Structure-function Studies and *in silico* Drug Discovery Targeting the Liver Oncoprotein Gankyrin

by
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Abstract

Gankyrin is an ankyrin repeat protein known for its oncogenic effects and its up-regulation in the early stages of almost all cases of hepatocellular carcinoma, as well as other cancers. It mediates its oncogenic effects via a series of protein-protein interactions with several key regulators of the cell cycle including, but not limited to, pRb, CDK4 and Mdm2. A better understanding of these interactions, including their structural details, would facilitate the development of novel therapeutics targeting gankyrin, as well as improving our understanding of gankyrin and the cell cycle control mechanisms it alters.

The nature of gankyrin's interaction with pRb is the subject of some dispute within the literature, including whether gankyrin is in a folded or unfolded state when interacting with pRb. Previous studies have identified that hydroxylation of ankyrin repeat proteins by FIH-1 requires substrate unfolding. Therefore, an investigation of the hydroxylation of gankyrin and other substrates demonstrates that gankyrin does not unfold when in complex with the C-terminal domain of the proteasomal S6 ATPase subunit. Molecular dynamics simulations of FIH-1 in complex with substrates derived from ankyrin repeats suggest that local sequence effects, as well as the tendency of the repeat to unfold, can determine whether or not an ankyrin repeat protein can be hydroxylated by FIH-1. Using hydroxylation to detect protein unfolding, this study provides further evidence that gankyrin interacts with pRb in a folded state, and via the use of in silico methods, proposes a structural model of the interaction. Mutations in gankyrin and pRb that are predicted to disrupt the interaction on the basis of the model do not result in altered binding when subjected to *in vitro* testing. An *in silico* screen to identify small molecules that can disrupt the gankyrin-CDK4 interaction was undertaken to identify lead compounds for the development of novel therapeutics for hepatocellular carcinoma and as a case study in the development of protein-protein interaction inhibitors. No lead molecules were identified, but the study provides evidence that the structural properties of gankyrin are amenable to the discovery of lead

molecules with affinities in at least the low micromolar range. By attempting to recapitulate the findings of an *in vitro* lead discovery screen targeting the ankyrin repeat domain of RNaseL using in silico techniques, the conclusion was drawn that such techniques, without further improvements, are unlikely to be successful in identifying small molecule ligands of ankyrin repeat proteins like gankyrin.

On the basis of this study, we can conclude that the discovery of small molecule inhibitors of gankyrin remains a valid and plausible objective, but that typical in silico techniques are unlikely to fulfil it. While the details of the interaction between gankyrin and pRb remain uncertain and thus inaccessible to drug design efforts, it can now be concluded with greater certainty that gankyrin participates in this interaction in its folded form. It therefore must do so via a mechanism that differs from other proteins containing an LXCXE motif.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Iain Murchland

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