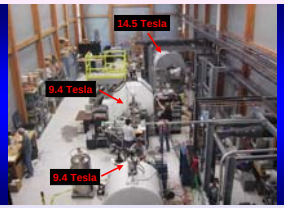


Proteomic Analysis of Brain Tumor Cells by use of FT-ICR MS

Charles Carpenter
Lawton Chiles High School Tallahassee, FL

Overview:

Of the estimated 17,000 primary brain tumors diagnosed in the United States each year, approximately 60% are gliomas. Glioblastoma multiforme (GBM) is by far the most common and most malignant of the glial tumors. The treatment of glioblastomas is palliative and includes surgery, radiotherapy, and chemotherapy.

No significant advancements in the treatment of glioblastoma have occurred in the past 25 years. Without therapy, patients with GBM's uniformly die within 3 months. Patients treated with optimal therapy, including surgical resection, radiation therapy, and chemotherapy, have a median survival of approximately 12 months, with fewer than 25% of patients surviving up to 2 years and fewer than 10% of patients surviving up to 5 years.

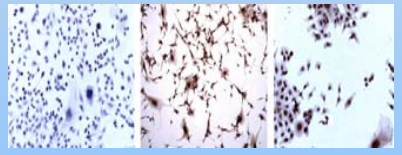
There are many factors that complicate effective treatment, such as their resistance to undergo apoptosis as well as their invasive nature. In fact, a correlation seems to exist between cells that are actively invasive and mobile and their inability to trigger apoptosis after being exposed to either ionizing radiation or chemotherapy.

It has recently been discovered that transfecting glioma cells with a wild type p53 protein and a cytotoxic agent produces almost complete cell cycle arrest followed by almost complete apoptosis of over 90 percent of cells. (See figure below) However, the exact mechanism of this activation is still unknown.

In order to study differentially expressed proteins in glioma cell lines, a proteomic approach was employed. Understanding of the molecular biology of gliomas is thus a clear and urgent necessity as the determination of the effectors of apoptotic resistance mechanisms in gliomas could lead to future therapies.

p53 is administered through an adenoviral vector construct

In vitro staining of glioblastoma cells after adenovirus therapy



Untreated cells - anti-galectin antibody | **Untreated cells + anti-galectin antibody** | **Adenovirus treated cells + anti-galectin antibody**

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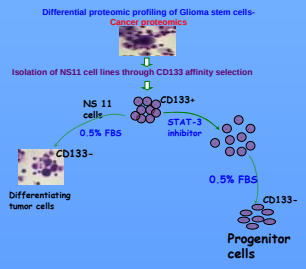
After the above experiment was performed and analyzed using proteomic methods, one protein, galectin-1, was found to be dramatically modulated. The treatment of wt-p53 transfection resulted in a notable down-regulation of galectin-1.

A molecular model of Galectin-1

http://www.cermav.cnrs.fr/lectines/images/pdb1slt.gif

Galectins are a family of structurally related carbohydrate-binding proteins. Galectin-1, a member of this family, contributes to different events associated with cancer biology, including tumor transformation, cell cycle regulation, apoptosis, cell adhesion, migration and inflammation. In addition, recent evidence indicates that galectin-1 contributes to tumor evasion of immune responses. There is increased interest of tumor biologists and clinical oncologists in this field and the potential use of galectins as novel targets for anticancer drugs.

In addition, researchers have identified stem cells in brain cancer tumors that replenish the tumors and keep them growing. Snuffing out these cancer stem cells could lead to a raft of new treatments for various cancers. It has been found that these stem cells initiated and sustained new brain tumor growth in mice. The identification of cancer stem cells is a significant step in the fight against these dreaded diseases. Because self-renewal is essential if tumors are to grow, agents that target such cells may be effective. If one cell can incite tumor growth, researchers know that's the cell to target if they want to stop the tumor's growth. Often, cancer tumors respond initially to chemotherapy and radiation treatments, only to grow back again later. Researchers now think that stem cells are responsible for tumor regeneration. If a drug could kill the tumor stem cells specifically, perhaps the tumor would not have the means to grow back. Cancer stem cells make up a tiny percentage (2 to 5 percent in breast cancer) of all the cells in a tumor, so it's possible scientists have been studying the wrong cells about 95 percent of the time. The next step is to find out what makes these cancer stem cells tick.



The research team here at the NHMFL facility has been given the opportunity to examine the proteomics of these stem cells. A sample of tumor stem cells was received and treated as shown in the diagram to the left. The differentiated cells and some of the non-differentiated cells have been prepared and analyzed using the methods described in the column to the right. When the data is analyzed, the different proteins, including Galectin-1, present in the samples can tell us a lot about what makes these cells grow and develop.

Current strategy

Step 1: Separation of proteins from soluble proteins- Ultracentrifugation

In this process, the proteins are dissolved and separated from the unwanted precipitate which contains the membrane lipids.

