2017 Research Fellowship Application : Entry # 10946

Project Information

**Project Title**
Role of BAP1-modulated long non-Therapeutic role of long noncoding RNA NEAT-1 in cholangiocarcinoma coding RNA as a determinant of drug sensitivity in cholangiocarcinoma

**Amount Requested**
$50,000

Applicant Information

**Applicant Name**
Mansi Parasramka

**eRA Commons Username**
PARASRAMKAM

**Degree (Select all that apply)**
- PhD
- Other

**Other Degree**
M.S.

**Academic Level (Select all that apply)**
- Post-Doc Fellow

Institution Information

**Institution Name**
Mayo Clinic

**Institution City**
Jacksonville

**Institution State**
FL

**Institution Country**
United States

**Email**
Although long noncoding RNAs (lncRNAs) have recently been identified to play a critical role in many human diseases, their role in cholangiocarcinoma (CCA) is not well known. Our aim is to evaluate the role of lncRNA dependent cellular processes in responses to therapy, and to use this knowledge to improve therapeutic outcomes for CCA. Alterations in BRCA-1 Associated Protein-1 (BAP1) are amongst the most frequent alterations in CCA. We have identified that loss of BAP1 is associated with an increase in lncRNA nuclear paraspeckle assembly transcript-1 (NEAT-1). Furthermore, NEAT-1 is induced in response to chemotherapeutic stress, and knockdown of NEAT-1 reduces cell survival and anchorage dependent growth. Based on this, we hypothesize that NEAT-1 dependent cellular responses can be targeted to improve responses to therapy in CCA patients with alterations in BAP1. We will evaluate this hypothesis using CCA cells with high or low BAP1 expression, and genetically engineered haploid cells with CRISPR/Cas9 knockdown of BAP1 in three aims: to (1) evaluate the role of NEAT-1 as a downstream mediator of BAP1, (2) evaluate downstream gene expression by NEAT-1 in response to chemotherapy, and (3) target lncRNA NEAT-1 for CCA therapy. These studies will allow us to evaluate the effect of modulating NEAT-1 on responses to gemcitabine and cisplatin that could ultimately result in new and more effective therapeutic options for CCA.

Cholangiocytes are constantly exposed to environmental stress that can result in cellular responses with alterations in cell survival signaling. Mutations in BAP1 are amongst the most commonly observed alterations in CCA. In these studies, we will identify the role of a long non-coding RNA, NEAT-1, that is a commonly upregulated IncRNA in many cancers. Our preliminary studies suggest an inverse relation between BAP1 and NEAT-1. Loss-of-function of NEAT-1 decreases CCA proliferation and migration. Furthermore, NEAT-1 is increased during exposure to chemotherapeutic stress. Thus, we hypothesize that targeting NEAT-1 dependent cellular responses in context of BAP1, could improve responses to therapy in CCA patients.

We have a particular interest in studying long noncoding RNA (lncRNA)-mediated effects on cancer of the biliary tract. A balance of IncRNA levels is important for cellular stability and disturbing this could lead to cancer. One of the most commonly mutated genes in cholangiocarcinoma (CCA) is BAP1, which plays a critical role in DNA repair pathways. For the purpose of this fellowship award, we will focus on the combined role of BAP1 and IncRNA as a driver of CCA and response to therapy for the following reasons: First, a loss-of-function mutation is observed in BAP1 gene suggesting a tumor suppressor role in CCA and its presence to be biologically relevant. Second, there is little information on how BAP1 interacts with IncRNAs in CCA. Finally, to our knowledge there are no current therapeutic molecules targeting BAP1-IncRNA mediated regulations due to lack of scientific evidence. These studies are driven by our desire to find more effective ways to treat CCA, and could result in more personalized treatment options.
Our overall goal is to identify targetable cellular mediators and mechanisms to improve outcomes of treatment in patients with CCA. Recent studies have identified chromatin modulators such as BAP1 as the most commonly mutated genes in CCA causing alterations in expression of genes in tumor formation, progression or response to therapy [1]. The Patel laboratory has identified long non-coding RNA (lncRNA) as mediators of drug sensitivity in other liver cancers [2]. However, there is limited knowledge of their role in CCA. In preliminary studies, the lncRNA NEAT-1 was altered in a BAP1 dependent manner in human CCA cells. We also observed NEAT-1 to be induced in response to chemotherapeutic stress. Based on this, we hypothesize that NEAT-1 dependent cellular responses can be targeted to improve responses to therapy in CCA patients with alterations in BAP1. We will test this hypothesis in the following three aims:

SA 1: To evaluate the role of NEAT-1 as a downstream mediator of BAP1. Using CCA cells with high or low BAP1 expression, or genetically engineered haploid cells with knockdown of BAP1 using CRISPR/Cas9, we will evaluate the effect of modulating NEAT-1 on BAP1-dependent and -independent responses to gemcitabine and cisplatin.
SA 2: Evaluate downstream gene expression by NEAT-1 in response to chemotherapy. We will assess NEAT-1 dependent gene expression and identify cell survival pathways and mediators following experimental manipulation of NEAT-1 in CCA cells, and will verify their involvement in NEAT-1 dependent survival signaling in CCA.
SA 3: Targeting IncRNA NEAT-1 for CCA therapy. We will evaluate the effect of knockdown of NEAT-1 on the responses to gemcitabine and cisplatin using 3-dimensional spheroid cultures of CCA cells. These studies will also be performed using cells with normal or reduced BAP1 expression, and will identify if NEAT-1 can be directly targeted to improve responses to chemotherapy.

