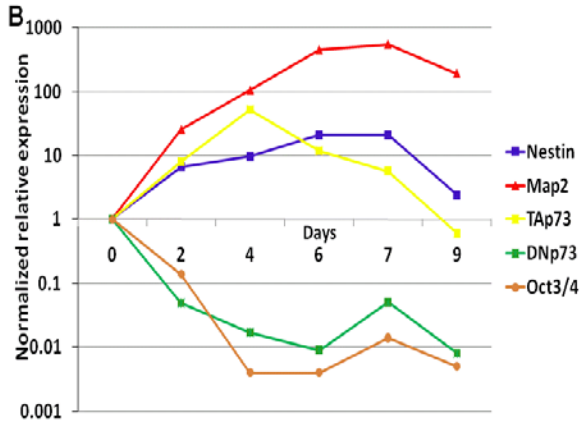

Figure 1B: mRNA expression of ES, Neuronal precursor and Neuron cell markers, along with TAp73 and DDNp73 during P19 differentiation.

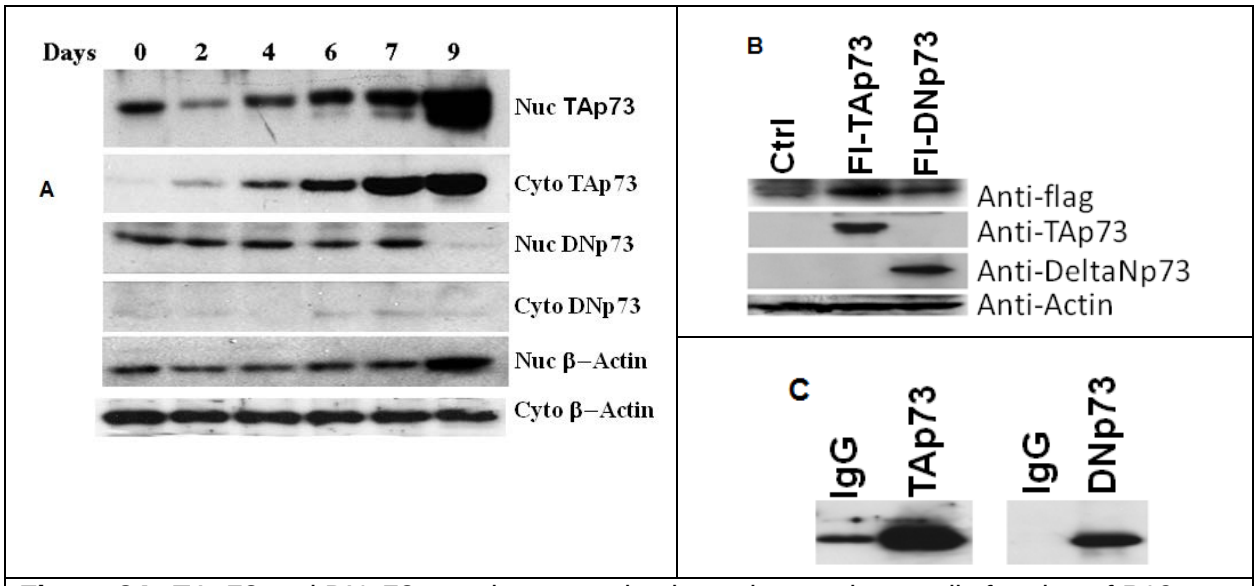

Figure 2A: TAp73 and DNp73 protein expression in nuclear and cytosolic fraction of P19 cells at different stages of differentiation.

Figure 2B: 293T cells were transfected with plasmids expression flag tagged either TAp73 or DNp73 for 48 hours before cell extracts were prepared and analyzed by Western blotting with indicated antibodies.

Figure 2C: P19 nuclear extracts were subjected to immunoprecipitation with either TAp73 or DNp73 antibodies before detection by Western blotting.

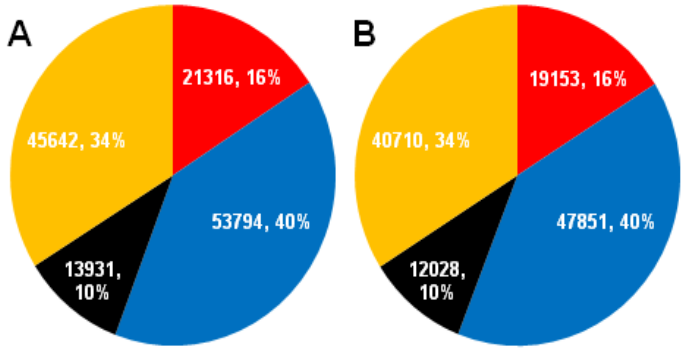

Figure 3: The distribution of genome wide TAp73 (A) and DNp73 (B) binding in TSS proximal region (-5Kb to +1Kb in red), TSS distal (-5Kb to -25Kb in black), intragenic (blue) and intergenic (yellow).

