ABSTRACT

REVERSING RESISTANCE IN MELANOMA AND MALARIA: TARGETING AUTOPHAGY WITH LYSOSOMOTROPIC AGENTS

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The autophagic pathway is a conserved cellular process whereby portions of the cytosol are targeted for lysosomal degradation. Under times of stress, autophagy is upregulated in order to promote cell survival. Accordingly, this process has been shown to be exploited by cancers and contribute to their progression and therapeutic resistance. Autophagy can be inhibited by lysosomotropic agents, such as chloroquine (CQ). CQ has long been used for malaria and more recently investigated in cancer therapy. Unfortunately, CQ is a relatively ineffective autophagy inhibitor requiring high concentrations. To address this, we screened related anti-malarial molecules and discovered quinacrine (QN) has sixty-fold greater potency at autophagy inhibition than CQ; however, it is also fairly cytotoxic. A quinacrine scaffold was used to synthesize a novel series of analogues and their autophagy inhibition screened. Several potent analogues were created including VATG032, which showed little cytotoxicity (see Chapter 2). We were also able to demonstrate that VATG032, along with a cytotoxic VATG014, prevented lysosomal turnover by increased pH. We next tested these inhibitors in patient-derived melanoma cell lines with oncogenic BRAF, shown to have high basal autophagy. We discovered that both VATG014 and VATG032 sensitized melanoma cells to the BRAF-V600E-selective inhibitor, vemurafenib (PLX-4032), in a more than additive fashion (Chapter 4).

Other mechanisms besides autophagy are known to contribute to therapeutic resistance, such as mutations that directly impair the activity of molecularly targeted drugs. Although

resistance mutations in BRAF have yet to be identified, therapeutic resistance to vemurafenib represents a major clinical problem. Vemurafenib functions by preferentially binding to the active form of the BRAF kinase domain, BRAF V600E is constitutively active, making vemurafenib a BRAF V600E selective inhibitor. We investigated a BRAF-V600E melanoma case study that did not respond to vemurafenib, and was later found to harbor a second kinase domain mutation, L567V (see Chapter 5). We determined that the dual V600E/L567V mutation diminished kinase activity as compared to the V600E mutation alone. Moreover, the BRAF double mutant was less sensitive to vemurafenib in vitro than BRAF V600E. We hypothesize that the L567V mutation, which restores V600E kinase activity to wild-type levels, disrupts preferential binding of vemurafenib and may represent a classical revertant mutation. Resistance in malaria is also extremely problematic. With the development of novel autophagy inhibitors derived from quinacrine, we questioned whether these agents may also have potential antimalarial activity. We tested blood cultures containing the malaria parasite, P.falciparum, with VATG014 and VATG032 and found that both were effective in reducing parasite viability. Both compounds maintained well-tolerated cytotoxicity profiles in mammalian cells that did not kill at concentrations that were sufficient for parasite toxicity. Taken together, these molecules may offer a therapeutic window in the treatment of malaria (see Chapter 3). Lastly, given the highly dynamic nature of autophagy, monitoring autophagic flux is a challenging issue. Many assays are currently used to measure autophagy; however, not all are suitable. We created a novel autophagy reporter assay utilizing the photoconvertable protein, mEOS2, fused to the autophagosome marker, LC3 (see Chapter 6). We demonstrate that this assay can be used to accurately monitor whole autophagosome populations, single or multiple autophagosomes, as well as translocation of cytosolic LC3 to autophagosomes. In sum, this

multifaceted assay represents a flexible and tractable tool for investigating autophagy.