# Geisinger Clinic

# Annual Progress Report: 2012 Formula Grant

**Reporting Period**

July 1, 2013 – June 30, 2014

**Formula Grant Overview**

The Geisinger Clinic received $110,249 in formula funds for the grant award period January 1, 2013 through December 31, 2014. Accomplishments for the reporting period are described below.

**Research Project 1: Project Title and Purpose**

*Role of PP2A Antisense RNA in Human Glioblastoma* – Global transcriptome analysis provides evidence that a large proportion of the genome can produce transcripts from both strands. Antisense transcription (transcription from the opposite strand to a protein- coding or sense strand) has been ascribed roles in gene regulation involving degradation of the corresponding sense transcripts (RNA interference from small and long non-coding RNAs), as well as gene silencing at the chromatin level. The role of antisense transcription in human glioblastoma (GBM) has not been studied so far. The purpose of this proposal is to study the role and regulation of protein phosphatase-2A (PP2A) long antisense RNA in human GBM and how we can exploit the PP2A antisense RNA for therapeutic purposes.

**Anticipated Duration of Project**

1/1/2013 – 12/31/2014

**Project Overview**

Despite the advances in surgical, radiation and chemotherapy treatments of human malignant brain tumors the prognosis remains poor with fewer than 3% of glioma patients alive 5 years after diagnosis. Molecular targeted therapies hold the promise of providing new anticancer treatments that are more effective than traditional therapies. In this context, understanding the transcriptional regulation of tumor suppressor genes in cancer stem cells will provide insights into the mechanism that leads to cellular transformation and oncogenesis. Protein phosphatase 2A (PP2A) plays the role of tumor suppressor through the modification of phosphorylation of several signaling proteins that regulate oncogenic transformation. Recently, we have generated an antisense cDNA library from human glioblastoma (GBM) stem cell RNA and we identified a portion of a PP2A-specific antisense RNA transcript. With this grant application we propose to clone the full-length PP2A antisense transcript and study its role in regulating the oncogenic transformation of human GBM stem cells. This study will provide novel therapeutic targets to prevent oncogenesis through the manipulation of antisense transcription.

The specific aims of this proposal are:
*Specific Aim 1: To isolate and clone the PP2A long antisense RNA from human GBM cancer stem cells.*
*Specific Aim 2: To identify the role of the PP2A antisense RNA in the generation and propagation of human GBM.*

To perform these aims we will employ a 5'- and 3'- RACE protocol to identify and eventually sequence the full-length PP2A antisense transcript. We will then clone it into an expression vector to transfect human GBM stem cells in order to examine if the PP2A antisense regulates the expression of the PP2A mRNA and protein. Finally, we will clone the antisense into a loxP retrovirus and inject it under stereotactic guidance into the supraventricular zone (SVZ) of *nestin-cre* mice to examine if the PP2A antisense transcript drives oncogenic transformation of the endogenous pool of neural stem cells in mice.

**Principal Investigator**

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**Other Participating Researchers**

None

**Expected Research Outcomes and Benefits**

Genome-wide analysis of the human transcriptome has suggested that virtually the entire genome is transcribed in both sense and antisense directions. Indeed much of the "junk DNA" is actively transcribed and roughly 40–50% of protein coding genes appear to exhibit long antisense non-coding RNAs. To date, there are only a few examples where long antisense non-coding RNAs have been shown to epigenetically control transcription at non-imprinted loci. Interestingly, these bidirectionally transcribed genes have been predominantly found to be tumor suppressor genes. It is noteworthy that several tumor suppressor genes become epigenetically silenced during the progression to cancer. We expect that our research proposal will identify the role of antisense RNA in the epigenetic silencing of the PP2A tumor suppressor gene in human glioblastoma (GBM) stem cells. This will provide new information on how epigenetic mechanisms act upon the imprinted genetic variances, which have already been described in neural stem cells to initiate the transformation to cancer stem cells and eventually the progression to GBM tumors. Finally, our research will reveal new therapeutic targets through the targeted regulation of antisense transcription in patients with GBM.

**Summary of Research Completed**

In order to demonstrate that expression of the PP2A long antisense RNA affects the PP2A

protein levels, we infected human neural stem cells (hNSCs) with a lentivirus carrying the PP2A-AS RNA (pLVX backbone from Clontech). While experiments are ongoing, the current trend ($p=.21$) shows that PP2A-AS induces a reduction in the expression of PP2A protein by an average of 86% in hNSCs (Figure 1).

