### LAY SUMMARY

**Brief overview**

We proposed basic research on a novel approach to attack metastasis in Black patients with triple-negative breast cancer (TNBC). The work focuses on newly discovered molecules that regulate protein abundance. These molecules are small RNAs, 18-40 nucleotides in length, and do not code for proteins. The molecules include microRNAs (miRNAs) and their isoforms (isomiRs), transfer RNA-derived fragments (tRFs), and ribosomal RNA-derived fragments (rRFs). IsomiRs, tRFs, and rRFs are recent developments in the field of small non-coding RNAs (sncRNAs) that were made possible by the increasing availability of deep sequencing data.

All three classes were initially considered aberrant or degradation products. Our laboratory was the first to show that all three classes are produced in a controlled manner and that their abundance levels and messenger RNA (mRNA) targets depend on sex and ancestry. These observations lead to an important conclusion: *people who differ by sex or ancestry modulate protein abundance differently in the same tissue*.

Our studies of TNBC tumors and normal-tissue-adjacent-to-the-tumor (NAT) from Black and White patients showed extensive molecular differences between the two ancestries. Specifically, the identities and abundances of isomiRs and tRFs and their linkages to mRNAs differed between Black and White patients. In other words, for a given tissue, there are regulatory events that result from the action of an isomiR or tRF on an mRNA and are present in patients of only one ancestry. More recently, we found that the same observations hold for rRFs.

The analyses led us to several dozen miRNAs/isomiRs, tRFs, and rRFs whose abundance increases or decreases significantly in TNBC tumors compared to NAT, but *only* in Black patients. Our analyses also suggested that these differences predispose Black TNBC patients to more aggressive biology and may be critical promoters of metastases in these patients. We proposed to investigate several of these regulators *in vitro* and focused on tRFs and rRFs since they are the most novel, their functions are uncharacterized, and they capture biology of which the community has been unaware.

**Summary of important findings in the first period**

Technical difficulties resulted in a delay in our launching of the project by 1.5 years. During that time, we worked extensively with two service providers to develop the custom assays we needed for this project. Neither provider was successful despite multiple attempts. This forced us to use a third provider and an alternative approach, which proved successful.

Having overcome the technical problems, we made great strides in the last few months. First, we evaluated the impact of modulating the abundance of four sncRNAs and controls in two TNBC model cell lines, one from a Black (MDA-MB-468) and the other from a White (MDA-MB-231) donor. Of the four sncRNAs tested, two can alter cell proliferation: one affects the proliferation of MDA-MB-468 cells only, whereas the other affects the proliferation of both tested cell lines. Second, we tested how modulating the abundance of the two sncRNAs that impact proliferation affects the speed of wound healing. We found that both sncRNAs speed up wound healing in both cell lines. Based on the observed results, we prioritized the study of these two sncRNAs and are awaiting the results of deep sequencing MDA-MB-231 and MDA-MB-468 cells following modulation of the levels of these two molecules and of a negative control.

**Summary of planned work for the next period**

In the months ahead, we will analyze the results of the deep sequencing datasets mentioned above. We expect to gain insights that will allow us to begin defining the mechanisms in which these sncRNAs participate. We will also test more sncRNAs from our prioritized list of tRFs and rRFs that show ancestry-specific expression in TNBC to determine if they can modulate cell proliferation and cell migration.

We will also be using the results generated in the first period of the METAvivor project as preliminary data in an application focused on the molecular biology of health disparities that we will be submitting to the National Cancer Institute this June.

**Other**

In the process, we initiated a collaboration with Dr. Danny Welch at Kansas University Medical Center, who is an expert on the molecular biology of metastasis and will be assisting us in evaluating the results of our experiments.

**Clinical relevance**

We are pursuing research activities that are at the beginning of the discovery/translational spectrum. We are focusing on regulatory molecules whose expression and mRNA targets are ancestry-specific. We believe that by focusing on these molecules it will be possible to attack the metastasis problem at its root.