Preclinical evaluation of protein disulfide isomerase inhibitors for the treatment of glioblastoma

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Medicinal Chemistry Third Year Seminar
Tuesday, March 21st, 2017 at 4:00pm

Approximately 8000 unique proteins in the cell require proper disulfide bond formation to fold and function correctly. Disulfide bond formation of nascent polypeptides is catalyzed by protein folding chaperones such as protein disulfide isomerase (PDI). PDI is overexpressed in glioblastoma, and high PDI expression correlates with poor prognosis in brain cancer patients. Additionally, PDI knockdown suppresses the invasiveness and metastatic capability of cancer cells and sensitizes tumors to chemotherapy, making it a promising target for brain cancer treatment. While several PDI inhibitors have been discovered, none have reached clinical trials. Many preclinical PDI inhibitors have potentially undesirable characteristics including reactive substituents, irreversibility, and off-target inhibition and interact directly with the active site cysteine residues. Additionally, these compounds do not have favorable characteristics for blood-brain barrier permeability, a factor that severely hampers the discovery of CNS drugs.

From a high-throughput screen of 20,000 compounds, representing a diverse library of scaffolds, we identified **35G8** as the most potent PDI inhibitor to date, with an IC_{50} value of 170 ± 10 nM. **35G8** is predicted to cross the blood-brain barrier, with ideal CNS properties including a low molecular weight and LogBB > -0.5. **35G8** is also potent in a panel of brain cancer cell lines and destabilizes PDI in the thermal shift assay. Computational docking studies suggest **35G8** binds in the a’ domain of PDI near the active site. We attempted to identify the pathways by which **35G8** causes cell death, and found cell death caused by **35G8** was not dependent on apoptosis nor necrosis. To further determine the mechanism of action of **35G8**, we performed RNA sequencing of nascently transcribed RNA (Bru-Seq). This led to the discovery that **35G8** likely causes cell death via a combination of autophagy and ferroptosis, a novel form of iron-dependent cell death, distinct from apoptosis. Further validating our hypothesis, the iron chelator deferoxamine rescued cell death induced by **35G8**. Additionally, expression of the autophagy marker LC3B increased in response to **35G8** treatment. Many cancers become resistant to chemotherapies that cause apoptosis and agents that cause novel forms of cell death may be effective in cases where apoptosis-inducing chemotherapy is unsuccessful. These preliminary results from our lab suggest PDI inhibition causes ferroptosis, and this link will be explored in future work. Establishing a link between PDI inhibition and ferroptosis presents an exciting avenue for using PDI inhibitors not only as single agents for glioma therapy, but also in combination or as secondary agents in other cancers where resistance develops to apoptosis-inducing therapy.