1. Arresting cancer by energy starvation

“Cancer, a top killer, may be stopped from its deadly progression with the latest discovery by NUS researchers. They have found how a compound that is undergoing preclinical trials as a potential drug can "starve" cancer cells of energy, thus preventing them from developing into a tumour.”

(Extracted from the media coverage)

Prof. J. Sivaraman from the Department of Biological Sciences (DBS), in collaboration with Prof. Low BC from DBS and the Mechanobiology Institute at NUS, has unveiled the X-ray structure of glutaminase (KGA), a key enzyme in cancer metabolism, with its chemical inhibitor, BPTES (bis-2-(5-phenylacetamido-1, 2, 4-thiadiazol- 2-yl) ethyl sulfide) (Thangavelu *et al.,* 2012).

The kidney-type glutaminase (KGA) is a key enzyme responsible for the process of glutaminolysis, which is harnessed by cancer cells (the Warburg Effect) to thrive and become a tumour. However, little is known about the structural interaction between KGA and its inhibitor, BPTES. The investigators, in collaboration with A/P V. Suresh from Department of Chemistry, NUS, as well as other colleagues from the Karolinska Institute, Sweden, determined the mechanism by which BPTES binds to and inhibits KGA, abolishing the cancer cells' energy source and thus potentially blocking their growth. Most strikingly, they revealed that BPTES inhibits KGA by inducing a dramatic allosteric conformational change. This result will aid in the development of better small-molecule inhibitors and analogues of BPTES that target glutaminase activity with reduced toxicity. The research team also concluded that a combination of BPTES and another inhibitor of a glutaminase-activating component would be even more effective at reducing glutaminase activity and cell growth.

