
Project Summary

Title The Role of TGM2 and Calretinin in the Acquired Radiation Resistance of Pancreatic Cancer Cells.

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Classification: Sophomore

Area of Study: Biology

Grade Point Average: [REDACTED]

Among the most destructive cancers, boasting a devastatingly low five-year survival rate of six percent, is pancreatic cancer (American Cancer Society, 2013). The resistance of this cancer to common cancer therapies contributes greatly to this severely low survival rate (Kumar et al., 2010, Zhou and Du, 2012). To elucidate the molecular mechanisms underlying radio-resistance in pancreatic cancer, Dr. [REDACTED] laboratory has recently succeeded in creating a pancreatic cancer isogenic cell line that differs in radiosensitivity from the parental cells. Through a quantitative proteomic method, Dr. [REDACTED] lab found that the expression of TGM2 and Calretinin was dramatically up-regulated in the radiation resistant pancreatic cancer cells. Preliminary characterization has found that TGM2, a calcium-dependent enzyme, and calretinin, a calcium-binding protein, both individually influence the radiation resistance of pancreatic cancer cells, but these effects were moderate. The calcium-ion dependent nature of the functions of both TGM2 and calretinin suggests that these proteins may coordinate to cause radiation resistance in pancreatic cancer cells. Hence, this research projects aims to study the combined effects of TGM2 and calretinin on the radiosensitivity of pancreatic cancer cells. In the proposed work, I plan to generate a stable cell line that overexpress TGM2 and calretinin, and a cell line in which the expression of TGM2 and calretinin is silenced. The radiosensitivity of each cell line will be assessed through the measurement of PARP cleavage (a measure of cell apoptosis) and the conduction of a clonogenic assay. Understanding the mechanism underlying radio-resistance could lead to the design of novel therapeutic methods to treat pancreatic cancer.

The Role of TGM2 and Calretinin in the Acquired Radiation Resistance of Pancreatic Cancer Cells

Specific Aims

Cancer is a genetic disease that affects the life of many people and families. Among the deadliest cancers, claiming the lowest overall five-year survival rate of six percent, is pancreatic cancer (American Cancer Society, 2013). The resistance – both inherent and acquired – of this type of cancer to the prevailing treatments of chemotherapy and radiation therapy coupled with its metastasizing nature contributes greatly to this critically low survival rate (Kumar et al., 2010, Zhou and Du, 2012). To elucidate the molecular mechanisms underlying radio-resistance in pancreatic cancer, Dr. [REDACTED]'s laboratory has recently succeeded in creating a pancreatic cancer isogenic cell line that differs in radiosensitivity from the parental cells. The radioresistant cell line was generated by exposing pancreatic cancer cells to a total dose of 60 Gy of γ -radiation in 30 fractions over a period of 6 weeks, a protocol that is commonly used in clinical radiotherapy.

Through a quantitative proteomic method, Dr. [REDACTED] lab found that the expression of TGM2 and calretinin was dramatically up-regulated in the radiation resistant pancreatic cancer cells. TGM2 is a calcium-dependent cross-linking enzyme with variegated functions that prior studies have found to inversely influence two fundamental components of cancer: cell apoptosis and growth (Kumar et al., 2010). It has also been implicated in the increased metastasis of tumors and the resistance of cancer cells to chemotherapy and radiation therapy (Kumar et al., 2010, Agnihotri et al., 2012). Calretinin, on the other hand, is a multifunctional calcium-binding protein that is expressed in many human cancers and has been found to be involved in cell apoptosis (Lugli et al., 2003). Preliminary studies on the function of TGM2 and calretinin in Dr.

laboratory have shown that individual overexpression of TGM2 or calretinin in radio-sensitive parental cells has caused moderate radiation resistance in cells, but this resistance was not found to be on par with the level of resistance characteristic of selected resistant cells (those exposed to ionizing radiation). Furthermore, the individual silencing of each of these two proteins resensitized selected radiation resistant cells to radiation. However, this, too, did not reach the level of sensitivity characteristic of the parental cells.

These findings and the calcium-ion dependent nature of the functions of both TGM2 and calretinin have led to the *hypothesis* that these two proteins may coordinate to cause radiation resistance in pancreatic cancer cells. Consequently, this research project will assess the combined effects of TGM2 and calretinin on the radiosensitivity of pancreatic cancer cells. I plan to test this hypothesis through pursuing the following Specific Aims:

Specific Aim 1. Generating a stable pancreatic cancer cell line that simultaneously overexpresses TGM2 and calretinin, and a cell line that expresses a reduced level (knockdown) of both TGM2 and calretinin

Specific Aim 2. Examining the effect of the overexpression and knockdown of TGM2 and calretinin on the radiosensitivity of pancreatic cancer cells.

