Cancer- and Immunobiology

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Lipid signaling in cancer and inflammation – pharmacological targeting and spatial regulation

In normal tissue, cell fate is controlled by surface receptors triggering signaling events at the inner leaflet of the plasma membrane. Here, phosphoinositide 3-kinases (PI3K) produces PIP3 to initiate a kinase cascade culminating in the activation of protein kinase B (PKB/Akt) and mammalian target of rapamycin (mTOR). PI3Ks controls cell growth, proliferation, survival and migration. In cancer cells, multiple inputs trigger a continuous activation of the PI3K/mTOR pathway. PI3Ks are therefore considered as valuable drug targets in oncology and inflammatory disease.

BKM120 is one of the clinically most advanced PI3K inhibitors (PI3Ki), is currently in phase III clinical trials and listed in more than 80 clinical trials in oncology. In the framework of PI3K and mTOR-targeting projects supported by the CTI, we have elucidated the mechanism of a BKM120-mediated cell cycle arrest (Bohnacker *et al.* 2015). Interestingly, BKM120 increased mitotic markers such as phospho-Histone H3 in a large cancer cell panel, suggesting that the drug acted by a mechanism that is distinct from other PI3K inhibitors (Fig. 1). Typically, PI3K inhibitors such as PQR309, and GDC0941 and GDC0980, arrest cancer cells in the G1/S cell cycle phase. BKM120 was found to interact with tubulin and PI3K. To further monitor the two inherent biological actions of BKM120 we investigated structurally related molecules and generated a potent BKM120-derived microtubule (MT) disruptor MTD147. This chemical split of PI3K and MT activities inherent to BKM120 allowed a functional profiling associating BKM120 with mitotic arrest and Histone H3 phosphorylation. Interestingly, the BKM120-induced mitotic arrest was detected below reported AUC0-24 levels currently used in clinical studies. This suggests that there is no valid therapeutic window for PI3K inhibition without interference with MT stability.

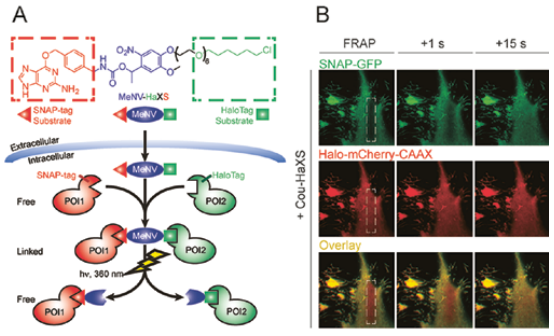

Fig. 1: A) Typically, PI3K inhibitors (PI3Ki) block the cell cycle in G1, and act cytostatic. When treated with a PI3Ki (here PQR309), cancer cells in mitosis can be rarely detected (mitotic marker: phospho-histone H3, pHistone H3). **B)** In contrast, BKM120 triggers a mitotic arrest with an accumulation of pHistone H3 positive cells, which accumulate in G2/M, condense nuclear DNA, and/or undergo apoptosis. At the same time cellular microtubules are disrupted.

A combination of chemical, cell biology and X-ray crystallography studies further elucidated why BKM120 blocked MT polymerization: BKM120 binds to the colchicine-binding pocket on β-tubulin. Binding depends on the orientation of BKM120's core pyrimidine ring. Moreover, biochemical, cellular and structural data suggests that the initially proposed orientation of BKM120 in PI3K is inverted. The in depth analysis of BKM120's activities mechanistically defined its dominant anti-tumor activity, and explains why PQR309 with a triazine core lacks the off-target effect on MT (Bohnacker *et al.* 2015).

The above structure function study also provided starting points for the production of very potent PQR309 follow-up molecules and mTOR kinase-selective inhibitors (which entered the drug development program of PIQUR Therapeutics (Beaufils *et al.* 2016; see sidebar), and potent microtubule polymerisation blockers with novel pharmaceutical characteristics. Altogether, the studies provide access to improved rational drug trials in drug combination studies, when combining PI3Ki and MT targeting drugs.

