An Approach to Arrest Cancer by Energy Starvation

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Cancer cells thrive very well by adapting their metabolic regimes and they use glucose and the amino acid glutamine as their energy sources. Glutaminase is required to break down glutamine in a process called glutaminolysis. This process provides a cell with energy and as such is exploited by cancer cells to meet their growing energy demands. Feeding off the breakdown of glutamine, cancer cells are able to grow and divide into a tumor. Glutaminase therefore makes a promising therapeutic target for the prevention of tumor progression. Inhibition of this enzyme could effectively starve the cancer cells of their energy source.

Human glutaminase is the first enzyme in glutaminolysis. Human body primarily produces the KGA (kidney-type) and the LGA (liver-type) forms of glutaminase; however it is the KGA form (which is also expressed in many other tissues) that is becoming a hot target for cancer therapy owing to the availability of the specific small-molecule inhibitor BPTES [bis-2-(5-phenylacetamido-1, 2, 4-thiadiazol-2-yl) ethyl sulfide], currently under preclinical trial in the USA. However, how this potential drug lead compound works to inhibit KGA activity and how KGA function is controlled by hormones and signaling pathways inside human body remains a big mystery. The understanding of how these processes work and be regulated, will lead to develop more potent and specific drug with lesser side effects. We have used X-ray crystallography to elucidate the structure of glutaminase when bound to its inhibitor BPTES, revealed that binding of BPTES triggers dramatic structural changes near the catalytic site and

thus rendering it inactive (Figure 1). Moreover, we provide the molecular basis of BPTES specificity to KGA, through the mutants that mimic LGA isoform, and these mutants are shown to be resistant to BPTES. These novel findings on how and where BPTES binds glutaminase provides information vital to the development of improved versions of this inhibitor that will lead to target-specific therapeutic inhibitors to tackle glutamine-addicted cancers with greater efficacy. For this project we have collaborations with A/P Valiyaveettil Suresh from Chemistry department and Prof Herwig Schüler from Karolinska Institutet, Sweden.

