DRUG DISCOVERY PROGRAMME

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Drug Discovery sits at the interface between bench science and clinical development. Our remit is to provide a mechanism by which we can translate the fundamental biology being undertaken within the Beatson into new medicines for patients where there is a clear unmet medical need.

Exploiting the basic research themes of the Institute's scientists we have made significant advances in our portfolio targeting novel approaches to modulate disease-relevant processes.

Metastasis is responsible for approximately 90% of cancer deaths, however there are currently no therapeutic agents available to combat this process. One of the proteins we have chosen to target is fascin, an actin bundling protein that plays a key role in the movement of cancer cells and whose upregulation is known to correlate with poorer overall survival in severe cases of pancreatic ductal adenocarcinoma. We have continued to utilise our fragment-based hit identification expertise to target other protein-protein interactions since these are very challenging biological targets but with a high degree of validation as cancer targets. In particular we are targeting RAS, one of the most highly validated cancer targets that is mutated in over 30% of all human cancers.

Fascin

Fascin is a migration promoting protein that is frequently upregulated when epithelial tissues become malignant. 80-90% of cancers are of epithelial origin and fascin is overexpressed in a variety of tumour types including bladder, colon, lung and pancreas. Thus, it is not only a prognostic marker for multiple types of cancer but is also a compelling drug target.

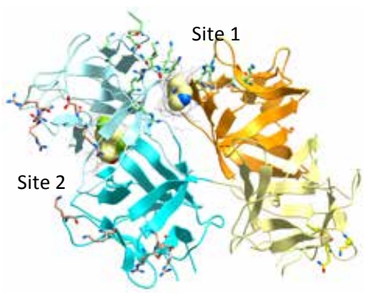

Figure 1
Structure of fascin indicating two ligand binding sites (Site 1 and 2) and their proximity to functionally important residues displayed in green and salmon.

Fascin is a uniquely folded actin bundling protein whose regulation by PKC is tightly coupled with integrins and the extracellular matrix. It exists in equilibrium between the cytoplasm and cytoskeleton where it is bound to actin. It has at least two binding sites for filamentous actin and crosslinks these filaments into tightly packed parallel bundles, oriented with their growing ends toward the plasma membrane. Since fascin is found in actin-rich protrusive membrane structures (microspikes and filopodia) and degradative structures (invadopodia and podosome), which are all pro-migratory, it is proposed that by stabilising actin, fascin provides cells with invasive properties that may confer increased metastatic potential.

We have taken a fragment based approach to identify novel binders of fascin; coupled with a highly successful crystallography campaign this has enabled us to progress initial fragment hits through to compounds with low µM binding. To date, over 100 new small molecule/fascin crystal structures have been generated that have aided hit validation and helped to guide structure-based hit-to-lead chemistry (Fig. 1).

Significant progress has been made in the equally challenging area of biochemical assay development. We have developed two new screens based on highly validated bundling techniques, which has enabled quantitative and reproducible assessment of novel compounds (Fig. 2 and 3). Combining biochemical data with crystallography analysis has enabled us to understand the functional implications of different binding modalities and focus optimisation to the most promising chemical structures.

Figure 2
Optimised robust bundling assay demonstrating concentration response of a BDP fascin inhibitor compound on percentage of bundling. In the absence of inhibitor, >90% of actin is in the pelleted fraction (P), with little in the supernatant (S). This is reversed upon addition of the inhibitor.

Figure 3
Bead-based fascin actin bundling assay. His tagged fascin is pulled down with nickel coated beads and bound actin detected with a fluorescent phalloidin. In the presence of the BDP fascin inhibitor compound, bundling by fascin is inhibited and the amount of phalloidin-labelled actin reduced.

Figure 4
Evolution of a weak binding fragment hit (NMR Kd >1000mM) to more potent analogues (Kd 41 & 30mM); small molecules that directly bind to KRAS (protein in grey).

Figure 5
The GDP-GTP catalytic cycle; b) The KRAS-GDP:SOS1 nucleotide exchange assay; c) Two compounds that inhibit nucleotide exchange in the assay represented in 5b.