We have a long standing interest in localized lipid signalling, as this is crucial to PI3K signalling in cancer and inflammation. In the ESF-funded project "Tracking of Phosphoinositide Pools – Key Signaling Components in Cell Migration and Polarisation", we have initiated the generation of novel tools to control subcellular signalling enzyme localization. To be able to dock signalling enzymes to any location in a cell, we have produced so-called chemical-inducers of dimerization (CIDs). These molecules have two reactive groups that specifically form covalent bonds with proteins fused to a SNAP- or a HaloTag (Fig. 2). When one of the proteins is targeted to a cell organelle with a suitable anchor, a target protein can then be associated with this organelle by the addition of a cell permeable CID. To make this association reversible, photocleavable groups were incorporated into the CIDs. This resulted in molecules that allow the dynamic, time resolved and localized dissociation of target enzymes from selected membranes (Zimmermann *et al.* 2014, example in Fig. 2B).

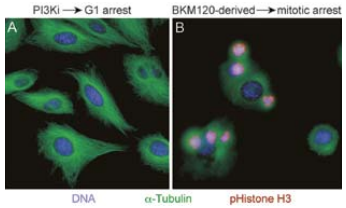

Fig. 2: A) The manipulation of the site of action of proteins of interest (POI) is important in lipid signaling research. The bi-functional chemical-inducer of dimerization (CID) MeNV-HaXS is cell penetrable, and reacts specifically with intracellular SNAP- and HaloTag-fused POIs. The covalent association of two tagged proteins can be reversed by illumination, where a 360 nm light pulse cleaves the MeNV group (Zimmermann *et al.* 2014). **B)** To improve the spectral properties of the CID, novel photocleavable molecules were produced: Cou-HaXS can be efficiently and rapidly cleaved at 405 nm, and is well suited for FRAP microscopy setups. The depicted TIRF images of HeLa cell plasma membrane demonstrate how a laser pulse within the highlighted section (broken lines) can dissociate a target protein (here SNAP-GFP) in a spatially controlled fashion from a membrane anchor (Halo-mCherry-CAAX). The lateral membrane diffusion of the CID-linked proteins is monitored by the recovery of the fluorescence intensities after the FRAP pulse.

Connection to Clinical Practice

PI3K/mTOR inhibitors – moving forward in clinical trials

Mainly due to its importance in cancer growth control, the phosphoinositide 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) pathway is a field of intense efforts in pharmaceutical industry. PIQUR Therapeutics, a spin-off company of the University of Basel, has successfully completed phase I clinical trials with its lead compound PQR309. PQR309 is a potent, brain penetrable pan-PI3K inhibitor with moderate action on mTOR kinase activity. Phase II studies have been initialized in lymphomas and solid tumors. A first drug combination trial using PQR309 with eribulin, a microtubule-depolymerizing agent, has been initiated in a collaboration with Eisai in triple negative breast cancer patients (piqur.com; clinicaltrials.gov).

Support from the Swiss CTI spurred research at University of Basel to generate a number of novel lead compounds targeting PI3K and mTOR with defined selectivity and pharmacological profiles. Three compounds, a brain penetrant pan-PI3K/mTOR inhibitor, a highly potent pan-PI3K inhibitor and a very selective and brain accessible mTOR inhibitor, have already passed into preclinical validation, proof of principle, toxicity and preclinical safety studies. Besides the potential therapeutic value, the produced drug portfolio will provide opportunities to evaluate the biology of tumor drug resistance to PI3K/mTOR inhibition, and to develop tools for chemical genetics approaches.

Selected Publications

Smirnova NF, Gayral S, Pedros C, Loirand G, Vaillant N, Malet N, Kassem S, Calise D, Goudounèche D, Wymann MP, Hirsch E, Gadeau AP, Martinez LO, Saoudi A, Laffargue M. (2014) Targeting PI3Kg activity decreases vascular trauma-induced intimal hyperplasia through modulation of the Th1 response. J Exp Med 211, 1779–1792

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Beaufils F, Rageot D, Melone A, Lang M, Mestan J, Cmiljanovic V, Hillmann P, Hebeisen P, Fabbro D, Wymann MP. (2016) Structure-activity relationship studies, synthesis, and biological evaluation of PQR620, a highly potent and selective mTORC1/2 inhibitor. Cancer Research 1336A