Overall, these studies will have a scientific impact as well as clinical translational potential by exploring a unique class of IncRNAs, as regulators of gene expression in CCA; their association with chromatin modulators such as BAP1, and will provide new mechanistic concepts for these genes. Future studies could involve small molecule screens for candidate therapeutics to target NEAT-1 and optimizing efficient delivery methods. These studies will help develop unique therapeutic approaches for CCA.
Long noncoding RNA (IncRNA) are transcripts larger than 200nt that do not encode for cellular proteins. In recent years, IncRNAs have emerged as an important class of genes that can regulate expression of genes involved in cell growth and differentiation [3]. A role for many IncRNAs has been identified in human diseases, including cancers. However, their role in CCA is not known. Cholangiocytes are constantly exposed to external stimuli that can alter cellular homeostasis. The ability to survive under these conditions may determine how CCA responds to chemotherapy. BAP1 is often altered in CCA. As a chromatin modulator, BAP1 is positioned to epigenetically modulate expression of genes involved in cellular responses to adverse environmental conditions such as exposure to therapy, and that contribute to cell survival [1, 4, 5]. We have identified an inverse relationship between BAP1 expression and IncRNA NEAT1 in an analysis using the Tumor Cancer Genome Atlas dataset. Experimental knockdown of NEAT-1 decreased cell proliferation, migration and invasion in CCA cells. We have also found that NEAT-1 can be altered in response to alterations in BAP1. NEAT-1 is overexpressed in several human cancers including hepatocellular carcinoma [6], but there is no available information on the role of this IncRNA in cholangiocarcinoma. NEAT-1 is an essential architectural component of nuclear paraspeckles, and can serve as an interface between DNA repair pathways, chromatin modifications and cancer phenotype [7, 8]. However, the functional contribution of NEAT-1 to biliary pathobiology and tumorigenesis is unknown. NEAT-1 is induced in response to cell stress such as virus infection or immune stimuli such as poly I: C and can alter cell survival [9, 10]. Thus, we propose that alterations in expression of NEAT-1 could be an important determinant of the response of CCA cells to chemotherapy. A potential mechanism could involve nuclear paraspeckle formation dependent regulated release of preformed nuclear mRNAs, allowing for the rapid translation of cell survival mediators. Thus, understanding the mechanisms by which NEAT-1 alters gene expression and survival signaling may be useful in developing new approaches to enhance the effect of chemotherapy.

Gemcitabine and cisplatin are the conventional systemic treatments of CCA but are not very effective. Targeting NEAT-1 that regulates tumor cell responses to chemotherapy could be useful in improving response to conventional therapy. The proposed studies will enhance our knowledge of fundamental mechanisms of IncRNA mediated regulation of gene expression in response to environmental stress that could also be translated into new therapeutic strategies for CCA, or even other cancers with similar mutations. Future studies could include screening using small molecule inhibitors or delivery of antisense to NEAT-1 using highly efficient nanomedicine platforms.

Project Timeline (2500 Max Characters)

The project timeline for completion is one year. We have already obtained some preliminary data, upon which the application is based, and we have all the necessary reagents on hand. We aim to accomplish our goals listed under Specific Aim 1 and 2 in the first two quarters of the year, with the remaining time will be distributed to work on Aim 3 and preparing our work for dissemination. Under Specific Aim 1, we will screen a panel of CCA lines to identify ideal candidate cell lines with high or low BAP1 expression. Exogenous modification of NEAT-1 expression using siRNA to NEAT-1 and comparing it to nontarget siRNA in CCA cells with low versus high BAP1 will be conducted to test a dose response effect of gemcitabine and cisplatin therapy. Additionally, we will validate our findings using genetically engineered haploid cells with knockdown of BAP1 using CRISPR/Cas9. Concurrently under Specific Aim 2 we will conduct NEAT-1 dependent gene profiling and assess the effect of experimental manipulation of NEAT-1 on paraspeckles and stress responses. We will evaluate the role of NEAT-1-mediated nuclear retention of genes in CCA cell survival.

Specific Aim 3 will be implemented by developing a three dimensional spheroid culture of CCA and using this to test dose response effect of gemcitabine and cisplatin in cells with experimental modulation of NEAT-1 or BAP1 to identify and establish target specific response to these conventional CCA therapies. During the span of the project, we will assess our progress in a timely manner and make required adjustments depending on our experimental data to accomplish our proposed aims.

Literature Cited


Supporting Files - Please upload any supporting files such as charts or graphs in JPG or PDF format.

- Parasramka-figures-1-3.pdf

Budget

Impact or Collab Award

I want to apply for an Impact Award (up to $50,000)

Impact Applicant Name

Mansi Parasramka

Impact App Title

Research Fellow

Impact App Institution

Mayo Clinic

Impact App Personnel 1 Name

Mansi Parasramka

Impact App Per 1 Effort

70

Impact Per 1 Base Salary
### Impact Per 1 Salary Requested
35784

### Impact App Personnel 2 Name
n/a n/a

### Impact App Per 2 Effort
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### Impact Per 2 Base Salary
0

### Impact Per 2 Salary Requested
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### Impact App Personnel Total
35784

### Impact Equipment List

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### Impact Total Requested
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50000