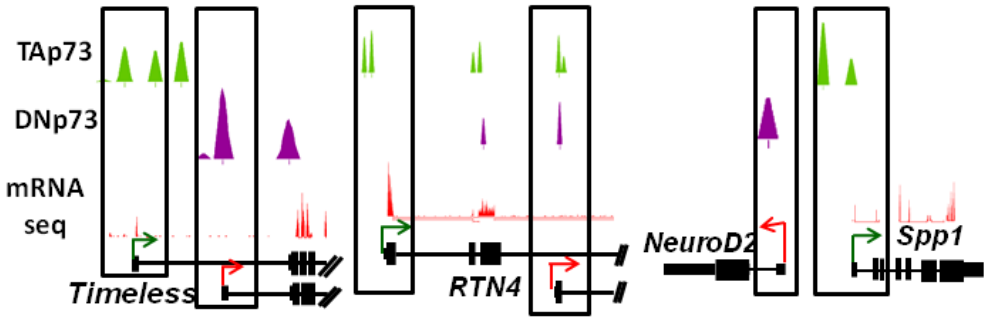

Figure 4A: Genomic binding profile of TAp73 (green) and DNp73 (purple),and mRNA-seq expression profile (red) on the promoters of Timeless, RTN4, NeuroD2 and Spp1 in undifferentiated day 0, P19 cells.

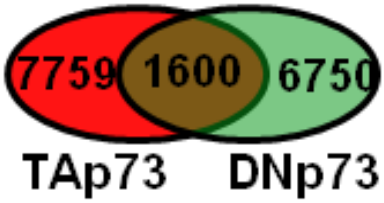

Figure 4B: Venn diagram shows the unique and overlapping target promoters of TAp73 and DNp73.

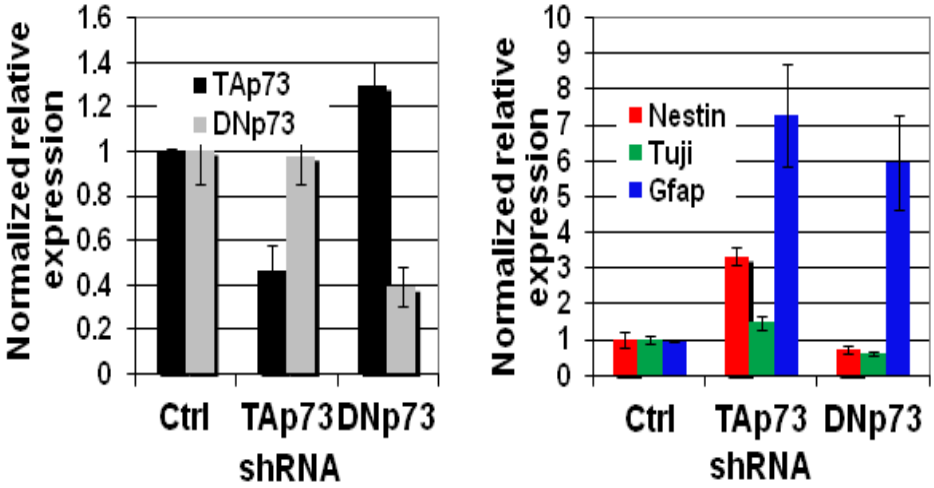

Figure 5: RT-PCR analysis on total RNA isolated from P19 cells that were infected with lentivirus containing the indicated shRNA for 48 hrs. The isoform specific shRNA show knockdown specificity and the observed knockdown is enough to cause aberrant expression of *Nestin* and *Gfap.*

