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651.MULTIPLE MYELOMA AND PLASMA CELL DYSCRASIAS: BASIC AND TRANSLATIONAL

Small Molecule Inhibition of MAGE-A3 Induces Cell Cycle Arrest and Apoptosis in Multiple Myeloma

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Background: The Type I Melanoma Antigen Gene-A3 (MAGE-A3) is commonly expressed in multiple myeloma (MM), a cancer of plasma cells. MAGE-A3 binds to Kap1 to form ubiquitin (Ub) ligase complexes and is associated with inhibition of apoptosis, resistance to chemotherapy, and cell cycle dysregulation. The highly conserved MAGE Homology Domain (MHD) undergoes a conformation change from "closed" to "open" in order to complex with Kap1, activate Ub ligase activity, and bind substrates. RNA interference (RNAi) knockdown of MAGE-A3 in MM cells results in upregulation of the endogenous CDK4/6 inhibitor P21 ^{Cip1} and pro-apoptotic BIM, resulting in cell cycle arrest and intrinsic apoptosis. Knockdown of MAGE-A3 also increased cell death in response to melphalan and panobinostat, but not to bortezomib or cis-platin. These findings lead to the hypothesis that small molecule inhibition (SMI) of MAGE-A3 Ub ligase activity will have therapeutic application in this disease.
Methods: We used the Schrodinger suite to perform *in silico* screening of a custom designed library of compounds using the crystal structure of the MAGE-A3 MHD to identify potential binding molecules that stabilize the closed MHD conformation. Candidates were then screened on human myeloma cell lines (HMCL) by CellTiter-Glo assay to identify compounds that reduced cell viability. Isothermal titration calorimetry was used to identify candidate SMI that bound to the MHD. HMCL were co-incubated with SMI; cell cycling was assessed by BrdU assay and apoptosis by Annexin V staining. Protein expression of p21 ^{Cip1}, BIM, and MAGE-A3 was assessed by Western blot. Combinatorial indexes in co-incubation experiments with ergotamine and chemotherapy agents were calculated with the Chou-Talalay method using CompuSyn.
Results: Structure-based and biochemical screening nominated ergotamine as a candidate MAGE-A3 SMI. Ergotamine bound to the MAGE-A3 MHD and induced cell cycle arrest and apoptosis in MAGE-A3-expressing human myeloma and lymphoma cell lines and increased expression of pro-apoptotic BIM and p21 ^{Cip1}, similar to results with RNAi knockdown. These effects were not observed in negative control MAGE-A3(-) lymphoma cells. Co-incubation of ergotamine with chemotherapy agents melphalan or panobinostat resulted in synergistic killing of MAGE-A3+ HMCL. Ergotamine did not synergize with bortezomib or cis-platin under these conditions.
Conclusions: These results support the combined structural and biochemical methods as an efficient means of screening for candidate inhibitors for MAGE-A3. Ergotamine recapitulated the results of RNAi knockdown of MAGE-A3 in cells that express the target, supporting a tool compound for further pharmacologic development. MAGE-A3 SMI have the potential to be a novel class of MM therapy that may be effective alone or in combination with chemotherapy or immune oncology agents. MAGE inhibitors may have broader application in other target-expressing cancers, including lung, melanoma, breast, and ovarian cancers, which are among the most common causes of cancer death.

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