Mentored Professional Enrichment Experience: REVISED

Applicant:

Name of Project/Experience:

Apoptotic potential of peritonealcarcinomatosis after hyperthermic intraperitoneal chemotherapy (HIPEC).

Impact of hyperthermic intraperitoneal chemotherapy (HIPEC) on PPARγ and apoptosis of colon cancer cells, potential peritoneal metastasis

Location where Project/Experience will take place:

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RATIONALE

Peritoneal cancer is a terminal condition of human gastrointestinal cancer, which is difficult to be treated and worsens the quality of patients' lives. Often peritoneal cancer arises from a metastasis of stomach, colon, ovary, pancreas, breast, sarcoma, and biliary cancer. Peritoneal cancer can also arise from a tumor blocking the duct of the appendix, called a pseudomyxoma and causing a rupture of the appendix and spread of the tumor cells. Another rare type of peritoneal cancer is mesothelioma, which is when the peritoneum is the origin of the cancer. HIPEC is a treatment method developed by Dr Sugarbaker in 1995 for cancer of the peritoneum. In this surgery, the abdominal cavity is assessed (if too much cancer is present or cancer is impossible to resect, the procedure is abandoned) then all visible tumors are removed (if this cannot be done, the procedure is abandoned), and then the liver undergoes an ultrasound (if any liver metastasis are found, the surgery is abandoned). Finally, Mytomycine C is poured into the abdomen at 41.5 C for 90 minutes. Pouring the chemotherapy directly into the abdomen affects few places outside of the abdomen unlike the traditional treatment of IV chemotherapy, which affects the entire body. Because of the direct effect of the chemotherapy, a much higher dose is able to be given (up to 100 times as much as can be given through IV). The temperature of the chemotherapy is raised to amplify effects as well as to penetrate deeper into the bowel than possible with room temperature chemotherapy. This

procedure has been shown to add a mean survival benefit of one year to the patient's life and some patients' gain long term survival, which has never been achieved with traditional treatment (Verwaal, et al., 2003).

This study will focus solely on the metastasis from colon cancer (15% of colon cancers metastasise to the peritoneum and 3% do not metastasise further, which are the group that can potentially be treated by HIPEC.) The tumor cells associated with peritoneal cancer from colon metastasis are Colo320 and HT29. These cells proliferate in the peritoneum due to many factors which may include a decrease in the levels of the tumor suppressor peroxisome proliferator-activated receptor gamma (PPARγ). A decrease of PPARγ is associated with peritoneal metastasis. Activation of PPARγ leads to apoptosis in the tumor cells Colo320 and HT29. Possibly, the reason that HIPEC has been shown to have positive outcomes is due to the effect on PPARγ. A study in 2008 showed that an effective treatment for lung cancer was due to the therapeutic effects of PPARγ and we believe this may also be the case in the peritoneum (Reddy, et al., 2008). We want to test whether HIPEC has any impact of PPARγ and on apoptosis in these cells.

GOALS

In this project I will cultivate HT29 or Colo320 human colon cancer cells and treat them by HIPEC. I will estimate the concentration of PPARγ and of other regulators of tumor growth such as p53, p27 etc. in these cells. In addition, I will determine apoptosis after HIPEC treatment. If HIPEC increases PPARγ and apoptosis in HT29 and Colo320, it will most likely block peritoneal metastasis by the same mechanism.

My goal is to better understand mechanisms of cancer metastasis and new methods of treatment. I will also take the opportunity to learn about surgical intervention for cancer, especially peritoneal cancer. I hope to be able to apply my past experience in genetics research and learn new protein isolation techniques in the wet lab.

METHODS

This is a molecular biology and genetics study. I will use cells from well defined colon cancer cell lines (namely Colo320 and HT29) and will analyse them for apoptosis and PPARγ concentrations. Because no patient cells will be used, human subjects approval is not necessary. Metastasis will not be measured directly. For estimating cellular steady state concentrations of PPARγ, cells will be lysed after HIPEC treatment and lysates analysed by Western blots. A specific antibody against PPARγ will be used to estimate steady state level of the protein in cell lysates. The amount of PPARγ will be assessed before and after treatment with HIPEC. The grade of apoptosis will be measured by the caspase-3/7 activity assay and by annexin-V-FITC staining. The amount of apoptosis before and after HIPEC treatment will also be assessed.

Statistical analysis will be done on the results of the study to determine if the effect of HIPEC and apoptosis is correlated with PPARγ activation.

ANALYSIS

The amount of PPARγ before and after treatment with HIPEC in the Colo320 and HT29 lines will be compared. If a significant difference is found between levels of

PPARγ before and after as well as a significant difference in the levels of apoptosis, the burden of this study will be met. The data will show PPARγ levels as well as apoptosis levels and a simple statistical analysis will attempt to correlate HIPEC with increased apoptosis and increased activation of PPARγ. The data will also be compared with studies published in PubMed. My goal will be met if I can analyse cells for apoptosis and PPARγ and compare the two. I hope to help begin a study that may expand past my eight weeks during the summer.

RERERENCES

Confuoro, G., Giuliano, M.E., Grimaldi, A., & Viviano, C. (2007). *Peritoneal carcinomatosis from colorectal cancer: HIPEC?* Surgical Oncology (16 Supplemental): 148-152.

Fuellbeck, M., Huang, X., Dumdey, R., Frommel, C., Dubiel, W., & Preissner, R (2005). *Novel curcumin- and emodin-related compounds identified by in silico 2D/3D conformer screening induce apoptosis in tumor cells.* BMC Cancer (5, *97*).

Pelz, J., Doeffer, J., Dimmler, A., Hohenberger, W., & Meyer, T. (2006) *Histological response of peritoneal carcinomatosis after hyperthermic intraperitoneal chemoperfusion (HIPEC) in experimental investigations*. BMC Cancer (6): 162.

Reddy, R., Srirangam, A., Reddy, K., Chen, J., Gangireddy, S., Kalemkerian, G., Standiford, T., & Keshamouni, V. (2008). *Chemotherapeutic drugs induce PPARγ expression and show sequence-specific synergy with PPARγ ligands in inhibition of non-small cell lung cancer.* Neoplasia (10, *6)*: 597-603.

Turner BioSystems. A Veritas Microplate Luminometer Method for Promega's Caspase-Glo 3/7 Assay.

Verwaal, V., van Ruth, S., de Bree, E., van Slooten, G., van Tinteren, H., Boot, H., & Zoetmulder, F. (2003). *Randomized trial of cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy and palliative surgery in patients with peritoneal carcinomatosis of colorectal cancer.* Journal of Clinical Oncology (21, *20*): 3737-3743.

SUPPORT

1. Do you request support funds? Yes

2. Would you be able to participate if a scholarship is not available? No