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From ebselen to hexylselen: Mechanisms of glutamate dehydrogenase and glutaminase enzyme inhibition

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Ebselen modulates target proteins through redox reactions with selenocysteine/cysteine residues, or through binding to zinc finger domains. Glutaminase (KGA/isoenzyme GAC) is an emerging drug target for cancer, and ebselen has been reported as a potent (9nM) KGA inhibitor. This stimulated our interest in investigating its inhibition mechanism. We developed an EZMTT-based KGA/GDH coupled assay to confirm KGA inhibition and discovered that ebselen is a GDH inhibitor, but not a potent KGA inhibitor. For further confirmation, fluorescein- or biotin-labeled ebselen derivatives were synthesized for direct binding assays. Biomolecular interaction analyses showed that GDH, KGA, and TrxR proteins bind to the biotinylated ebselen, but not GST protein and the gel shift assays showed that the fluorescein-labeled ebselen derivative co-migrates with *E. Coli* Hexameric GDH and monomeric/dimeric TrxR in a dose-dependent manner; it also co-migrated with KGA but disrupted the tetrameric form of its KGA enzyme at high compound concentrations. Further proteomic analysis demonstrated that the ebselen derivative cross-links w proteins through a specific cysteine at the active site of GDH and TrxR, but for KGA, the binding site is at the N-terminal appendix, outside of the catalytic domain. The binding sites were further validated by mutagenesis, and these results explain why ebselen is not a potent KGA inhibitor in functional assays. To develop ebselen as a potent KGA inhibitor, we designed and synthesized several benzo[d][1,2]selenazol-3(2H)-one dimers which were subjected to SAR analysis by several specific glutaminolysis assays both biochemical and cell-based. Hexylselen was found to be a dual KGA/GDH inhibitor that completely disrupts mitochondrial functions of cancer cell lines while demonstrating essentially no toxicity to normal cells up to a 10μM concentration; it completely inhibits the growth of many aggressive cancer cell lines. In an aggressive liver cancer xenograft model, hexylselen significantly reduced tumor size, caused massive tumor tissue damage, and prolonged survival rate. These results provide a solid foundation for further experiments aimed at creating an effective anticancer KGA allosteric inhibitor.

Biography

Benfang H Ruan holds a doctorate in Biochemistry and Bioanalytical Chemistry from Rice University and completed post-doctoral research in Molecular Biology and Biophysics at Yale University. From 2005 to 2013, she actively worked as a scientist/project leader in the therapeutic area of drug discovery at Wyeth/Pfizer and then at Forma Therapeutics. In 2013, she won the Distinguish Global Expert Award from Zhejiang Province and accepted a full professor position at Zhejiang University of Technology. Now she has published 50 research articles in the reputed journal and leads a 20-member research team working on tumor metabolism and drug discovery.

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