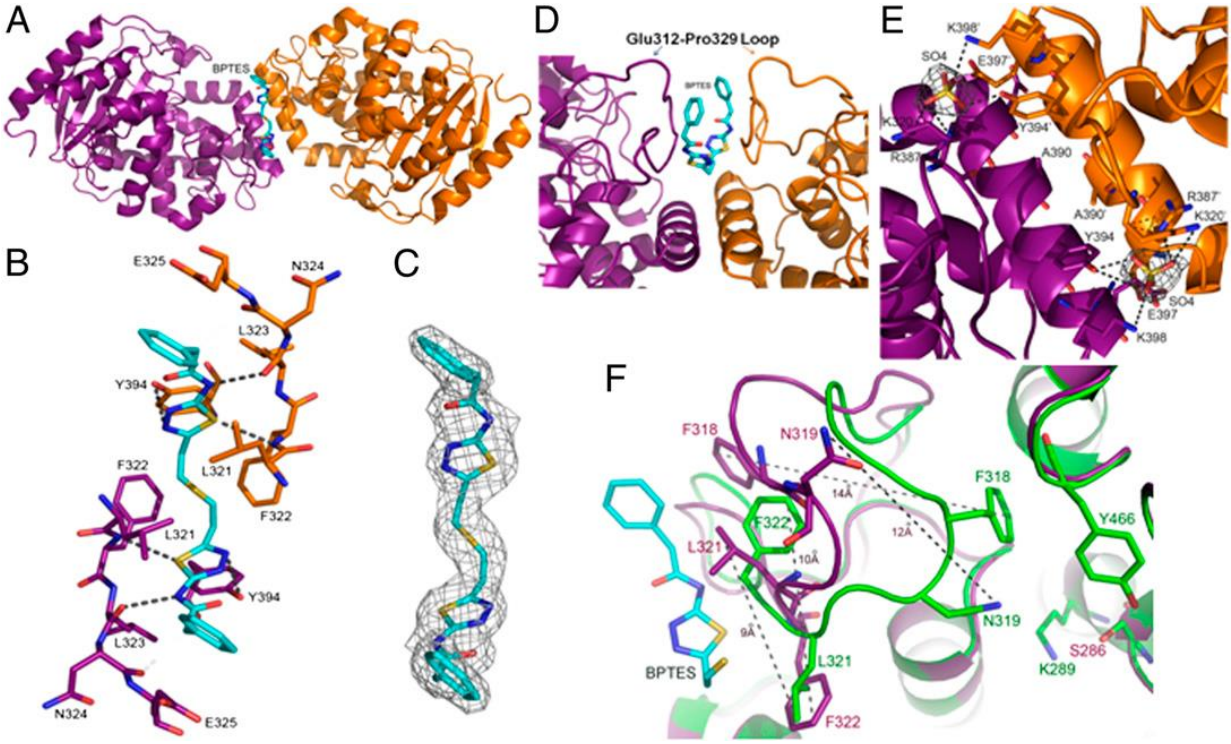

Figure 1. Structure of cKGA: BPTES complex and the allosteric binding mode of BPTES. (A) Structure of cKGA dimer and BPTES is shown as a cyan stick. (B) A close-up view of the interactions of BPTES in the cKGA allosteric inhibitor binding pocket. (C) Electron density map (*2Fo – Fc* map, contoured at 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).

These results were published in the journal *Proceedings of the National Academy of Sciences (**PNAS**)*.

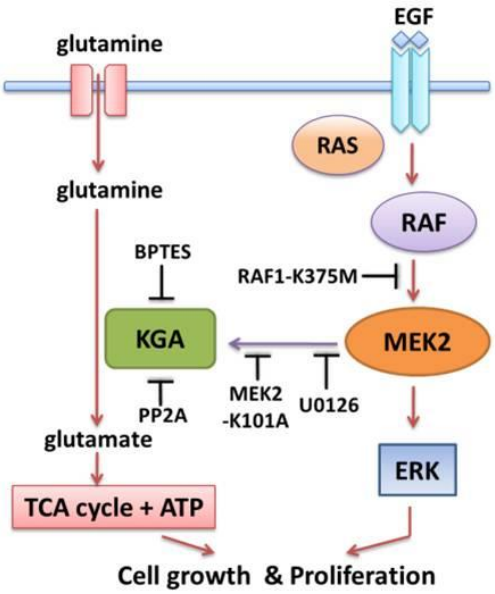

Figure 2. Schematic model depicting the synergistic cross-talk between KGA-mediated glutaminolysis and EGF-activated Raf-MEK-ERK signaling. Exogenous glutamine can be transported across the membrane and converted to glutamate by glutaminase (KGA), thus feeding the metabolite to the ATP-producing tricarboxylic 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.

These findings could offer a more potent but less cytotoxic cancer treatment regime and show promise for a new dual-drug cancer treatment that may be more effective with fewer side effects, especially for patients with cancers, such as lymphoma, prostate cancer, glioblastoma, breast cancer and kidney cancer.

2. Discovery of HYB fold involved in cancers

Sivaraman' s group is also interested in elucidating the structural and molecular basis for the link between ubiquitination (a process which controls the levels of protein expression) and metabolic enzymes involved in cell proliferation and cancer metabolism. Recently, they discovered a third phosphotyrosine (pTyr) domain fold associated with a signal transduction pathway previously linked to cancer.