Next we sought to identify the distribution of PP2A-AS expression in human glioblastomas and determine whether the expression correlates with PP2A protein expression. To perform this experiment we used human glioma tissue arrays (U.S. Biomax) and performed *in situ* hybridization with a PP2A-AS specific probe, a PP2A mRNA-specific probe and an antibody against PP2A protein (Cell Signaling Technology). The hybridization and the protein staining was performed on consecutive serial sections of the human glioma tissue array. The results were analyzed by Student's t-test and we demonstrated that increased expression of the PP2A-AS in human gliomas correlates with decreased PP2A protein expression (*: $p<0.05$) (Figure 2).

Since PP2A functions as a tumor suppressor (Mumby, 2007) we examined whether inhibition of PP2A expression in normal neural stem cells deregulates the cell cycle and induces cellular transformation. To perform these experiments we infected hNSCs with the PP2A-AS lentivirus and 2 days later we isolated RNA to perform a cell cycle quantitative RT-PCR array (SABiosciences). We demonstrated that expression of the PP2A-AS RNA in hNSCs induces the expression of genes responsible for the metaphase-anaphase transition and components of the 9-1-1 checkpoint complex (responsible for DNA repair) (Table 1). Both these gene families are expressed during the initiation of transformation (Wäsch & Engelbert, 2005; Bao et al., 2004) and suggest that expression of the PP2A antisense RNA drives the cells towards a transformed phenotype.

*References*

Mumby, M. (2007). PP2A: unveiling a reluctant tumor suppressor. *Cell*, *130*(1), 21-24.

Wäsch, R., & Engelbert, D. (2005). Anaphase-promoting complex-dependent proteolysis of cell cycle regulators and genomic instability of cancer cells.*Oncogene*, *24*(1), 1-10.

Bao, S., Lu, T., Wang, X., Zheng, H., Wang, L. E., Wei, Q., ... & Li, L. (2004). Disruption of the Rad9/Rad1/Hus1 (9–1–1) complex leads to checkpoint signaling and replication defects. *Oncogene*, *23*(33), 5586-5593.

*Table 1:*

| Gene | Function | Fold Change |
|---|---|---|
| ANAPC2 | Promotes metaphase-anaphase transition via ubiquitination | 30.34 |
| HUS1 | Response to DNA damage, DNA repair | 4.98 |
| RAD9A | Response to DNA damage, DNA repair | 3.03 |

*Figure 1*

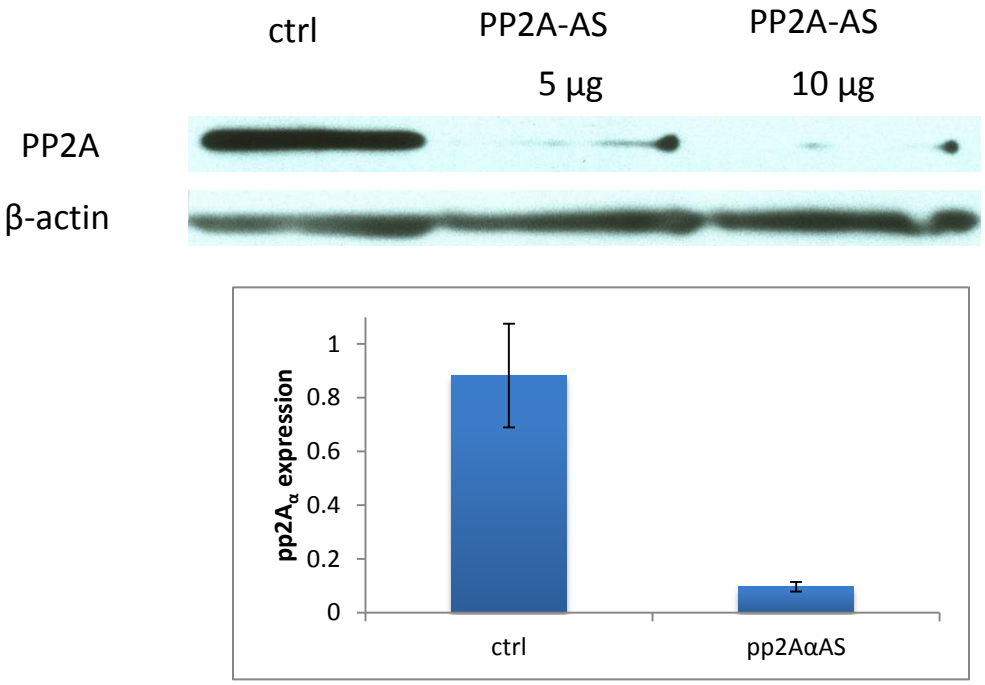
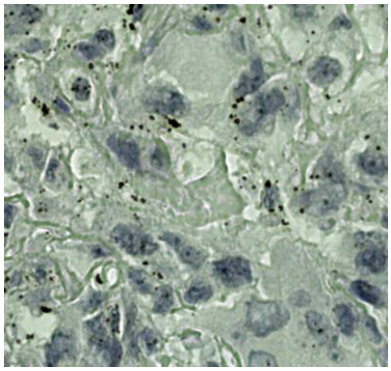
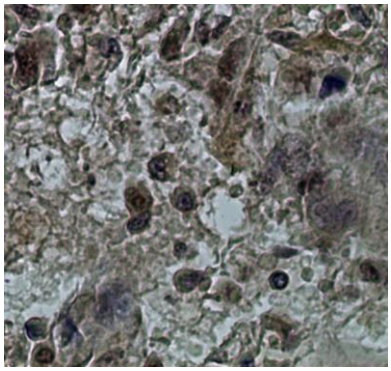