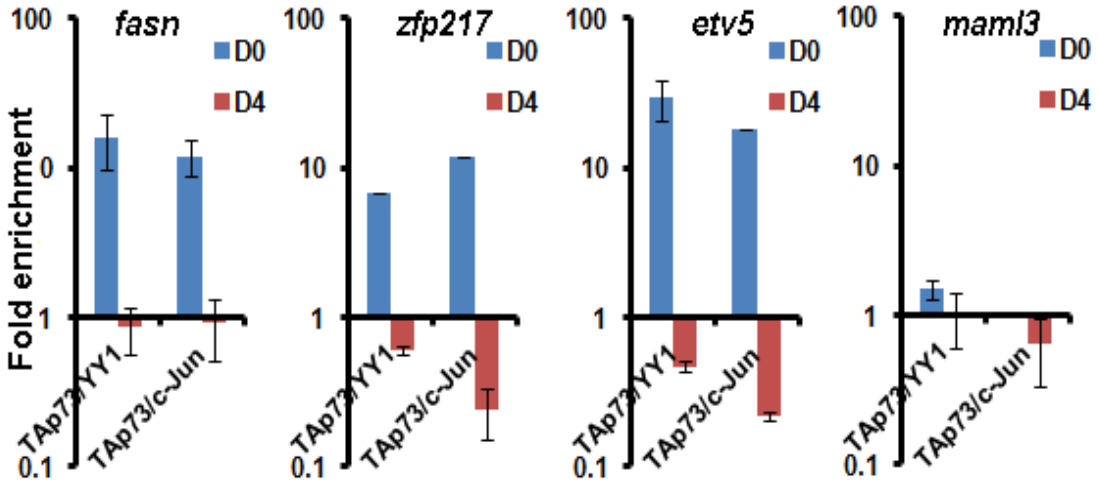

Figure 6: YY1 and c-Jun are TF partners for TAp73 in undifferentiated P19 cells. (A) Undifferentiated (day0) and specified (day4) P19 cell chromatin was subjected to first immunoprecipitation with anti-TAp73 antibodies and bound nucleoprotein complexes were eluted. These nucleoprotein complexes were subjected to a second round of immunoprecipitation with either anti-nonspecific IgG, anti-YY1, or anti-c-Jun antibodies to isolate the DNA fragments with bound TAp73/YY1 or TAp73/c-Jun proteins together. The co-recruitment on the indicated gene promoters was determined by real time PCR.

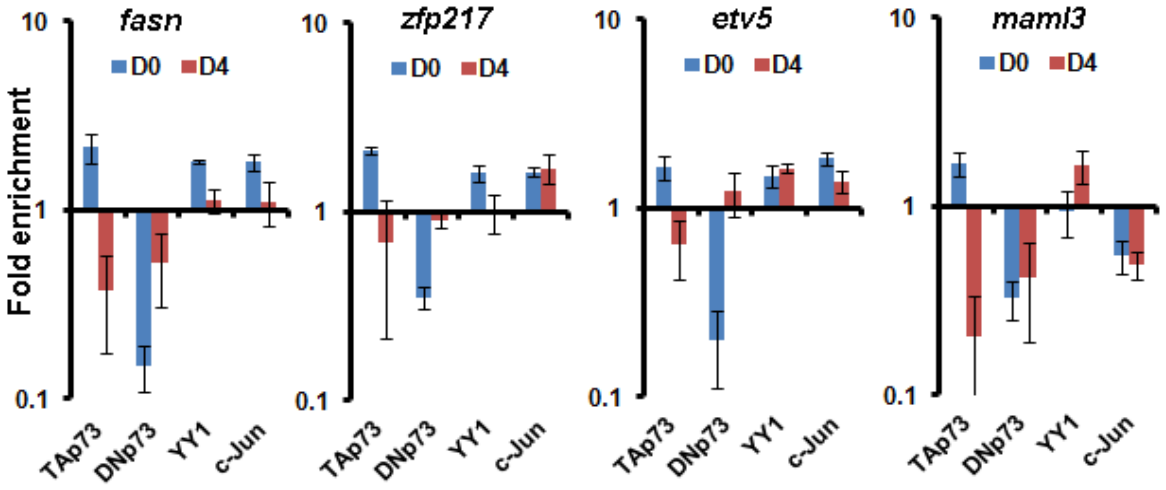

Figure 7: ChIP experiments were performed on day 0 and 4 chromatin using either anti-nonspecific IgG, TAp73, ΔNp73,YY1, orc-Jun antibodies individually and recruitment was determined on eluted DNA by PCR for selected targets. ld enrichment was calculated relative to the IgG control samples in both panel A and B. We observe that TAp73 recruitment is lost by day4 on all four promoters while c-Jun and YY1 protein is retained on select promoters. Day0 corresponds to blue bar and day4 to red bars.

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, listed in the table, in a PDF version 5.0.5 format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For

example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

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):
1. Alternative transcription and alternative splicing in cancer	Pal, S., Gupta, R. and Davuluri, R.V.	*Pharmacology & Therapeutics*	May 2012	☐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:

We are planning to submit the following manuscript: "Isoform-specific transcritptional networks of TP73" (In preparation).

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.

None.

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.