Phosphotyrosine (pTyr)-binding domains, typified by the SH2 (Src homology 2) and PTB domains, are critical upstream components of signal transduction pathways are often implicated in various cancers. The SH2 was the first pTyr binding domain to be identified over two decades ago in 1986, and was later followed by the discovery of the PTB domain in 1994. Since their discoveries, more than 100 SH2 domains and over 40 PTB domains have been revealed. These two domains are prominent mediators in signalling pathways frequently exploited by cancer cells and, up to now, were the only major phosphotyrosine binding domains in existence.

In a recent breakthrough, Sivaraman' s lab, in collaboration with Prof. Graeme R. Guy, IMCB, Singapore, discovered a new pTyr binding domain fold in protein called Hakai, an E3 ubiquitin ligase so named due to its role in E-cadherin ubiquitination and degradation (Hakai means destruction). They coined this new domain the HYB domain, which stands for Hakai pTyr-binding (HYB) domain (Mukherjee *et al*, 2012, 2014). This pTyr binding domain bears a novel structure, with a completely unique fold, and lacks structural homology with any of the known 75,000 structures in the Protein database (PDB). Moreover,

sequence analysis shows that the HYB domain is found in approximately 70 species, thereby making it the third largest pTyr binding domain after the SH2 and PTB domains.