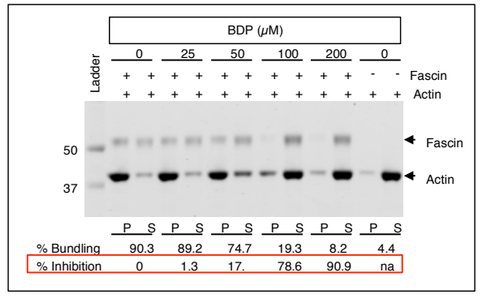

Figure 2

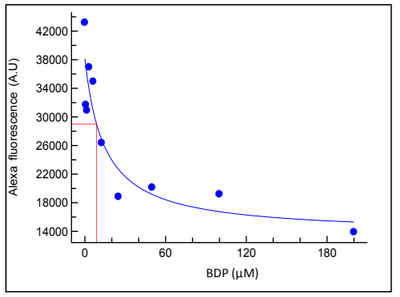

Figure 3

RAS

Approximately a third of all human cancers and the majority of pancreatic, colorectal and lung cancers are driven by mutations in RAS genes. The RAS gene family has three main members; KRAS, HRAS and NRAS, and all play a pivotal role in cell signalling. Under circumstances where RAS genes are mutated, cells multiply uncontrollably and escape cell death signals. The significance of KRAS mutation in driving and maintaining oncogenesis is well recognised. KRAS mutations are associated with over 20% of all human cancers, and in particular are associated with the vast majority of pancreatic ductal adenocarcinomas and a significant number of other tumour types including colon and lung. In the GTP-bound and active state KRAS signals from the plasma membrane through a functionally diverse set of downstream effector proteins including PI3K, RAF and RALGDS to pathways that control cellular growth, apoptosis, survival and differentiation.

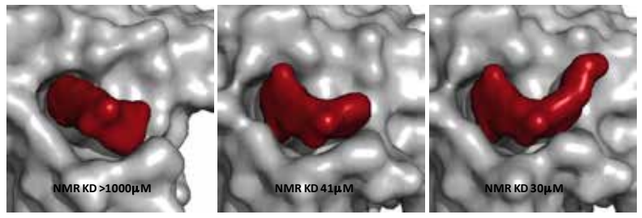

Figure 4

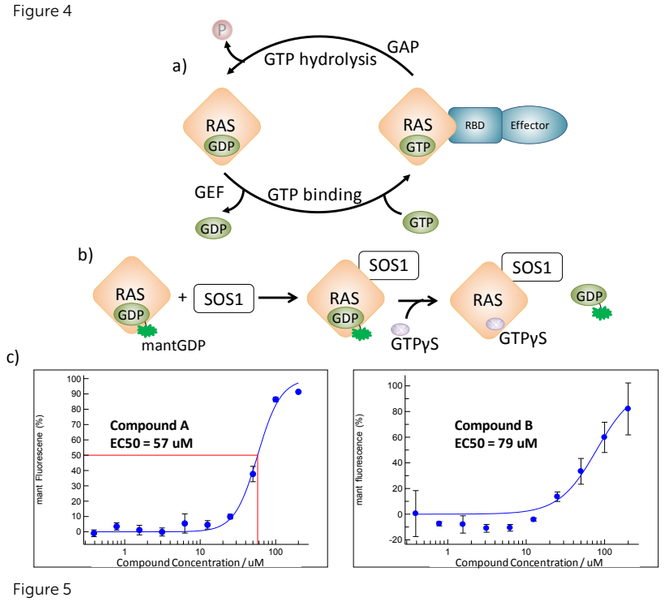

Figure 5

Our approach to identifying start points from which to build a drug discovery project capitalised on our strengths in fragment screening and structure-based drug design. We performed a primary screen of our fragment library against a mutant variant of KRAS using high field NMR experiments, identifying a number of weak binders. After confirmation of binding, crystal structures were obtained that have been instrumental in enabling us to improve potency (Fig. 4) and a strategy to increase this further, towards molecules that will be able to probe inhibition of KRAS effector protein interactions at the cellular level.

In order to assess the ability of our compounds to inhibit one of the functions of KRAS, we have assayed multiple examples in a nucleotide exchange assay (Fig.5).

Through the application of structural biology and medicinal chemistry the project has improved potency of the initial fragment hits, delivering compounds that bind directly to KRAS and inhibit the function of nucleotide exchange. Future strategies will build on this, utilising a combination of chemistry, structural biology and biology to generate potent KRAS binders that can inhibit the interaction between GTP-bound KRAS and its effector proteins.

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