None

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_____ No___X_____

If yes, please describe your plans:

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. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH

Provide the following information for the 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 **DAVULURI, Ramana V.**	POSITION TITLE **Associate Professor**
eRA COMMONS USER NAME **DAVULURI10**	

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

INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
Nagarjuna University, India	B.S.	1985-1988	Mathematics
Indian Agricultural Statistics Research Institute, IARI, New Delhi, India	M.S.	1989-1991	Statistics & Computer Applications
Indian Agricultural Statistics Research Institute, IARI, New Delhi, India	Ph.D.	1991-1996	Statistics & Computer Applications
Dept. of Plant Genetics, VIB, University of Ghent, Gent, BELGIUM.	Post Doc. Fellow	1998	Bioinformatics
Cold Spring Harbor Laboratory (CSHL), Cold Spring Harbor, NY 11724, USA	Post Doc. Fellow	1999-2001	Computational Biology

A. *Personal Statement*

Dr. Davuluri has worked in the areas of bioinformatics and computational genomics since 1998, starting with the postdoctoral training in Dr. Michael Zhang's laboratory at CSHL, NY. Research in his laboratory is primarily focused on developing statistically rigorous data-mining algorithms and informatics platforms for better understanding gene regulatory mechanisms in mammalian cells at isoform-level, and more importantly, how dis-regulation of these mechanisms leads to cancer. His research program is interdisciplinary in nature with a complement of experimental investigation. Coming from a background in quantitative sciences, he gained synergistic expertize in statistical modeling, genomics and bioinformatics, while leading several projects over the past 11 years. During his tenure at OSU, he was a Co-PI of an NSF's Biological Infrastructure grant; and Project Leader (Project #3) and Core Director (Data Management & Computational Modeling Core) of a P50 grant supported by NCI's Integrative Cancer Biology Program (OSU center for Cancer Epigenetics). He was also awarded an NIH R01 and American Cancer Society Research Scholar Grant to study the epigenetic modifications across alternative promoters and associated isoform-level gene signatures in normal and cancer genomes. His group develops computational models and bioinformatics tools for systems biology research and uses integrative bioinformatics and NextGen sequencing methods to study brain development and brain tumors (e.g., Pal, et al. 2011, *Genome Research;* Pal, et al. 2012, *Pharmacology & Therapeutics*). He is well qualified to lead the proposed research project.

B. *Positions and Honors*

Professional Experience

1993-1998 Asst. Professor of Statistics, ANGR Agricultural University, Hyderabad, INDIA

1998-1999 Special Project Scientist (Bioinformatics), International Crops Research Institute

	for the Semi Arid Tropics, (ICRISAT), Patancheru, AP, INDIA
2001	Research Investigator, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
2001-2007	Assistant Professor, Bioinformatics & Computational Biology, Human Cancer Genetics (HCG) Program, Department of Mol. Virology, Immunology and Medical Genetics, OSU, Columbus, OH
2001-2007	Adjunct Assistant Professor, Department of Biomedical Informatics, OSU, Columbus, OH
2002-2008	Head of Bioinformatics Unit, HCG, Comprehensive Cancer Center, OSU, Columbus, OH
2003-2008	Mentor in Biosciences, Mathematical Biosciences Institute, OSU, Columbus, OH
2007-2008	Associate Professor (tenured), Bioinformatics & Computational Biology, HCG Program, Dept. of Mol. Virology, Immunology and Medical Genetics, OSU, Columbus, OH
2008-Present	Associate Professor; Director of Computational Biology, Center for Systems and Computational Biology, The Wistar Institute, Philadelphia, PA
2008-2011	Scientific Director, Bioinformatics Facility, The Wistar Institute Cancer Center, Philadelphia, PA
2008-Present	Wistar Associate Professor, Dept of Genetics, University of Pennsylvania, Philadelphia, PA
2012-Present	Scientific Investigator, Penn Brain Tumor Center, University of Pennsylvania, Philadelphia, PA

Honors and Professional Service

1989-1993	IASRI (Indian Council of Agricultural Research) junior & senior research fellowships
1997	Young Scientist Award (Statistics): 1996-97 by *Indian Science Congress Association*, 84th annual session, Delhi, India. [A prestigious award presented by the prime minister of India]
2003-2005	V Scholar Award, The V foundation for Cancer Research, USA
2006-2009	Ad Hoc Reviewer, National Science Foundation, USA; National Institutes of Health, USA.
2007	Program Committee Member, *Interface 2007: the 39th Symposium on the interface of statistics, computing science, and applications*, May 23-27 Philadelphia, USA
2007-08	Program Committee Member, *PSB 2008, 2009: (Pacific Symposium of Biocomputing)*
2007-present	Editorial board member, *Cancer Research* Official journal of AACR; *Advances in Bioinformatics*
2008-present	Editorial board member, *Journal of Nucleic Acids Investigation*
2007-present	Ad hoc member of various NIH Special Emphasis Panel Study Sections (BDMA, BST, GGG)
2012-2016	Member, Tumor Biology & Genomics Peer Review Committee, American Cancer Society
2008-present	Philadelphia Healthcare Trust Endowed Chair, The Wistar Institute, Philadelphia.