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Kinetic modelling of *in vitro* data of PI3K, mTOR1, PTEN enzymes and on-target inhibitors Rapamycin, BEZ235, and LY294002

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ABSTRACT

The phosphatidylinositide 3-kinases (PI3K) and mammalian target of rapamycin-1 (mTOR1) are two key targets for anti-cancer therapy. Predicting the response of the PI3K/AKT/mTOR1 signalling pathway to targeted therapy is made difficult because of network complexities. Systems biology models can help explore those complexities but the value of such models is dependent on accurate parameterisation. Motivated by a need to increase accuracy in kinetic parameter estimation, and therefore the predictive power of the model, we present a framework to integrate kinetic data from enzyme assays into a unified enzyme kinetic model. We present exemplar kinetic models of PI3K and mTOR1, calibrated on *in vitro* enzyme data and founded on Michaelis-Menten (MM) approximation. We describe the effects of an allosteric mTOR1 inhibitor (Rapamycin) and ATP-competitive inhibitors (BEZ235 and LY294002) that show dual inhibition of mTOR1 and PI3K. We also model the kinetics of phosphatase and tensin homolog (PTEN), which modulates sensitivity of the PI3K/AKT/mTOR1 pathway to these drugs. Model validation with independent data sets allows investigation of enzyme function and drug dose dependencies in a wide range of experimental conditions. Modelling of the mTOR1 kinetics showed that Rapamycin has an IC_{50} independent of ATP concentration and that it is a selective inhibitor of mTOR1 substrates S6K1 and 4EBP1: it retains 40% of mTOR1 activity relative to 4EBP1 phosphorylation and inhibits completely S6K1 activity. For the dual ATP-competitive inhibitors of mTOR1 and PI3K, LY294002 and BEZ235, we derived the dependence of the IC_{50} on ATP concentration that allows prediction of the IC_{50} at different ATP concentrations in enzyme and cellular assays. Comparison of drug effectiveness in enzyme and cellular assays showed that some features of these drugs arise from signalling modulation beyond the on-target action and MM approximation and require a systems-level consideration of the whole PI3K/PTEN/AKT/mTOR1 network in order to understand mechanisms of drug sensitivity and resistance in different cancer cell lines. We suggest that using these models in a systems biology investigation of the PI3K/AKT/mTOR1 signalling in cancer cells can bridge the gap between direct drug target action and the therapeutic response to these drugs and their combinations.

1. Introduction

The kinases, the mammalian target of Rapamycin (mTOR1/2), and phosphoinositide-3 kinase (PI3K) are central hubs in the regulation of anti-apoptotic and proliferation signalling in the PI3K/PTEN/AKT/mTOR1/S6K1 network (Cully et al., 2006; Laplante and Sabatini, 2012). Given this regulatory role, they are considered pivotal targets in cancer drug therapy (Zoncu et al., 2011). The fundamental goal in targeted therapy drug development is high therapeutic efficacy, and this efficacy is characterised not only by inhibition of the targeted protein but also by the response of the whole signalling network to on-target inhibition.

Predicting the response of the PI3K/PTEN/AKT/mTOR1/S6K1 signalling network to targeted therapy is made difficult because of pathway complexities. These complexities include multiple extra- and intra-cellular stimuli, extensive crosstalk with other signalling pathways, multiple feedforwards and feedbacks within pathways, and aberrations in different proteins (Chandarlapaty, 2012). These complexities and protein mutations within the pathway combine in ways that can induce pathway hyperactivation in cancer cells and lead to *de novo* and acquired drug resistance in different cancers (Wang et al., 2009).

