Aim: This study aimed to investigate whether autophagy could confer acquired resistance to TKI in NSCLC, and the role of TME in autophagy induction and TKI sensitivity.

Methods: Two NSCLC cell lines (HCC827 and HCC4006) with EGFR mutations (exon 19 deletions) were selected. MTT and annexin-V binding assays were performed to determine cell proliferation and apoptotic cell death upon TKI treatment respectively. Autophagy was determined by conversion of LC3I to LC3II, formation of ATG5/12 conjugation and p62 degradation using Western blot. Acidic vesicular organelle (AVO) formation was shown by acridine orange staining. Autophagy inhibitor (chloroquine) was used to study the functional significance of TKI-induced autophagy. TME was established by co-culturing human lung fibroblast MRC-5 with HCC827 cells directly. The cells were separated by cell sorter after staining surface marker for HCC827 cells. Then, ELISA and real time PCR were performed to detect the production of cytokines, mRNA and autophagic molecules respectively.

Results: MTT assay after 72 hours of treatment confirmed that both cell lines were sensitive to erlotinib (IC50 < 0.5 µM). Erlotinib increased LC3II expression, ATG5/12 conjugation, p62 degradation and formation of AVO, compatible with induction of autophagy. Combination of chloroquine (autophagy inhibitor) with erlotinib for 48 hours increased apoptotic events compared to TKI or chloroquine alone. Co-culturing MRC-5 cells with HCC827 cells produced an autophagic TME and cytokine storm. Cytokines (IL-6 or IL-8) induced autophagy in NSCLC and MRC-5 cells, and inhibition of cytokine in co-cultured system partially reversed autophagy. TKI sensitivity within the TME is not affected, and combination of chloroquine with erlotinib works better than single treatment, even under TME.

Conclusions: EGFR-TKI induced both apoptosis and autophagy in EGFR-mutated NSCLC cell lines. Inhibition of autophagy with chloroquine enhanced TKI-induced cell death. In the presence of TME, autophagy was induced in both cancer and stromal components, partially mediated via inflammatory factors. Autophagy inhibition under TME could also enhance apoptotic effect of TKI.

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