

PUBLIC/LAY ABSTRACT

We propose basic research and 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.

Cells are fundamental blocks from which living things are built. Cells group together into tissues. Tissues group together to build organs. Organs group together to build organisms. Cells are autonomous units that live, replicate, and die by following instructions written in the *organism's blueprint* ("DNA"). Cells also contain other smaller building blocks, including the essential class of molecules known as *proteins*. Cells make the proteins they need by following instructions in the organism's DNA. Cells convert the instructions into an intermediary template, the 'messenger RNA' (mRNA), then use the template to make proteins. Human cells can make more than 20,000 different proteins. These proteins have diverse roles and are essential for the cell's well-being. The set of proteins that the cell produces at any given moment, and their relative levels, determine the cell's behavior and how it interacts with neighboring cells. Not surprisingly, disruption of protein levels and functions can lead to pathological conditions and disease. Because proteins are essential, the cell controls their levels with exquisite accuracy.

Researchers typically study cells by viewing them as "bags of parts." These parts give cells their properties. Therefore, determining what each part does and how parts interact with one another helps distinguish health from disease, understand how cells work, and figure out how organisms live and die. For decades, researchers studied cells by focusing solely on proteins and their interactions. This approach led to breakthrough advances and therapies for many human diseases. Still, there were many phenomena that proteins could not explain.

The first hints that something important was missing came exactly 30 years ago when scientists discovered the first member of a new category of molecules that differ from proteins. These molecules are known as microRNA (miRNA) and are crucial to a cell's well-being. MiRNA are essential because they regulate the levels of proteins in the cell. We know now that miRNA level disruptions lead to protein level disruptions and are responsible for the onset of many cancers, resistance to chemotherapy and radiation, and metastasis.

Fifteen years ago, a technological advance occurred destined to change the collective understanding of how cells work. This advance, known as "deep sequencing," allows scientists to take a snapshot of all the non-protein molecules in a cell. This snapshot allows one to "see" and "count" all these molecules at once. Initially, deep sequencing was used to confirm the importance of miRNA. Then, the surprises began.

First, we learned that the locations of the organism's blueprint that describe how to make miRNA do not produce a single miRNA regulator but *multiple regulators* at the same time. Each such regulator controls the abundance levels of a *different* set of proteins. Then, we learned that two types of molecules believed to play housekeeping roles, the transfer RNA (tRNA) and the ribosomal RNA (rRNA), also produce small molecules: these are called "tRNA fragments" (tRF) and "rRNA fragments" (rRF). Very little is known currently about the tRF and the rRF. Even so, there is strong evidence that they regulate the levels of proteins in the cell, just like miRNA.

How do these findings relate to cancer, TNBC, metastasis, and ancestry disparities? Our laboratory was the first to discover and report several fundamental properties of miRNA, tRF, and rRF: 1) We showed that cell type, tissue type, and disease type determine which miRNA, tRF, and rRF the cell makes and their abundance. 2) Importantly for this Project, we also showed that a person's ancestry, sex, and age modulate the abundance levels of these regulators. These properties are critical: **people who differ by ancestry, sex, or age produce different pools of regulators that affect protein levels differently and lead to differences in cell behavior.** As we also showed, these findings hold in multiple cancers. **Specifically, for TNBC, we showed that these differences predispose Black patients to more aggressive cancer biology and metastases.**

We analyzed normal breast and TNBC tumors from White and Black patients with TNBC. We identified specific miRNA, tRF, and rRF whose levels change significantly in TNBC tumors compared to normal breast, but only in Black patients. These molecules are entirely novel, uncharacterized, and capture biology of which we had been unaware. Our studies suggest that these molecules are key promoters of metastases in Black TNBC patients. Having already prioritized them, we propose to investigate several of these regulators in vitro. The research that we propose is at the beginning of the discovery/translational spectrum. Thus, we believe it will be possible to leverage our findings to attack the metastasis problem at its root.