

# PEDF: An Angiogenesis Inhibitor and Its Role in Glioblastoma Multiforme

Wisconsin Virtual Learning SMART Team: Colette Fox, Emily Billin, Hardy Liesener, Holly Van Gorden, Kiera Yohe, Michael Mitchell

Teacher: Mrs. Becki Van Keuren

Mentor: Shama P. Mirza, Ph.D., Department of Biochemistry, Medical College of Wisconsin

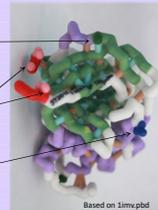
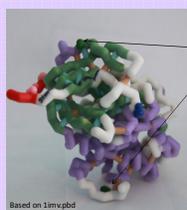
## Abstract

Glioblastoma multiforme (GBM) is a cancerous brain tumor with almost 100% recurrence rate even after surgery, radiation and chemotherapy. Pigment epithelium-derived factor (PEDF) has been found in areas where these tumors do not grow as aggressively. PEDF slows the growth of tumors by inhibiting angiogenesis, a physiological process involving growth of new capillaries from pre-existing blood vessels in the body. Restricting blood flow to the tumor starves it of oxygen and nutrients. The mechanism of PEDF-mediated inhibition of angiogenesis is unknown. Research has shown that PEDF undergoes posttranslational modifications (PTM), chemical changes to a protein after translation, such as the addition of carbohydrates (glycosylation) or phosphate groups (phosphorylation), which may occur during various cellular events in tumors. PEDF is phosphorylated at Ser227, Ser114 and Ser24 and glycosylated at Asn285. Glycosylation may also occur on amino acids within a specific region of the protein (amino acids 371-383). The Wisconsin Virtual Learning SMART (Students Modeling A Research Topic) Team modeled PEDF using 3D printing technology. Identifying the PTMs of PEDF in GBM tumors and plasma samples may further the understanding of angiogenesis inhibition and in turn, may lead to the development of treatments for these lethal cancers.

## PEDF: A Potent Anti-Angiogenic Agent<sup>3</sup>

- PEDF, a serpin protein, is transcribed from the gene located on chromosome 17 and translated into a chain of 418 amino acids, folded into beta sheets and alpha helices.
- Exhibits multiple and varied biological activities such as neuroprotective, neurotrophic and tumor cell apoptosis.
- Has been researched as a potential anti-tumor agent by inhibiting angiogenesis through its ability to bind to collagen and its phosphorylation states in Ser24, Ser114, and Ser227.
- GBM tumor patients who have larger amounts of PEDF proteins expressed have tumors that grow less aggressively.
- The exact mechanism of inhibiting angiogenesis is not yet known.
- PEDF undergoes post-translational modifications (PTMs) during various cellular events involved in tumor growth and the spread of cancer and are thought to regulate the activity of PEDF, producing anti-angiogenic results.
- PTMs include glycosylation, when carbohydrates are added to the protein, or phosphorylation, when phosphate groups are added to the protein

PTM Types	PTM Sites
Phosphorylation	Ser227, Ser24, Ser114
O-Linked Glycosylation	Any Serine or Threonine residues in the sequence of 371-383
N-Linked Glycosylation	Asn285

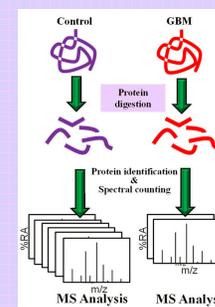


## Biological Significance of PTMs on PEDF:

- PEDF phosphorylation at Ser24 and Ser114 increases anti-angiogenic activity, while phosphorylation at Ser227 reduces anti-angiogenic activity<sup>4</sup>. Thus, it is assumed that changes in phosphorylation on different residues of PEDF controls the tumor progression in GBM.
- In general, glycosylation changes are common in tumor progression and malignant transformations. However, the role of PEDF glycosylation in GBM progression has yet to be studied.

## Mass Spectral Analysis for Differential Protein Expression in GBM

### Spectral Counting Quantification

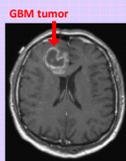


- Tissue lysates from GBM biopsies and epilepsy samples are analyzed by mass spectrometry. Proteins identified are quantified based on the number of spectra each protein is identified from.
- Identified proteins in each sample are quantified based on the spectra from which they are identified. As shown in the figure to the left, the protein identified in GBM has two spectra and the same protein in the control has six spectra. This shows that the protein identified is three times more expressed in the control compared to GBM.
- Using this approach of spectral counting, 883±71 proteins are differentially expressed in GBM vs. control epilepsy tissues.
- PEDF protein is identified to be down-regulated in GBM samples.
- Further validation of PEDF in GBM samples is performed by Western Blotting.

