Estrogen receptor (ER) positive luminal breast cancer represents approximately 70% of all newly diagnosed breast cancers. ER is known to drive breast cancer development and progression, and targeting ER has been one of the most successful targeted therapies in oncology. However, a portion of ER expressing breast tumors does not respond to endocrine therapy and the tumor regrows rapidly (de novo resistance). The potential genomic changes underlying resistance to endocrine treatments are still unclear.

A number of studies have implicated that genomic variation in ER binding sites can affect breast cancer susceptibility and progression. This prompted us to develop a pipeline to perform an extensive analysis of potential regulatory single nucleotide variants (SNVs) in ER chromatin immunoprecipitation-sequencing (ChIP-seq) data from hormone responsive breast cancer cells and tumors. This led to the discovery of 5,389 motif altering SNVs in the first phase of our study. One of the top candidates was a SNP within intron 2 of the IGF1R gene, rs62022087, which was predicted by our data to increase the affinity to ER. Our further in-vitro functional studies confirmed that ER is more preferentially binding to mutant allele vs wild-type allele on this locus and therefore can increase the expression of IGF1R gene. Application of our pipeline to all publicly available ER ChIP-seq data highlights large numbers of putative regulatory SNVs associated with ER target genes.

Although it was known for decades that ESR1 gene is not mutated in primary breast tumors, there has been an explosion of the studies in the last two years describing ER as being highly mutated in metastatic breast cancer. Given the clinical relevance of ESR1 mutations as potential drivers of resistance to endocrine therapy, we used sensitive detection methods to determine the frequency of ESR1 mutations in a panel of primary tumors (n=43), bone (n=12) and brain metastases (n=38), and cfDNA (n=29) were collected from 121 patients. Five ESR1 mutations (S463P, Y537C, Y537N, Y537S, D538G) were assessed by digital droplet PCR (ddPCR). ESR1 mutations were detected at very low allele frequencies in 3 primary breast cancers (0.07%-0.2% allele freq), and at high allele frequency in 4 metastases (1.4%-44.9% allele freq), suggesting that in some tumors rare ESR1 mutant clones are enriched by endocrine therapy. In cfDNA cases, treatment was associated with changes in the allele frequency in 7 patients with the mutation in their blood. This study shows that sensitive detection of ESR1 mutations in primary breast cancer and in serial blood draws may be predictive for development of resistant disease.

In conclusion, our studies have suggested SNVs in ER pathway are associated with breast cancer progression and metastasis in ER+ disease. In our experimental model, the SNVs include ESR1 gene mutations and genetic polymorphisms in ER binding sites. We believe such functional SNVs will affect ER-cofactors interaction, ER binding sites, the expression of downstream targets and eventually signaling pathways activated by ER. Our better understanding of ER associated DSVs will inform us about the biology existing behind resistance against endocrine therapy.