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IRB: RAGE and Pancreatic Adenocarcinoma

Scientific Abstract
The receptor for advanced glycation end-products (RAGE) is a newly recognized factor regulating cancer cell invasion and metastasis. In part via mitogen-activated protein (MAP) kinase activation and matrix metalloproteinase (MMP) induction, RAGE influences cell growth and invasion. While other reports examine the differential expression of RAGE in pancreatic cancer cell lines, no report specifically explores RAGE expression and stage of disease. In the United States, pancreatic cancer is the fourth leading cause of cancer deaths and is an almost universally fatal disease. Surgical resection provides the only curative treatment, yet only a small percentage of patients are good candidates for surgery. Furthermore, pancreatic cancer is markedly resistant to both radiation and chemotherapy. These facts highlight the need to explore pancreatic tumor biology, identify patterns and predictors of progression and relapse, and hone adjuvant therapy in order to improve long-term survival. This study plans to examine retrospectively approximately 150 specimens of pancreatic adenocarcinoma previously resected at Columbia University Medical Center. We will grade these tumors and then use immunohistochemistry to assess RAGE and RAGE ligand expression. We hypothesize that increased RAGE and RAGE ligand expression will correlate with increased stage of disease, identifying a novel indicator of malignant potential and a possible therapeutic target in cases of pancreatic adenocarcinoma.

Lay Abstract
The receptor for advanced glycation end-products (RAGE) is a cell membrane protein that among other functions helps regulate cell growth. Several reports on the relationship between RAGE expression and cancer have been published to date, including renal, gastric, and colon cancer. The pancreas serves both an exocrine function that aids in digestion and an endocrine function that regulates blood sugar. Like other areas of the gastrointestinal tract, it is susceptible to the development of adenocarcinoma. Given the poor response of pancreatic cancer to standard forms of therapy, recent efforts have focused on the application of novel, biologically targeted agents aimed at cancer mechanisms. No report specifically evaluates RAGE expression and the degree of disease in adenocarcinoma of the pancreas. This study plans to examine retrospectively approximately 150 specimens of pancreatic adenocarcinoma previously excised at Columbia University Medical Center. We will evaluate the microscopic architecture of these tumors to grade the degree of malignancy and then use staining techniques to determine the degree of RAGE expression. We hypothesize that increased RAGE expression will correlate with increased stage of disease, identifying a new indicator of malignant potential and a possible therapeutic target in cases of pancreatic adenocarcinoma.

Study Design:
1.) Study Purpose and Rationale:

The purpose of this study is to establish a relationship between RAGE expression and pancreatic cancer and lay the groundwork for future investigations into diagnostic and therapeutic options for patients with this disease.

In the United States, pancreatic cancer is the fourth leading cause of cancer deaths, accounting for more than 35,000 deaths annually (1). It is an almost universally fatal disease, with a 5-year survival rate of less than 5%. Over 95% of patients diagnosed with pancreatic adenocarcinoma will die of the disease, with more than half within 6 months (1). Surgical resection is the only curative treatment. However, at the time of diagnosis, only 10% to 20% of tumors are resectable. Furthermore, pancreatic cancer is highly resistant to both radiation and chemotherapy (2). These data highlight the need to investigate tumor biology, identify patterns and predictors of tumor progression and relapse, and develop beneficial adjuvant therapy in order to impact long-term survival after surgical resection. The receptor for advanced glycation end-products (RAGE) is a starting point for addressing these needs.

Several reports on the relationship between RAGE expression and tumor biology have been published to date (3). In renal cell carcinoma, RAGE is associated with cancer cell growth (4). Kuniyasu et al demonstrated that RAGE expression is closely associated with invasion of colon cancer cells and is correlated with metastasis. In their sample of 119 patients, RAGE positivity in Dukes' B, C, and D cases was 19%, 81%, and 100% respectively ($p<0.0001$) (5). With respect to pancreatic cancer, Takada et al demonstrated that RAGE expression is concordant to the metastatic ability of representative human pancreatic cancer cell lines (6, 7). Arumugam et al found that S100P, a RAGE ligand, plays a major role in the aggressiveness of pancreatic cancer that is likely mediated by its ability to activate RAGE (8). These researchers also demonstrated that Cromolyn, an anti-allergy drug, binds S100P, prevents activation of RAGE, inhibits tumor growth, and increases the effectiveness of gemcitabine in experimental models (9). From these data, we hypothesize that RAGE is involved in the development and progression of pancreatic adenocarcinoma. We have designed the following study to further explore this hypothesis.

