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604.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

Autophagy Induction Constitutes a Targetable Vulnerability in FLT3-Mutant Acute Myeloid Leukemia Cells Treated with FLT3-Inhibitors

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Recently, we and others identified autophagy induction as a protective mechanism in *FLT3*-ITD mutant acute myeloid leukemia (AML) cells upon treatment with FLT3-inhibitors (FLT3i), constituting an increasing issue in clinical practice. Here, we investigated the molecular consequences of autophagy induction upon FLT3i treatment, exploring resistance mechanisms and interrogated whether autophagy inhibition can improve FLT3i antileukemic therapy. Drug response of primary *FLT3*-ITD AML samples *ex vivo* treated with FLT3i, quizartinib (AC220, n= 59) and midostaurin (PKC412, n=64), was associated with their transcriptional profile to unravel which molecular programs were enriched in poor responders. Both gene set enrichment analysis (GSEA) and single sample GSEA (ssGSEA) revealed enriched molecular signatures compatible with autophagy activation in poor responders to FLT3i, while sensitive samples enriched molecular signatures consonant with mitochondrial function. Enrichment scores for programs associated with "regulation of autophagy" and "autophagosome organization" were positively correlated to *ex vivo* response area under the curve values for AC220 and PKC412. To functionally address the molecular pathways associated with autophagy activation upon FLT3i treatment, MOLM-13 and MV4-11 AML cells (*FLT3*-ITD) and primary AML samples, were treated with AC220 and PKC412 alone or in combination with the autophagy inhibitor chloroquine (CQ, 5-10μM). In *FLT3*-ITD AML models, treatment with FLT3i significantly induced the formation of acidic vesicular organelles, suggesting autophagy induction. We also observed a reduction of autophagy regulators in a dose- and time-dependent manner, evidenced by the conversion of LC3B-I to LC3B-II, with degradation of LC3B-II and p62. Furthermore, FLT3i treatment resulted in phosphorylation inhibition of AKT ^{Ser473}, mTOR ^{Ser2448} and its downstream protein p70S6K $^{Thr421/Ser424}$, and phosphorylation reduction of ULK1 ^{Ser757}. Combination of FLT3i with CQ (10 μM) significantly reduced cell viability and survival for both PKC412 and AC220 inhibitors. To rule out the off target effects associated with CQ treatment, we performed a knockdown (KD) on the key autophagy mediator ATG5 using shRNA-mediated inhibition in *FLT3*-ITD cells. In MOLM13 cells, FLT3i treatment in ATG5-KD cells significantly reduced the IC50 (control: 31nM vs. ATG5-KD: 17nM) and increased apoptosis induction (control: 41% vs. ATG5-KD: 58%). We validated these observations in *ex vivo* treated primary AML samples, where we observed that in *FLT3*-ITD but not in *FLT3* wild-type samples, the combination of FLT3i (PKC412 and AC220) plus autophagy inhibitors (CQ) reduced cell proliferation and survival. In summary, we demonstrated that autophagy induction is a biological process intrinsically associated with drug resistance in *FLT3*-ITD mutant AMLs when treated with FLT3i. Hence, autophagy inhibition can be leveraged to increase the antileukemic effect of FLT3i and highlights the autophagy as a major acquired biological process associated with FLT3i therapy.

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