Background: Many mouse strains are currently in use to examine breast cancer, and a portion of these strains progress to metastatic cancer. However, it is not clear how appropriate many of these mouse models are for comparison to human breast cancer. Accordingly, we built and analyzed a database of all publicly available gene expression data for mouse models of breast cancer and compared these samples to human breast cancer (Hollern and Andrechek, Breast Cancer Research 2014). In part of our analysis of this database we predicted which key signaling pathways were activated in the various models of breast cancer. Interestingly, we predicted a role for the E2F transcription factors in the MMTV-PyMT model of breast cancer, a model with nearly complete metastasis to the lung. We tested the prediction that E2Fs were involved in PyMT induced metastatic cancer by interbreeding E2F knockout mice with the PyMT transgenic mice. Surprisingly, we recently demonstrated that mice missing E2F1 and E2F2 resulted in a vast reduction in metastasis in the PyMT induced tumors (Hollern *et al*., MCB, 2014). With the analysis of these tumors, we were surprised to note that there were a reduced number of genetic alterations, it appeared that the genetic material had fewer deletions and other events. Thus, we have identified a critical need to understand how E2F transcription factors regulate metastasis through the regulation of stability of the genome.

Rationale: Given that the E2F transcription factors regulate metastasis, the rationale for this project is built on the hypothesis that the genomic events regulated by the E2Fs are responsible for the block in metastasis. Thus, it is of utmost importance to uncover these genetic events to determine how the E2Fs regulate metastasis with the ultimate goal of blocking metastasis in human cancer.
Goals: *The primary goal of this proposal is to find the genetic sequence changes regulated by E2F transcription factors that cause metastasis*. To test the hypothesis that E2Fs regulate metastasis through defined genetic targets we have devised a series of specific aims;

Specific Aim 1 – Sequence and compare tumors that do and do not metastasize. In the background we described tumors from the PyMT mouse model that are highly metastatic and the other strain of PyMT mice that lacked E2F transcription factors that did not metastasize. The goal of this aim is to sequence the full genome from primary tumors from these two types of tumors and to compare them to identify genes that are gained, lost or mutated.

Specific Aim 2 – Identify genes driving tumors to metastasize. The sequence data from the first aim for genes that are gained / lost / mutated will be put together with gene expression data we have already generated from the same tumors. The combined analysis of sequence and expression data will allow us to select for genes that have potential roles in metastasis. This will result in a list of potential metastasis driver genes which will then be filtered through human cancer data where metastatic outcome is known. The goal of this aim is to generate a ranked list of metastasis driver genes.

Specific Aim 3 – Validate that the putative driver genes have a role in metastasis. Using both tissue culture and mouse model systems we will confirm that the potential genes that we identify in the first two aims have a role in metastasis. We will accomplish this by removing the potential driver genes in metastatic cells, then directly testing for a role in metastasis by implanting these cells into the mammary gland and monitoring the lungs as the tumors develop. In this way we will functionally test the driver genes for a role in metastatic cancer.

Expected Outcomes: At the conclusion of the work proposed in this grant we expect to have obtained, analyzed and compared the complete genomic sequence from metastatic and non-metastatic breast cancer to identify potential metastasis driving genes. These genes will also be compared for gene expression data in mouse tumors and in human metastatic breast cancer samples. This list of genes will then be functionally validated through experiments where we remove the gene and test for metastasis. Taken together, this work will result in a significant body of work that will point towards new potential therapeutic targets and results that will function as preliminary data for a planned R01 submission.

Significance: While the proposed work is basic, it is critically important to establish the mechanisms by which metastasis occurs. The successful understanding of the molecular mechanisms that regulate metastasis is critical for new biomarkers and identification of new potential therapeutic targets.