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Bortezomib combines with suberoylanilide hydroxamic acid (SAHA) to synergistically induce caspase-dependent apoptosis and blocks SAHA's activation of EBV lytic cycle in nasopharyngeal carcinoma

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Histone deacetylase inhibitor, suberoylanilide hydroxamic acid (SAHA), could induce Epstein-Barr virus (EBV) lytic cycle and apoptosis in EBV-positive nasopharyngeal carcinoma (NPC) cells. In this study, we investigated the effects of combining a proteasome inhibitor, bortezomib, with SAHA in the treatment of NPC cells. Synergistic killing of a panel of EBV-positive NPC cells upon treatment with various combinations of bortezomib (0, 7.5, 15, 30, 60 and 120 nM) and SAHA (0, 0.625, 1.25, 2.5, 5 and 10 uM) was demonstrated by MTT assay and isobologram analysis. The synergistic killing was due to apoptosis as demonstrated by markedly increased sub-G1, annexin V-positive and TUNEL-positive cell populations. Strong proteolytic cleavage of PARP, caspase-3, -7 and -9 and increased reactive oxygen species (ROS) level were detected concomitantly in the NPC cells. ROS scavenger, N-acetyl cysteine, diminished the apoptotic effects of combined bortezomib and SAHA whilst caspase inhibitor, Z-VAD-FMK, significantly suppressed the apoptosis without decreasing the generation of ROS. Interestingly, bortezomib inhibited SAHA's induction of EBV lytic protein expression and abrogated production of infectious viral particles in NPC cells. Combined bortezomib and SAHA, when compared with either drug alone, induced a stronger suppression of growth of NPC xenografts in nude mice. In conclusion, bortezomib combines with SAHA to synergistically induce caspase-dependent apoptosis of NPC cells by generation of ROS and blocks SAHA's activation of EBV lytic cycle. The drug combination should be further investigated as a novel therapeutic strategy in the treatment of nasopharyngeal carcinoma.

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Development of small molecule inhibitors of LMP1-TRAF2 interaction that specifically block B-cell transformation by EBV

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EBV induces malignant diseases in human immune and epithelial cells. To date, no drug is available that specifically targets EBV or its transforming functions. The viral LMP1 oncoprotein is expressed in virtually all EBV-associated malignancies and is essential for viral cell transformation and pathogenesis. LMP1 activates signal transduction by interaction with cellular signaling molecules of the TRAF family. The CTAR1 domain of LMP1 induces non-canonical NF-kappaB signaling by recruiting TRAF2 through a conserved PxQxT motif within CTAR1. Here, we describe the development of an ELISA-based protein-protein interaction assay of LMP1 and TRAF2 which allowed us to screen compound libraries for inhibitors of this interaction. The assay fulfils the requirements for a high throughput assay according to NIH guidelines and has a z-value larger than 0.5. In a library of 800 natural small molecules, pre-selected for drug-likeness, we identified a hit cluster of two structurally closely related compounds, E11 and F07, which blocked interaction of LMP1 and TRAF2 with IC50 values in the low micromolar range. Both compounds only differ in a methyl side group. As demonstrated by STD-NMR analysis, E11 directly but non-covalently interacts with the target protein TRAF2. E11 treatment results in a delay of CTAR1-induced, TRAF2-mediated TRAF3 degradation and, thus, an inhibition of non-canonical NF-kappaB activation by LMP1. Most strikingly, both compounds specifically blocked proliferation of human EBV-transformed B-cells but not of EBV-negative B-cells. Moreover, proliferation of P493-6 B-cells, which can proliferate under the control of two different conditional proliferation programs (EBNA2/LMP1 or Myc), was only blocked by E11 in the LMP1-, but not in the Myc-driven proliferation mode. This result further demonstrates the specificity of the compound for LMP1 and shows that effects of E11 on B-cells were not due to cytotoxicity. In summary, we identified pharmacologically active compounds directed against EBV-induced transformation.