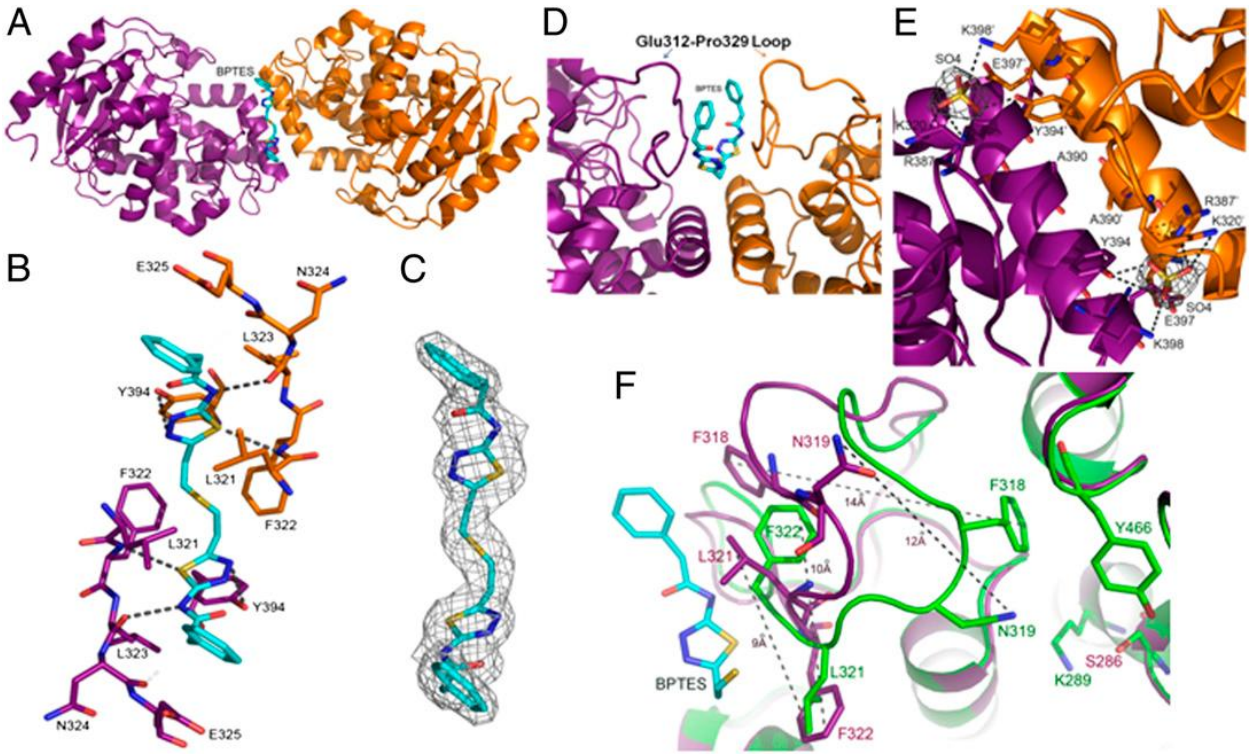

Figure 1. Structure of cKGA: BPTES complex and the allosteric binding mode of BPTES. (A) Structure of cKGA dimer and BPTES is shown as a cyan stick. (B) A close-up view of the interactions of BPTES in the cKGA allosteric inhibitor binding pocket. (C) Electron density map (*2Fo – Fc* map, contoured at 1.0σ) for BPTES is shown. (D) A close-up view of the BPTES binding pocket on the surface exposed region of the loop Glu312-Pro329 at the dimer interface. (E) Perpendicular view of dimer interface formed by the sulphate ion, hydrogen bonding, salt bridge, and hydrophobic interactions between residues from each monomer. (F) Conformational changes on cKGA induced by binding of the BPTES. For clarity only half of the BPTES is shown. Structure superposition of monomeric BPTES complex (magenta) and

apo cKGA (green), showing conformational changes of key residues on the loop Glu312-Pro329. The BPTES binding site is located~18 Å away from the active site (Ser286).

Furthermore, we reveal that KGA activity can be upregulated by phosphorylation (a form of modification by kinases) upon stimulation of cells with epidermal growth factor (EGF), a hormone that is linked to cancer growth and metastasis in various tumours. We further show that this is mediated by a key signaling node, the Ras/Raf/Mek/Erk serial kinase pathway (Figure 2). Most interestingly, treatment of cells with drug that blocks Mek with BPTES that blocks KGA shows synergistic inhibition, raising the hope of developing a novel dual-hit treatment regimes for cancer treatments.

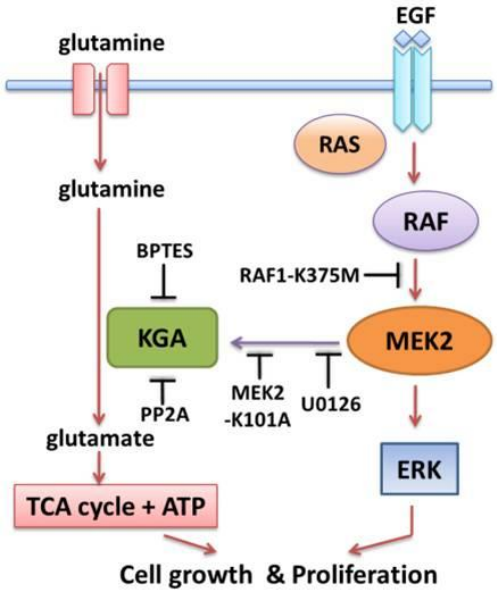

Figure 2. Schematic model depicting the synergistic cross-talk between KGA-mediated glutaminolysis and EGF-activated Raf-Mek-Erk signaling. Exogenous glutamine can be transported across the membrane and converted to glutamate by glutaminase (KGA), thus feeding the metabolite to the ATP-producing tricarboxylic acid (TCA) cycle. This process can be stimulated by EGF receptormediated Raf-Mek-Erk signaling via their phosphorylation-dependent pathway, as evidenced by the inhibition of KGA activity by the kinase-dead and dominant negative mutants of Raf-1 (Raf-1-K375M) and Mek2 (Mek2-K101A), protein phosphatase PP2A, and Mek-specific inhibitor U0126. Consequently, inhibiting KGA with BPTES and blocking Raf-Mek pathway with Mek2-K101A provide a synergistic inhibition on cell proliferation.

Reference

Thangavelu K, Pan CQ, Karlberg T, Balaji G, Uttamchandani M, Suresh V, Schüler H, Low BC, Sivaraman J (2012) Structural basis for the allosteric inhibitory mechanism of human kidney-type glutaminase (KGA) and its regulation by Raf-Mek-Erk signaling in cancer cell metabolism. **PNAS** (2012) May 15;109(20):7705-10.