Uncovering vulnerabilities in triple-negative breast cancer

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Summary

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Triple-negative breast cancer (TNBC) constitutes a small subtype (~15%) of breast cancer, but causes the majority of breast cancer-related deaths. As defined by the absence of ER and PR expression and HER2 overexpression, TNBC is not curable by hormone receptor or HER2-targeted therapies. Furthermore, TNBC is highly heterogeneous and most aggressive. To date, cytotoxic chemotherapy remains the mainstay in the management of TNBC. Despite the initial response to the standard-of-care chemotherapy, TNBC often exhibits intrinsic or acquired drug resistance, and subsequently, recurs in local and distal organs. Targeted therapies have long been pursued for the treatment of TNBC, but rarely demonstrate satisfactory clinical outcomes. Therefore, improved understanding of the intricate biological basis underlying TNBC insensitivity to targeted agents and defining new therapeutic opportunities are of the upmost importance. The aim of the studies presented in this thesis was to systematically identify gene/kinase susceptibilities of refractory TNBC cells, and reveal novel potent targeted therapies for TNBC as monotherapy or in combination with approved kinase drugs.

Chapter 2 exploited a FRET (fluorescence resonance energy transfer)-based high throughput imaging approach to quantitatively monitor ERK and AKT dynamic activity in MEKi-resistant and AKTi-resistant TNBC cells in response to the 378 kinase inhibitors. By deriving a mathematical model to integrate proliferative response profiling and ERK- and AKT-based kinase activity dynamics analysis, I revealed unique kinase dependencies on RTK/MAPK and PI3K/AKT pathways that are distinctly targetable in the resistant TNBC cells. Specifically, MEKi-resistant cells were responsive to inhibitors against PI3K pathway but refractory to EGFR-targeted inhibitors, whereas AKTi-resistant cells were sensitive to EGFR/MAPK pathway blockade but showed resistance against mTOR inhibitors. The work provides new opportunities to explore effective therapeutic kinase targets in treatment-refractory cancer cells, as well as assess the drug efficacy and possible off-target effects of clinically used drugs.

By carrying out kinome-scale siRNA screen, **Chapter 3** identified specific vulnerable kinase targets in EGFRi- and mTORi-resistant TNBC cells. Pharmacological inhibition of these targets greatly suppressed TNBC cell proliferation in different resistant scenarios, highlighting the potential of targeting these kinase vulnerabilities to combat the hard-to-treat disease. Moreover, a kinome-wide siRNA screen was performed in EGFRi-resistant TNBC cells in combination with lapatinib treatment. The combination screen investigated the synthetic lethality interactions with EGFR-targeted inhibition. The results have demonstrated that, a Src family member FYN, conferred TNBC resistance against

EGFR kinase-targeted inhibition via negatively regulating EGFR/PI3K/AKT signaling. Targeting FYN released the activity of downstream PI3K and AKT signaling, rationalizing the co-targeting strategy to subvert drug resistance against inhibitors targeting EGFR/PI3K/AKT signaling axis in cancer cells with elevated EGFR expression, including TNBC.

In **Chapter 4,** a broad kinase inhibitor library screening was carried out across ~20 TNBC cell lines representative for six main TNBC subtypes. The research demonstrated a poor correlation of TNBC molecular subtypes with their proliferative responses to various kinase inhibitors. This study explored effective combined drug treatment to overcome TNBC resistance to mTOR inhibitors. The targets of the identified synergistic kinase inhibitors were predicted by cheminformatics-based survey and functionally validated by siRNA-mediated gene suppression. The AEE788 + rapamycin combination identified in this chapter represents a novel therapeutic strategy to combat TNBC. The putative targets of AEE788 have been revealed in determining rapalog combination efficacy. The combination, by targeting multiple kinases, not only sustains inhibited MAPK activity, but also effectively suppresses mTOR signaling, thereby eliciting synergistic anti-proliferative effects in TNBC. In addition, the findings are complementary to the presently published target spectrum of the kinase drug AEE788. **Chapter 4** revealed the synergistic effects of multi-kinase targeted inhibitor AEE788 on rapalogs treatment in TNBCs. Chemoinformatics-guided target prediction and validation further pronounced the polypharmacology mechanisms underlying the synergy.

Genetic alterations are thought to be favored during the initiation, development and progression of cancer in an evolutionary fashion. Over 80% of TNBCs exhibits TP53 mutation, being a major reason for causing gene instability in this disease. Insightful analysis of genomic sequencing data in (triple negative) breast cancer exploiting bioinformatics holds the promise for the identification of novel therapeutic targets. **Chapter 5** has exploited the robust ADMIRE algorithm to analyze the copy number and gene expression profiles across a set of triple-negative tumors. The analysis prioritized 148 candidate genes driving TNBC cell growth and proliferation. siRNA-based functional screen further validated, besides known EGFR and MYC oncogenes, novel driver genes including ASAP1 which showed high amplification frequency and gene expression in TNBC cohorts. Of relevance, high level of ASAP1 expression correlates with poor prognosis in patients with TNBC. TempO-Seq-based targeted whole genome RNA sequencing analysis concluded that, the novel TNBC driver gene ASAP1, regulates various cytokine and apoptosis signaling components that are significantly associated with TNBC prognosis, supporting the potentiality of ASAP1 as a therapeutic target for the dismal disease.

In summary, the work presented in this thesis has identified important kinase signaling addictions of drug-resistant TNBC cells and key regulators conferring kinase inhibitor resistance, and discovered novel driver genes and combinatorial targeting strategies to subvert drug resistance. These studies provide new insights into the molecular basis underlying TNBC responses to clinical kinase drugs and provoke potential therapeutic targeting strategies for the incurable TNBC.