From a functional perspective, the HYB domain plays a vital role in cellular physiology, acting as a key regulator of E-cadherin, the most essential adhesion molecule in epithelial tissues. In addition to E-cadherin, the HYB domain interacts with Cortactin and DOK1. Cortactin is a structural protein involved in coordinating actin rearrangement during cell movement, whilst DOK1 is a scaffolding protein that assists in the assembly of signalling complexes. Both of these proteins may have important functional contributions to the progression of cancer, and both are regulated by the activity of Hakai. Furthermore, recent studies show the involvement of Hakai in human colon and gastric adenocarcinomas. The novel features of the HYB domain and its interaction with key molecules reveals the physiologically important role of Hakai in cancer. This study opens up new avenues for specific therapeutic interventions.

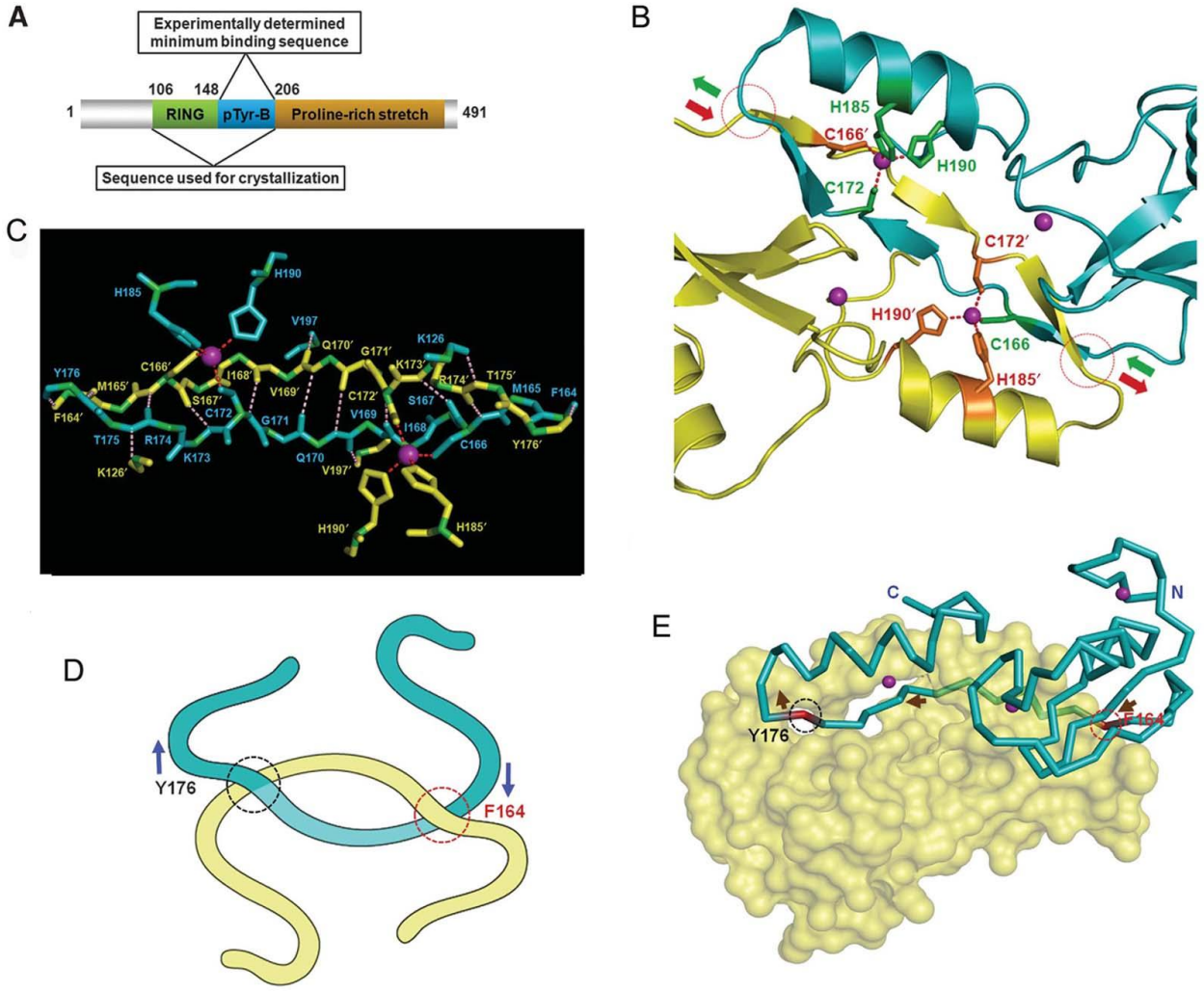

Figure 1. A novel protein fold in Hakai. (A) A schematic diagram of the Hakai protein. (B) The Hakai dimer forms an intertwined configuration spanning the points indicated in circles, with the entry and exit paths shown in green and brown arrows. The zinc-interacting side chains are shown as green and brown sticks. (C) The backbone of the Hakai (aa 106–206) residues involved in intermolecular main-chain H-bonding and the zinc-coordinating side chains of adjacent monomers at the dimer interface are shown in cyan and yellow. The pink dots indicate the main-chain H-bonds; the red dots indicate the zinc coordination bonds. (D) A

schematic diagram of the novel Hakai interlinked arrangement (E) The monomers of the interlinked Hakai dimer are shown in surface representation and Cα trace, respectively. The Cα trace monomer enters and exits the other monomer at the red and black circles, respectively. Brown arrows show its entry and exit path.

