**Therapeutic Co-Targeting of the BTK/HER2 Axis Induces Iron Accumulation to Overcome Trastuzumab Resistance in HER2-Positive Breast Cancer**

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**Abstract**

**Background:** Trastuzumab is a leading treatment for HER2-positive breast cancer, but many patients develop resistance and experience disease recurrence. Bruton's tyrosine kinase (BTK) plays a crucial role in the B-cell antigen receptor (BCR) signaling pathway and is emerging as a promising target for cancer therapy. Acalabrutinib may irreversibly inhibit BTK, which negatively affects Nrf2 translocation to the nucleus. We hypothesize that by inhibiting BTK, acalabrutinib prevents Nrf2 from translocating to the nucleus and activating Nrf2-dependent antioxidant genes under oxidative stress. This mechanism ultimately enhances the sensitivity of HER2+ breast cancer cells to ferroptosis. **Materials and Methods:** Expression profiles of BTK in publicly available breast cancer samples were analyzed. The role of the phenotype of trastuzumab-resistant breast cancer cells (SKBR3-IR and BT474-IT) was assessed using wound healing, Matrigel invasion, and growth inhibition assays. In addition, immunohistochemistry analysis was used to confirm the effects of shBTK, acalabrutinib, and trastuzumab on therapy-resistant breast cancer cells. **Results:** We demonstrated that BTK was aberrantly expressed in HER2+ breast cancer cells and patients’ samples. HER2 overexpression was associated with therapy resistance. When shBTK was successfully transfected in trastuzumab-resistant cells, the major EMT markers associated with therapy resistance and immune checkpoint were downregulated, and tumor migration and invasion abilities of the cells were inhibited. Acalabrutinib enhances the efficacy of trastuzumab in trastuzumab-resistant HER2+ breast cancer cells. This combination induces apoptosis and ferroptosis, marked by ROS accumulation, extensive lipid peroxidation, and depletion of both oxidized and reduced glutathione. Compared with single-agent treatment, the combined use of acalabrutinib and trastuzumab had significant tumor suppression effects on trastuzumab-resistant cells in vivo. **Conclusion:** The results demonstrated that acalabrutinib effectively inhibited the therapy-resistant phenotype of trastuzumab-resistant HER2+ breast cancer cells by disrupting the BTK/HER2+ signaling. Mechanistically, disrupting BTK and HER2 triggers ferroptosis by downregulating NRF2 and HMOX1 while inactivating GPX4. In vivo, the combination of acalabrutinib and trastuzumab significantly inhibits tumor growth. Our findings demonstrate that acalabrutinib and trastuzumab synergistically inhibit HER2+ breast cancer cells by inducing both apoptosis and ferroptosis.

***Keywords***: Iron Accumulation; HER2+; acalabrutinib; therapy-resistance; breast cancer cells；Ferroptosis