The schematic in Fig. 1 shows the pathways involved in the activation of the PI3K/PTEN/AKT/mTOR1/S6K1 network by receptor tyrosine

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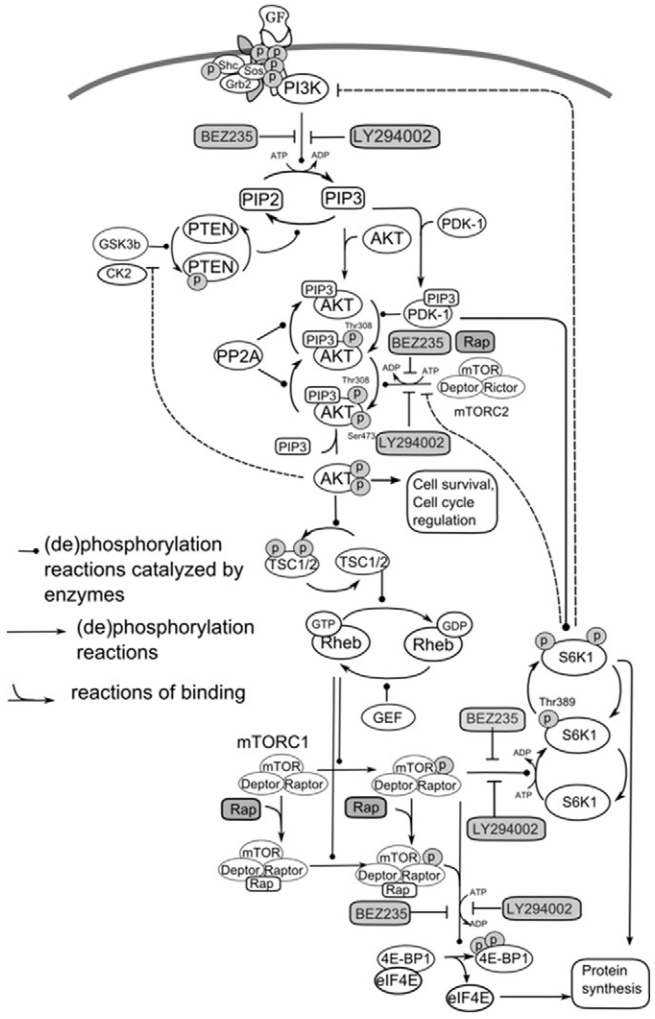

Fig. 1. Scheme of PI3K/PTEN/AKT/mTOR1 pathway activated by growth factor (GF) and inhibited by allosteric inhibitor, Rapamycin, and ATP-competitive inhibitors BEZ235 and LY294002.

kinase (RTK) signalling. Upstream stimulation of the network results in recruitment and phosphorylation of PI3K, which then phosphorylates phosphatidylinositol 4,5 bisphosphate (PIP2) to phosphatidylinositol 3,4,4-triphosphate (PIP3). The second lipid messenger PIP3 in turn recruits downstream proteins to the plasma membrane and this leads to phosphorylation of AKT and PDK1 kinases. Phosphatase and tensin homolog deleted on chromosome ten (PTEN) dephosphorylates PIP3 into PIP2. A normal function of PI3 kinase and phosphatase PTEN strictly control the response of AKT signalling to RTK stimuli (Goltsov et al., 2012). The most common aberrations in the PI3K/PTEN cycle, observed in 80% of cancers, are loss of *PTEN* and/or *PIK3CA* mutations that cause activation of the PI3K/PTEN/AKT/mTOR1/S6K1 signalling network independently of RTK signals, which can lead to tumourigenesis and drug resistance (Nik-Zainal et al., 2016; Stemke-Hale et al., 2008).

mTOR acts as a key downstream integrator of PI3K/PTEN/AKT signalling and the serine/threonine protein kinase assembles into two complexes, mTOR1 and mTOR2: mTOR1 is a major regulator of cap-dependent translation and elongation via phosphorylation of 4EBP1 and S6K1 respectively (Siddiqui and Sonenberg, 2015); mTOR2 phosphorylates AKT(Ser473) (Cully et al., 2006). Besides kinase catalytic domains, mTOR has several domains (FAT, HEAT, FRB) acting as structural scaffolding for different subunits of the mTOR1 and mTOR2 complexes (Raptor, Rictor, PRAS40, and mSLT8) which regulate its binding to substrate/activator, catalytic activity and localisation (Yang et al., 2013). Phosphorylation of S6K1 at multiple phospho-sites by both mTOR1 and PDK1 (Phosphoinositide-dependent kinase-1) forms feedforward and feedback loops in the PI3K/PTEN/AKT/mTOR1/S6K1 pathway (Fig. 1). These feedback loops, together with crosstalk with the MAPK pathway and mTOR1 autoregulation through protein-protein interactions, mean that drug action must account for system-scale properties. That is, the response of the output signal is defined by properties of the whole signalling network, since it is the whole network that modulates functioning of the specific drug targeted proteins.