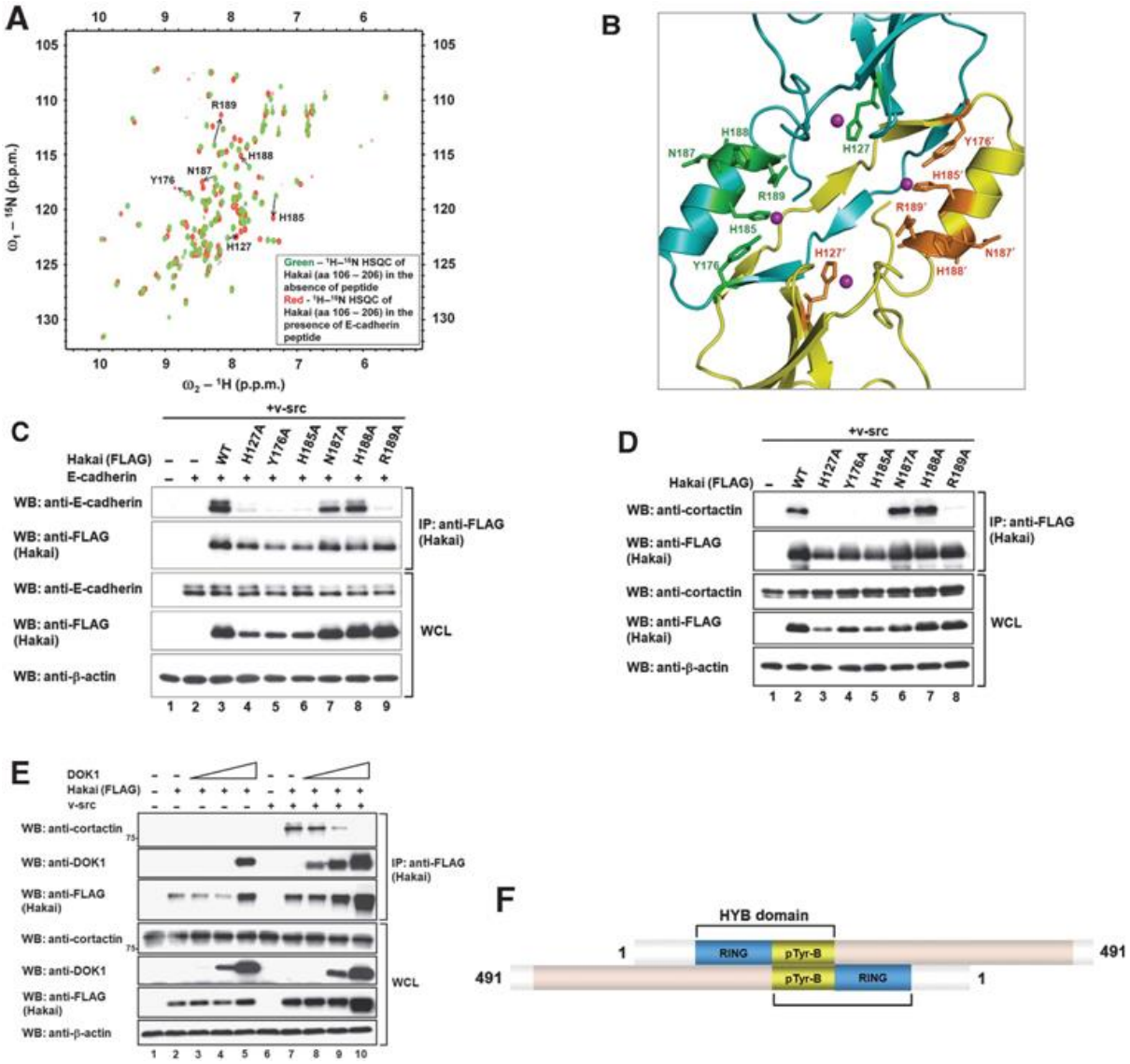

Figure 2. (A) An overlay of the 1H–15N-HSQC spectra of Hakai (aa 106–206) in the absence (green) or the presence (red) of an tyrosine-phosphorylated E-cadherin peptide. (B) The six potential E-cadherin-interacting residues in Hakai (aa 106–206) are highlighted as sticks in the ribbon representation of the crystal structure. (C) The interaction between E-cadherin and the Hakai mutants of the residues identified by NMR was analysed by immunoprecipitating FLAG-tagged Hakai. (D) HEK293 cells were transfected with the identified Hakai mutants, and their interaction with endogenous cortactin was studied. (E) DOK1 was co-transfected into HEK293 cells with Hakai to study its competition with endogenous cortactin for binding to Hakai. FLAG immunoprecipitates were immunoblotted for cortactin. (F) Schematic representation of the Hakai dimer and the HYB domain.

Related publications

1. Ng, C., Jackson, R.A., Buschdorf, J.P., Sun, Q., Guy, G.R. and Sivaraman, J., (2008) Structural basis for a novel intrapeptidyl H-bond and reverse binding of c-Cbl-TKB domain substrates. **EMBO J.**, 27, 804-816.
2. Sun, Q., Jackson, R.A., Ng, C., Guy, G.R. and Sivaraman, J., (2010) Additional serine/threonine phosphorylation reduces binding affinity but preserves interface topography of substrate proteins to the c-Cbl TKB domain. **PLoS One.**, 5, e12819.
3. Sun, Q., Ng, C., Guy, G.R. and Sivaraman, J., (2011) An adjacent arginine, and the phosphorylated tyrosine in the c-Met receptor target sequence, dictates the orientation of c-Cbl binding. **FEBS Letters.,** 585, 281-285.
4. Mukherjee M, Chow SY, Yusoff P, Seetharaman J, Ng C, Sinniah S, Koh XW, Asgar NF, Li D, Yim D, Jackson RA, Yew J, Qian J, Iyu A, Lim YP, Zhou X, Sze SK, Guy GR, Sivaraman J., (2012) Structure of a novel phosphotyrosine-binding domain in Hakai that targets E-cadherin. **EMBO J**., Jan 17; 31(5):1308-19.
5. 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.,** May 15; 109(20):7705-10.
6. Mukherjee M, Jing-Song F, Ramachandran S, Guy GR, Sivaraman J (2014). Dimeric switch of Hakai-truncated monomers during substrate recognition: insights from solution studies and NMR structure **J Biol Chem**., Sep 12; 289 (37):25611-23.