Long Non-Coding RNAs as Biomarkers for Multiple Myeloma Progression

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ABSTRACT

Multiple myeloma is one the most common hematologic malignancies, accounting for approximately 13% of all hematologic malignancies and 1% of overall cancer. Despite advances in treatments, myeloma is still incurable and the lack of reliable biomarkers to predict its development is a critical barrier. Myeloma is always preceded by a premalignant phase, called monoclonal gammopathy of undetermined significance (MGUS), and can then progress to smoldering multiple myeloma (SMM) and/or malignant myeloma.

Although survival of myeloma patients has improved with new treatments, most patients suffer fatal relapse. Long non-coding RNAs (lncRNAs), which are longer than 200 base pairs, have important regulatory functions by binding to proteins, and have proven to play roles in promoting cancer. Due to tissue specificity of lncRNAs, they show promise as prognostic and diagnostic biomarkers for myeloma.

This project aims to address the critical gap in understanding the malignant evolution of myeloma by identifying and characterizing the mechanisms of lncRNAs for use as biomarkers for prognosis of disease progression. Thereby, we compared singlecell RNA sequencing data from plasma and B cells from a publicly available dataset of normal (n=11), MGUS (n=?), SMM (n=6), and myeloma (n=12) patient samples and a validation cohort of 18 myeloma patient samples from the Multiple Myeloma Research Foundation's CoMMpass Study. We identified six differentially expressed lncRNAs comparing MGUS to SMM samples, 14 lncRNAs comparing SMM to myeloma, and 19 lncRNAs comparing normal to myeloma, which we term *Multiple Myeloma Progression-associated lncRNAs (MMPals).*

We focused on the top most differentially expressed lncRNA, *MMPal1,* also known as *NEAT1.* We detected little to no *MMPal1* expression in normal samples, and saw an increase of expression in MGUS patient samples to myeloma samples. Silencing *MMPal1* expression with silencer RNAs shows a decrease in proliferation and viability.

To determine if *MMPal1* is associated with drug resistance, we treated cells with melphalan, a chemotherapy drug used as the conditioning agent in autologous stem cell transplantation. Melphalan-sensitive MM.1S cells showed less *MMPal1* expression when compared to melphalan resistant U266B1 cells. Next, we assessed if *MMPal1* binds to Chromobox 4 (CBX4) protein, due to its similar cellular location in nuclear speckles, epigenetic regulation, and known binding to lncRNAs. We conducted RNA immunoprecipitation and individual-nucleotide resolution cross-linking immunoprecipitation (iCLIP) qPCR to determine that indeed CBX4 binds to *MMPal1.* We show that *MMPal1-CBX4* interaction only occurs in melphalan-resistant cells when treated with melphalan and not in melphalan-sensitive cells.

More recently, to determine clinical importance, we used multiplexed Fluorescent RNA *In situ* Hybridization (mFISH) to detect *MMPal1* in cells and patient samples. Additionally, we created locked nucleic acid antisense oligonucleotides (LNA ASOs) targeting *MMPal1* and saw a decrease in viability and increase in cytotoxicity and apoptosis with decreased *MMPal1* expression. *Our preliminary data*

serves as strong rationale for our hypothesis that MMPal1 binding to CBX4 plays a *role as a master epigenetic regulator to promote myeloma progression and that lncRNAs can be utilized as biomarkers for prognosis of myeloma disease progression.*

Our hypothesis will be tested in three specific aims.

- ***Aim 1 will* assess *interaction of MMPa/1 RNA and CBX4 protein in patient and melphalan-treated cells.*** We hypothesize that *MMPal1* expression increases and interacts with CBX4 in melphalan-treated cells. To date, we have optimized detection of *MMPal1* expression in myeloma cells and patient bone marrow aspirate samples using mFISH. Next, we will use mFISH combined with immunohistochemistry to assess *MMPal1* RNA and CBX4 protein expression simultaneously in cells treated with and without melphalan and in myeloma patient samples.
- ***Aim 2 will identify CBX4-lncRNA interactions and their clinical importance in myeloma.*** We discovered binding of *MMPal1* RNA to CBX4 protein and hypothesize that other RNAs may also bind to CBX4 thereby promoting myeloma. Thus, we will conduct CBX4 iCLIP sequencing to identify all bound RNAs targets *in vivo.*
- ***Aim 3.*** *MMPal1* CRISPR and CBX4 knockdown, overexpression, or LNA ASOs, will be assessed to determine cell viability, cytotoxicity, and apoptosis using the ApoTox-Glo Triplex Assay. RNA will be isolated from respective cells for sequencing to identify gene regulation.

Overall, this proposal will be the first to assess the *MMPal1-CBX4* interaction in understanding multiple myeloma progression. Further, this proposal will fill a knowledge gap for the clinical significance of lncRNAs and have translational impact by evaluating lncRNAs as diagnostics and therapies to improve survival and longevity.