## Angiogenesis: The Root of Glioblastoma Multiforme

### Glioblastoma Multiforme (GBM)<sup>1</sup>

- 22% of brain cancers are GBM.
- These tumors are highly malignant (cancerous) and fast growing.
- The survival rate is less than two years after diagnosis.
- The incidence rate is 3-5 cases per 10,000 people in Europe and North America.
- There is almost 100% recurrence rate even after chemotherapy, radiation, and surgery

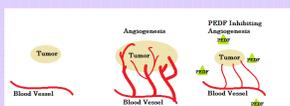


### Angiogenesis in GBM<sup>2</sup>

- A process in which new capillaries form from the pre-existing blood vessels in the body, bringing nutrients and oxygen to tumors (See figure below).
- Sustainable blood supply to tumor leads to further growth and metastatic potential.
- If inhibited, GBM tumor growth is slowed considerably.

### The Inhibitor of Angiogenesis

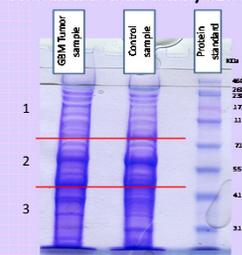
- Pigment epithelium-derived factor (PEDF) is a serpin protein, which end to play inhibitory roles of other proteins' functions and chemical reactions.
- PEDF is highly expressed in the eye's pigmented epithelium and in the brain; also distributed in areas such as the liver, testis, ovaries, placenta and pancreas.
- In the figure below PEDF proteins inhibit new capillaries from forming, preventing nutrients and oxygen to the tumor.
- Tumor growth is suppressed.



## References

1. American Cancer Society: Cancer Facts and Figures 2010. Atlanta, GA: 2010.
2. Angiogenesis in glioblastoma multiforme: AG Linkous, EM Yaxlovitskaya. Anticancer Agents Med Chem. 2011, 8; 712-8.
3. Pigment epithelium-derived factor inhibits glioma cell growth in vitro and in vivo. Zhang T, Guan M, Xu C, Chen Y, Lu Y. Life Sci. 2007;81(16):1256-63.
4. Extracellular phosphorylation converts pigment epithelium-derived factor from a neurotrophic to an antiangiogenic factor. Maik-Rachline G, Shaliti S, Seger R. Blood. 2005;105(2):670-8.

## Protein fractionation by SDS-PAGE followed by mass spectral (MS) analysis



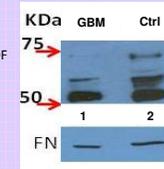
SDS-PAGE gel of whole tissue lysate. Proteins are stained using coomassie brilliant blue (CBB) stain and are separated by molecular weight. Column 1 is GBM tissue protein, 2 is control epilepsy sample and 3 is protein standard. The two red lines signify where the gels are cut into three fractions.

- Gel is cut into three fractions as shown in the figure. The red line signifies the place where the gels are cut to make three fractions.
- Proteins are digested in-gel using trypsin to make smaller peptides that are analyzed by MS.
- Peptides are desalted and analyzed by MS, which is used for protein identification and quantification.
- Protein quantification is carried out by spectral counting method.
- Proteins are quantified by comparing the GBM vs. control epilepsy tissues.
- SDS-PAGE separation followed by MS analysis gives comprehensive quantification of proteins in GBM vs. control epilepsy samples.

## Western Blot Analysis of PEDF

- Fibronectin(FN) is expressed equally in both samples and shows that similar amounts of each sample were added.

- PEDF is expressed in both GBM (lane 1) and control epilepsy (lane 2) samples. (Epilepsy is the non-tumorous tissue available from the human brain that can act as a control for GBMs.)



- The arrow at 50 kDa in the image is pointing to the location where PEDF is identified.

- Multiple bands represent that PTMs have occurred.
- The arrow at 75 kDa represents possible PTMs on PEDF.
- PEDF expression is less in GBM vs. control.

- PTMs on PEDF are possibly different in GBM vs. control.
- Differences in expression levels and PTMs are correlated to GBM tumor progression; studies on these mechanisms are underway.

## Summary

- GBM is a highly aggressive brain tumor with highly invasive and fast growing properties.
- Through mass spectrometry, it has been identified that proteins are differentially expressed in GBM tumors compared to control epilepsy tissues.
- PEDF protein is found to be down-regulated in samples from GBM compared to controls.
- Western blotting of PEDF identified potential PTMs on the protein.
- PEDF undergoes the PTMs of phosphorylation and glycosylation during various cellular functions.
- Understanding PEDF and its PTMs might lead to new therapeutics for GBM.
- The mechanism of these PTMs and their role in GBM progression will be studied further by researchers.