HOTSPOT MUTATIONS ON ESTROGEN RECEPTOR-A ARE MULTIMODAL AND CONTEXTUAL DRIVERS OF BREAST CANCER ENDOCRINE RESISTANCE AND METASTASIS

Zheqi Li

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Hotspot mutations in the estrogen receptor alpha (ERα) are frequently detected in ER+ metastatic breast cancer, and there is increasing evidence that these mutations confer endocrine resistance and additional metastatic capacities to breast cancer patients with advanced disease. However, their functional role remains largely unknown.

In the first part, we report the generation of genome-edited MCF7 and T47D cell lines harboring Y537S and D538G ESR1 mutations. ESR1 mutations confer ligand-independent growth and endocrine resistance. Transcriptomic analysis revealed highly mutation site- and context-dependent gene expression profiles. I also characterize the critical role of enhanced IGF1R signaling in ESR1 mutant cell lines through IRS1 upregulation and pointing towards a potential for co-targeting IGF1R and ERα in breast tumors with mutant ESR1.

In the second part of this dissertation, I comprehensively addressed a current critical question—whether these mutations contribute to actual metastatic process, or merely endocrine resistance. I show evidence from our in-house datasets for the presence of ESR1 mutations exclusively in distant but not in locally-recurrent tumors. In line with transcriptomic profiling of ESR1 mutant tumors, genome-edited ESR1 mutant cell line models have a reprogrammed cell adhesive gene network, which functionally confers enhanced cell-cell contacts while decreasing cell-ECM adhesion. Context and allele dependent migratory phenotypes revealed druggable
vulnerabilities, which could be exploited by combination of Wnt and ER targeting strategies. Global ER and FOXA1 binding sites complimentary with accessible genome data uncovers loss of FOXA1 dependency of D538G mutated ER and novel FOXA1-driven epigenetic reprogramming. Collectively, these data serve as essential evidence for ESR1 mutations-driven metastasis and provide guidance for future pre-clinical therapeutic strategies.

In the third part, I identified that basal markers were highly enriched in ESR1 mutant cells and metastatic clinical samples. This could be explained by dual mechanisms regulating induction of basal cytokeratins: a CTCF-driven chromatin loop and progesterone receptor-mediated transactivation. Clinically, tumors with high basal cytokeratins expression share multiple enriched immune pathways with ESR1 mutant tumors mainly correlated to enhanced S100A8/9-TLR4 signaling. Together, these observations show that activating ER mutations confer basal molecular feature mediated by epigenetic regulations and further implies immune therapeutic vulnerabilities.

In summary, we comprehensive decipher the multimodal and contextual role of hotspot ESR1 mutations in breast cancer endocrine resistance and metastasis, as well as the unique gain of basal molecular features in this thesis. Our study provides mechanistic and therapeutic insights to overcome these activating mutations in advanced breast cancer patients.