2.) Study Design and Statistical Procedures:

This study is a retrospective case series reporting the correlation between RAGE expression and stage of disease in pancreatic adenocarcinoma. We estimate that we have approximately 150 cases of pancreatic adenocarcinoma that have been prepared by the pathology department in a tissue microarray for immunohistochemical staining and other molecular procedures. A review of the literature revealed that similar studies examining the molecular biology of pancreatic adenocarcinoma report cases ranging from 37-71, making our estimated number of cases within the reportable range. Given the work of Kuniyasu et al in regards to colon cancer as cited above, we hypothesize that there will be similar differences in RAGE expression and stage of disease in pancreatic adenocarcinoma. Power analysis shows that we require a minimum of 28 samples to show a difference in RAGE expression of 10%, 20%, 70%, and 90% with stage I, II, III, and IV pancreatic cancer respectively with a power of greater than 85% and $p<.05$.

However, since we cannot be certain that RAGE expression in pancreatic cancer behaves similar to colon cancer; we propose to use 150 samples.

3.) Study Procedures:
Our proposed study is a retrospective analysis of RAGE expression in pancreatic adenocarcinoma specimens resected at Columbia University Medical Center. We will examine approximately 150 specimens. First, we will use notes from each patient's preoperative visit to determine multiple demographic variables, including age, sex, family history, occupation, and tobacco use. Given that RAGE expression has been implicated in several other disease processes, we also will examine the patient charts for comorbidities and previous surgery, as well as preoperative chemotherapy, radiation, and other neoadjuvant therapy status. We will use the operative reports to delineate the type and extent of resection. We will examine the pathology report for degree of cellular atypia, local invasion, and lymph node involvement. Using antibodies to RAGE, we then will stain the specimens to assess any correlation between RAGE expression and stage of disease. Using antibodies to RAGE ligands, we also will stain the specimens for the ligands S100P and amphoterin to determine if colocalization correlates with stage of disease. We will collect these data into an Excel spreadsheet and use appropriate biostatistical analyses to determine significance on several fronts. First, we will determine whether any of the demographic variables influence RAGE expression and /or stage of disease. Second, we will determine whether a relationship exists between RAGE expression and biology of the disease. Finally, we will determine if there is a correlation between RAGE ligands and biology of the disease and take direction for future research pursuits into prognostic markers and therapeutic targets for pancreatic cancer.

4.) Study Drugs or Devices:
No drugs will be given and no devices will be used in this study.

5.) Study Questionnaires:
No questionnaires will be used in this study.

6.) Study Subjects:
Study subjects will be patients who previously underwent resection of pancreatic adenocarcinoma at Columbia University Medical Center.

Description of the Disease:
The pancreas is a gland organ that serves both an exocrine function that aids in digestion and an endocrine function that regulates blood sugar. The pancreas is susceptible to both endocrine and exocrine tumors. However, exocrine tumors, specifically pancreatic ductal adenocarcinoma, represent 95% of malignant pancreatic neoplasms (10). In the United States, pancreatic cancer is the fourth leading cause of cancer deaths, accounting for more than 35,000 deaths annually (1). Pancreatic ductal adenocarinoma is a biologically aggressive disease with an extremely poor prognosis. Surgery remains the only curative treatment for pancreatic adenocarcinoma; however, at the time of diagnosis, only 10 to 20% of tumors are resectable (2). Even after radical pancreatic resection for localized pancreatic adenocarcinoma, the median survival is 12-18 months and the 5-year survival

is 15% (10). Given these results, there is a great need to understand the biological mechanisms behind the aggressiveness and resistance to therapy of pancreatic cancer. A rigorous examination of the RAGE-tumor relationship may aid our understanding of tumor biology in this region and help guide therapy.

Goals of Therapy: There is no therapy in this study because it is a retrospective analysis of existing data. We hope, however, that our results will lay the foundation for future research into RAGE as a prognostic marker and therapeutic target for patients with pancreatic cancer.

Inclusion Criteria: We will include all patients with primary adenocarcinoma of the pancreas that was resected, identified by pathological examination at Columbia University Medical Center, and included in the pathology department's tissue microarray in preparation for immunohistochemical staining.

Exclusion Criteria: We will exclude any patients with disease that is metastatic to the pancreatic region. We will not exclude patients based on demographic variables or coexisting medical conditions, though we will note patients with comorbidities known to be associated increased RAGE expression (e.g., diabetes, Alzheimer's disease).

7.) Recruitment:
This study is a retrospective analysis of existing data and therefore has no applicable recruitment methods.

8.) Confidentiality of Data:
We will de-identify data during the collection process prior to analysis. We will collect the minimum amount of information needed for the purposes of this study. Only the investigators in this study will have access to this data, and all of the information gathered will be used for this study only. The information will not be given to other investigators, institutions, or agencies.

9.) Potential Risks:
This study is a retrospective analysis of existing data, and we believe there are no potential risks to the patients that we can identify at this time. There will be no alteration of patient treatment and there are no potential adverse outcomes.

10.) Potential Benefits:
There are no immediate potential benefits to the patients whose specimens will be used for this study. This study offers potential benefits to future patients with pancreatic adenocarcinoma as RAGE and its ligand are potential targets for adjuvant therapy.

11.) Alternatives:
There are no applicable alternatives as this retrospective analysis of pre-existing data does not involve therapy or alteration of clinical treatment.

12.) References:

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