If successful, the results of this study: a more complete knowledge and understanding of the role of TGM2 and calretinin in the radioresistance/radiosensitivity of pancreatic cancer cells, could lead to the development of targeted novel therapeutic techniques that could improve the survival rate of pancreatic cancer patients.

Experimental Methods

Specific Aim 1. Generating a stable pancreatic cancer cell line that simultaneously overexpresses TGM2 and calretinin, and a cell line that expresses a reduced level (knockdown) of both TGM2 and calretinin.

1. The following cell lines are available in Dr. [REDACTED] laboratory:

Cell line 1: pancreatic cancer parental cells (MIA PaCa-2): expresses wild-type levels of calretinin and TGM2.

Cell line 2: overexpresses calretinin.

Cell line 3: overexpresses TGM2.

Cell line 4: expresses silenced (substantially reduced) level of TGM2

Cell line 5: expresses silenced (substantially reduced) level of calretinin.

Cell lines 2-5 were selected with the antibiotic neomycin phosphotransferase and, thus, confer G418 resistance.

Generation of Additional Stable Cell Lines: I plan to use the experimental protocols that have been established in Dr. [REDACTED] lab to generate the following two stable cell lines:

Cell line 6: Overexpresses both TGM2 and calretinin. I will use cell line 3 (overexpresses TGM2) as the starting material to generate the cells that overexpress both TGM2 and calretinin by transfecting the line 3 cells with the vector that expresses calretinin.

Cell line 7: The cell line cells in which the expression of TGM2 and calretinin is silenced. I will use cell line 4 (in which the expression of TGM2 is silenced) as the starting material to generate the cells in which the expression of both TGM2 and calretinin is silenced by transfecting the line 4 cells with the vector that expresses the RNA sequence that specifically targets calretinin.

Because cell lines 3 and 4 are G418-resistant, the vectors used in generating cell lines 6 and 7 will express the antibiotic puromycin. Thus, the stable cells will be selected using puromycin. Once the resistant clones are acquired, I will use Western blot analysis (according to the protocol already established in Dr. [REDACTED] lab) to screen the clones that express the desired levels of the proteins of interest. Western blotting is an experimental technique that involves fractionation of proteins with SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis), transfer of proteins to a membrane, incubation of the membrane with a primary and, then, secondary antibody, and, finally, detection of the protein of interest with a luminescence-based technique.

Specific Aim 2. Examining the effect of the overexpression and knockdown of TGM2 and calretinin on the radiosensitivity of pancreatic cancer cells.

1. Examining apoptotic responses of the established cell lines to gamma-radiation: After establishing cell lines 6 and 7, I will compare the radiosensitivity of cell lines 6 and 7 with cell lines 1 through 5. I will first expose the seven cell lines (cell lines 1-7) to gamma-ray radiation and then measure cell apoptosis (also called programmed cell death) of each line by examining the PARP cleavage using Western blotting (experiment of exposure of cells to gamma-radiation will be supervised by a Research Technician in Dr. [REDACTED] lab according to an established laboratory protocol).

Poly(ADP-ribose)polymerase (PARP) is an enzyme that functions primarily in the detection of DNA damage and its subsequent repair (Kraus and Lis, 2003). In normal cells, PARP is most prevalent in its 116 kDa form (Boulares et al., 1999). However, once cell apoptosis is triggered, activated caspases cleave this 116 kDa form to yield the 85 kDa form of PARP, which renders the enzyme inactive and is characteristic of apoptotic cells (Los et al.,

2002). Cells that show a larger amount of PARP cleavage indicate a higher level of apoptotic activity after treatment with radiation and, therefore, are said to be radiosensitive. Conversely, cells that show smaller amounts of PARP cleavage indicate a lower level of apoptotic activity after exposure to gamma-radiation and, thus, are said to be radioresistant.

2. Clonogenic Assay: I plan to further examine the radiosensitivity of the established cells using the clonogenic assay, which measures the respective reproductive capabilities of irradiated and untreated cells as assessed by their abilities to yield colonies of cells. In this procedure, after exposure to different doses of gamma radiation, the cells are plated on Petri-dishes. Following incubation at 37° C for 3 weeks, the cells will be fixed and stained, and the number of colonies in each plate will be counted. Radiosensitive cells will exhibit fewer colonies than resistant cells (Franken et al., 2006). Clonogenic assay will be performed using a protocol that has been established in Dr. [REDACTED] lab.

Research Plan: This study will tentatively follow the schedule listed below.

January, 2014 – March, 2014: Establish cell lines 6 and 7.

April, 2014 – August, 2014: Measure the apoptotic responses of the seven cell lines to gamma radiation by examining PARP cleavage using Western blotting.

September, 2014 – November, 2014 – Conduct clonogenic assays to assess radiosensitivity of the seven cell lines.

December, 2014 – Analyze data and read related literature

40 weeks, 10 hours a week = 400 hours

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