Multiple drugs targeting this pathway are in clinical practice or preclinical development (Kalachand et al., 2011; Markman et al., 2010), and here we focus on Rapamycin and BEZ235 (Mita et al., 2016). We also consider LY294002 because of its common use in studies of the response of PI3K/AKT pathway to inhibition in different cancer cell lines (Vlahos et al., 1994; Walker et al., 2000). Rapamycin is a mTOR-targeting molecule isolated from bacteria and used as an antifungal and immunosuppressive drug (Laplante and Sabatini, 2012; Wang et al., 2009). In preclinical studies, Rapamycin has shown anti-tumour effects against various tumour types including breast cancer, glioblastoma, rhabdomyosarcoma by controlling cell survival, angiogenesis and proliferation (Laplante and Sabatini, 2012; Wang et al., 2009). Temsirolimus, a rapamycin rapalog, was approved to treat progressive renal cell carcinoma in the clinic (Hudes et al., 2007). Although Rapamycin rapalogs have shown promising antitumour effects in preclinical studies, most of the clinical trial outcomes were disappointing. The existence of several feedback loops in the PI3K/AKT/mTOR pathway may have played an important role in the limitation of Rapamycin in addition to its inadequacy in blocking mTOR2 (Harrington et al., 2004; Jacinto et al., 2004; Yu et al., 2011).

Both PI3K and mTOR belong to the PI3K-related kinase (PIKK) family, and so both might be inhibited by the same ATP competitive inhibitors, resulting in dual activity against both targets. BEZ235 is a potent PI3K/mTOR dual inhibitor that blocks both class I PI3K and mTOR1/2 through competition for the ATP-binding sites of these enzymes (Maira et al., 2008). *In vitro* and *in vivo* studies have shown its high anti-proliferative activities in neoplastic cells as well as a reduction in the phosphorylation level of AKT and its downstream proteins (Jebahi et al., 2014; Maira et al., 2008; Serra et al., 2008). BEZ235 is currently in phase 1/2 clinical trials and is being tested against a number of solid tumours including breast, endometrial, renal and pancreatic tumours. Results show good dose toleration but limited clinical response as a monotherapy or in combination with other drugs (Bendell et al., 2015).

LY294002 is an ATP competitive inhibitor of PI3K (p110α/β) and, although it is not in clinical practice due its toxicity, it is widely used in numerous cell line studies to inhibit the PI3K/AKT pathway. Studies have revealed that LY294002 is not specifically selective for PI3K and can have off-target effects, and mTOR is among those off-targets impacted by LY294002 (DNA-dependent kinase (DNA-PK), casein kinase (2CK2), GSK3β) (Gharbi et al., 2007; Toral-Barza et al., 2005). Accordingly, some of these off-target effects of LY294002 relate to the PI3K/AKT/mTOR pathway and might contribute to the overall inhibition effect observed upon cell treatment. Toral-Barza et al. (2005) showed that LY294002 is a PI3K/mTOR dual inhibitor that blocks PI3K and mTOR kinase through an ATP-competitive mechanism.

Studies on the PI3K/PTEN/AKT/mTOR1/S6K1 pathway reveal clear discrepancies among drug efficacy readouts at the target, pathway and cellular levels for different cancer cell lines (Hassan et al., 2014; Santiskulvong et al., 2011; Shoji et al., 2012). These observed discrepancies are likely due to a mix of the complexity of the signalling network and its mutations together with fundamental differences in enzyme kinetics in various cellular environments. In particular, intracellular conditions in cancer cells may be significantly different to experimental conditions in enzymatic assays, e.g. substrate and cofactor concentrations (Acker and Auld, 2014). To inform the comparison of drug efficacies obtained in enzymatic and cellular assays, and to combine the data derived from these assays, we need data on protein and inhibition kinetics in different cancer cells in different conditions. In general, key