*Figure 2*

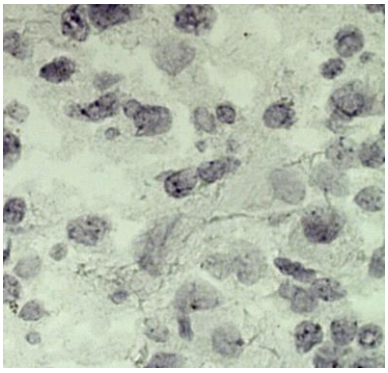
High PP2A-AS

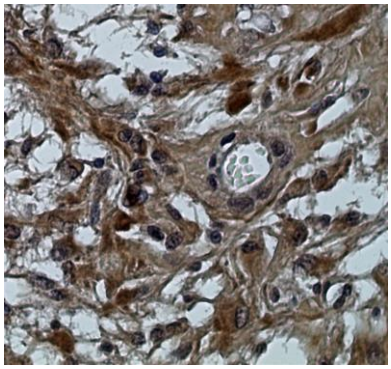
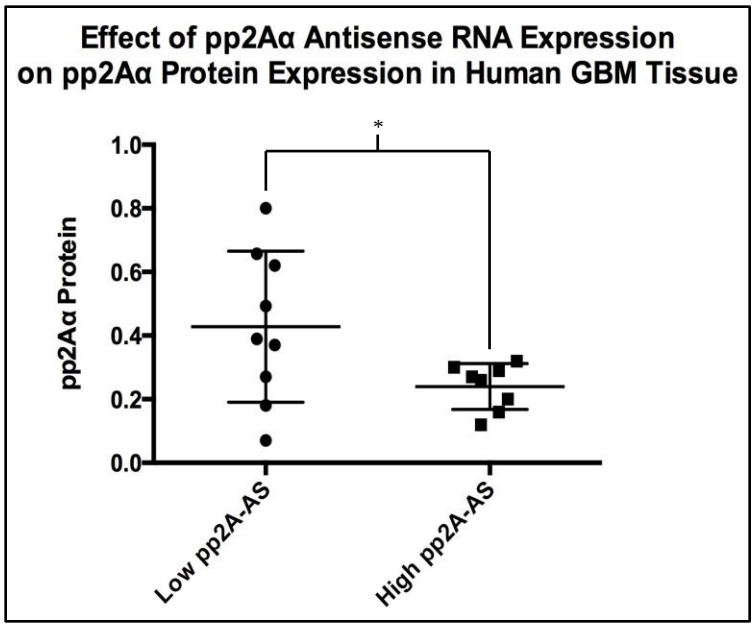




Low PP2A protein

Low PP2A-AS





High PP2A protein