

Sample P&F Research Proposal

Project #3 Proposal

TITLE: The Role of Raf-1 Kinase Inhibitory Protein (RKIP) in Colorectal Cancer Metastasis.

INVESTIGATOR: Eugene A Choi M.D. (Department of Surgery)

Metastasis, a process comprising discrete stages including early events such as epithelial-mesenchymal transition (EMT), invasion, intravasation, and late events such as extravasation and colonization at distant sites, is largely responsible for death in colon cancer patients. Therefore, a major goal of our proposal is to identify genes responsible for metastatic progression that can also serve as prognostic markers and potential therapeutic targets.

One approach to address this problem is to characterize the action of metastasis suppressors. Raf Kinase Inhibitory Protein (RKIP) has been implicated as a metastasis suppressor for many solid tumors including prostate and breast. RKIP is expressed in cells of normal colonic mucosa and the loss of RKIP expression has been reported to be a marker of tumor progression and distant metastasis as well as a predictor of survival. RKIP functions as an inhibitor of key signaling cascades including MAPK, G Protein-Coupled Receptors, and NF- κ B. Our laboratory recently showed that inhibition of Raf/MAPK by RKIP leads to induction of the microRNA let-7 and suppression of genes such as HMGA-2 that promote tumor invasion and metastasis in a breast cancer model. Similarly, ectopic expression of RKIP in a relatively aggressive and tumorigenic colorectal cancer (CRC) cell line induces let-7 expression, inhibits invasion and suppresses liver metastasis in an immunodeficient mouse xenograft model after intrasplenic injection of CRC cells. We therefore hypothesize that RKIP regulates hepatic metastatic genes in CRC through microRNA let-7 expression and its downstream targets. To validate our hypothesis that RKIP inhibits both early and late steps metastasis, we would like to extend our model by using an orthotopic colorectal cancer model with cecal implantation of CRC cells and demonstrate the role let-7 and HMGA-2 has directly on cell invasion in colorectal cancer.

Project # 3

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INVESTIGATOR(S): Eugene Choi. MD

Funding Period: 12-1/09-11/30/10

PROGRESS REPORT: The goals of this project were to define downstream mediators of RKIP regulated metastasis and to define if RKIP inhibits colorectal metastasis in an orthotopic colorectal cancer model. During the funding period, we determined that RKIP directly regulates the expression of microRNA, -let- 7 and induction of let-

7 inhibits HMGA2 expression. HMGA-2 is a chromatin-remodeling protein that is normally expressed in early embryonic development and in cancer cells, promotes epithelial-mesenchymal transition (EMT), which promotes tumor cell invasion and metastasis as well as resistance to chemotherapy agents and radiation. In our studies, over expression of mature let-7 or transient knockdown of HMGA-2 in HCT-116 inhibited invasion with the greatest effect seen with mutant RKIP (which prevents the release of Raf-1 in response to epidermal growth factor (EGF), acting as a stronger inhibitor of MAPK signaling. Further, RKIP expression down-regulated MAPK signaling. We are currently using MAPK signaling inhibitors to determine the effect on let-7 and HMGA-2 expression and cell invasion. In addition, we are using conventional chemotherapeutic agents such as 5-FU and gemcitabine to determine if RKIP and let-7 sensitizes colorectal cancer cell lines. To determine other RKIP regulated downstream mediators of invasion and metastasis, we took the U133plu2 affymetrix arrays (Human samples) from a published paper on colorectal cancer. The matrix of expression values were then tested using Gene Set Analysis (GAS) on established groupings of genes sets including let-7 targets to RKIP expression. We generated a list of potential RKIP and let-7 targets. We have selected a few of the putative targets such as MAP4K4 and COL5A to validate our methodology. Both MAP4K4 and COL5A are involved in tumor progression.

Core Facilities Used: (1) Cell and Microbiology Biology Core (now the Host-Microbe Core (2) Molecular and"

"Genetic Engineering Core (3) Tissue and Cell Analysis Core"

GRANTS: Planned submission of NIH K08 10/11

HONORS/AWARDS: NONE

ABSTRACTS: E. Choi, J. Menon, E. Eves, and M. Rosner, The Role of Raf-1 Kinase Inhibitory Protein (RKIP) in Colorectal Cancer Liver Metastasis presented at the Academic Surgical Congress, Huntington Beach, CA, February 2010.

PUBLICATIONS: E. A. Choi, E. M. Eves, J. Menon, M. R. Rosner. The Role of Raf-1 Kinase Inhibitory Protein (RKIP) in Colorectal Cancer Liver Metastasis. J SURG RES 01/2011; 165(2):291-291.

Please acknowledge support from the DDRCC (NIDDK P30DK42086) in your publications and presentations.