Step 2: Separation based on pI- Isoelectric focusing.

The proteins are re-hydrated in a buffer solution.

Step 3: Separation based on molecular weight.

In this process, the pI strips are placed on a SDS gel in a electrophoresis unit for 8 hours with an applied voltage of around 200 V. This allows the separated proteins to separate further by molecular weight.

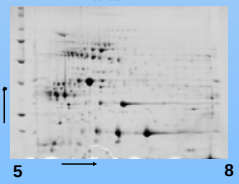
Electrophoresis unit.

Step 4: Staining of the Gel.

Depending on the target proteins, several different stains can be used. The stains are applied to the gels and allowed to stir for a couple of hours. The result is a gel as in the image below.

Stirring unit

2-D Gel Stain



Step 5: Image analysis (PDQuest)

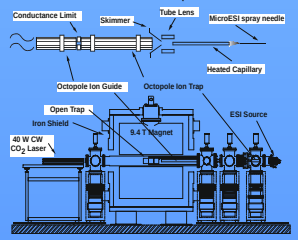
In this step, the gels are scanned and the images saved. Computer software then analyzes the Gels for the presence of differentially expressed proteins.

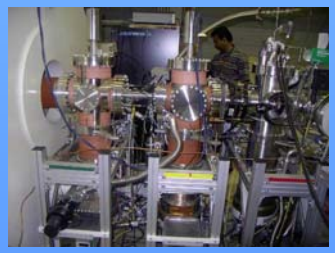
PDQuest Scanner

Step 6: FT-ICR MS and MS/MS (Fourier transform ion cyclotron resonance mass spectrometer)

The Gels are cut into slices and are digested further with trypson. Then, each part is run through the FT-ICR Mass Spectrometer. This is the part where the magnets are used. The high magnetic fields allow for more accurate MS than can be accomplished with a standard mass spectrometer.

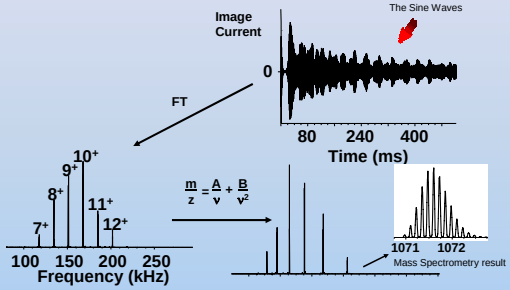
9.4 Tesla ESI FT-ICR Mass Spectrometer





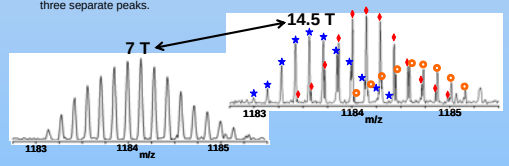
How it Works

Fourier transform ion cyclotron resonance mass spectrometer is a type of mass spectrometer for determining the mass-to-charge ratio (m/z) of ions based on the cyclotron frequency of the ions in a fixed magnetic field. The ions are trapped in a Penning trap (a magnetic field with electric trapping plates) where they are excited to a larger cyclotron radius by an oscillating electric field. The resulting signal consists of a superposition of sine waves. The useful signal is extracted from this data by performing a Fourier transform to give a mass spectrum.



The importance of the magnetic field

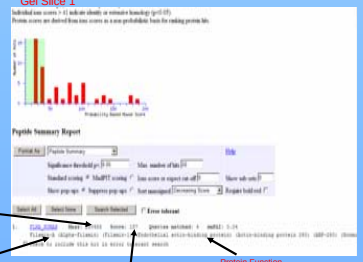
The image below is that of the mass spectrometer results for the same sample using two different FT-ICR superconducting magnets. Using the 7 Tesla field magnet, only one peak is found. However, using the 14.5 Tesla magnet, it can be seen that it is actually composed of three separate peaks.



How the Data is analyzed

The resulting mass spectrometry readings are then compared to a massive database of proteins using a software program call Mascot. The Mascot then creates a report that looks like the screen shot here to the right. Each matching protein is given a score. The higher the score, the more reliable the match is. This is done for each slice of the gel.

Gel Slice 1



Sample Data

Protein Category	Control Protein	Entry Name	MW	MS	#Peptides
IMMUNITY AND DEFENSE	HLA-DRB1 1505	P79494_HUMAN	10,515	55	2
TRANSPORT	ADP/ATP translocase	CAF00941	32,803	77	3
SIGNALING	14-3-3 eta	1433F_HUMAN	28,070	50	2

The above list is a small version of how the protein list is composed. The actual result will contain several hundred proteins on the list. Without FT-ICR, the highly reliable molecular weights would not be possible.

Acknowledgements

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Xu Wang, Graduate student

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http://www.cancer.gov/cancertopics/pdq/treatment/adultbrain/HealthProfessional/page9

http://www.genomenewsnetwork.org/articles/03_03/stem_c.shtml