Summary of LST funding at Imperial College London - 2016

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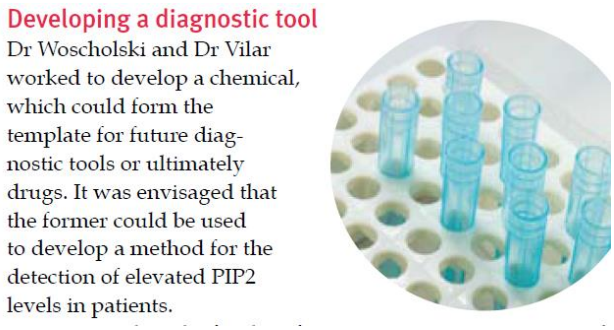

Lowe syndrome is a X chromosome-linked disorder caused by the loss of the OCRL gene, which affects many organs (eyes, brain, kidney, skin, etc.). Since the discovery of a genetic link in the 1980s and the subsequent identification of the OCRL gene in humans in 1992 by Nussbaum and co-workers, a lot has been learned about this rare disease and the gene responsible for the development of this disease. After an initial burst of research publications in the 1990s following the identification of the OCRL gene being an inositol 5-phosphatase, research output flat lined by the beginning of the millennium. It was at this time that the Lowe Syndrome Trust (LST) was formed in 2000 with one of the aims being the fostering of medical research of the disease development and potential treatments. Funding by the LST enabled researchers like myself to focus their research efforts on this inositol phosphatase and the lipids substrates and products it controlled, and how these in turn affect the cellular development and behaviour. From the beginnings the LST created a worldwide network of expertise that spread the message of the importance of medical research into this disease, which resulted in a recognisable increase in research output over the years. Without the LST this effort may have never materialised and it is the LST's legacy to have boosted research in this area at a crucial time.

One of the first funding the LST provided was aimed at finding a chemical approach to enable future drug discovery programmes. The cellular function of the OCRL inositol phosphatase is the removal of a phosphate (figure 1; red circles) of the tri-phosphorylated lipid PIP2. A loss of OCRL phosphatase activity will result in the abnormal accumulation of PIP2 in cells, which in turn is changing via signalling cascades the cellular behaviour and function, ultimately causing the symptoms of the disease. To bypass these effects in patients a chemical drug could be designed to bind to the elevated PIP2 and in doing so mask the elevated lipid from the signalling cascades controlling the cellular

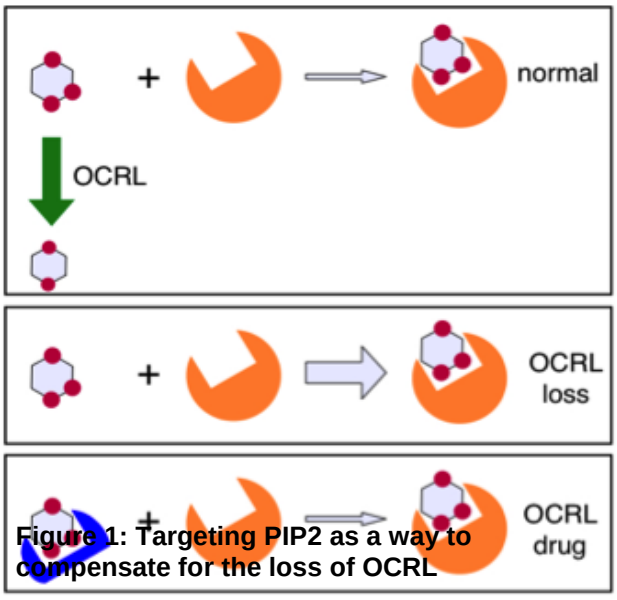

Figure 1: Targeting PIP2 as a way to compensate for the loss of OCRL

behaviour. This chemical would effectively mimic the action of the missing phosphatase, thus being the closest one could get of a chemical intervention being an enzyme replacement therapy. However, the chemical drugs would by design also be important research tools that could be used to diagnose and manipulate PIP2 levels in the cell and whole body. The first funding from the LST to us enabled the pursuit of this idea by generating a prototype molecule capable of binding the elevated inositol lipid PIP2. This work started out almost a decade ago and with the help of LST funding established now a wider set of chemical structures that are currently being characterised with respect to their lipid binding potential.

Previous funded work by the Lowe Syndrome Trust (£140k over the last 10-5 years via 2 grants and one extension) enabled us to obtain the chemical prototype molecule, capable of binding PIP2. We have established that this prototype can selectively bind PIP2 in the test-tube and in living cells. The latest funding (£200k) from the LST was invested in the widening of the chemical structures needed to obtain the necessary knowledge for making these molecules better leads for future drug discovery programmes. Therefore, a range of modified chemical compounds based on the original prototype were generated and characterised with respect to their lipid selectivity. The original prototype was recognising two PIP2 molecules in the membrane (dimeric), which was subsequently modified to recognise single PIP2 molecules, but with the added benefit of carrying a fluorescence reporter (see Figure 2). In addition, methods for lipid extraction and detection compatible with these new

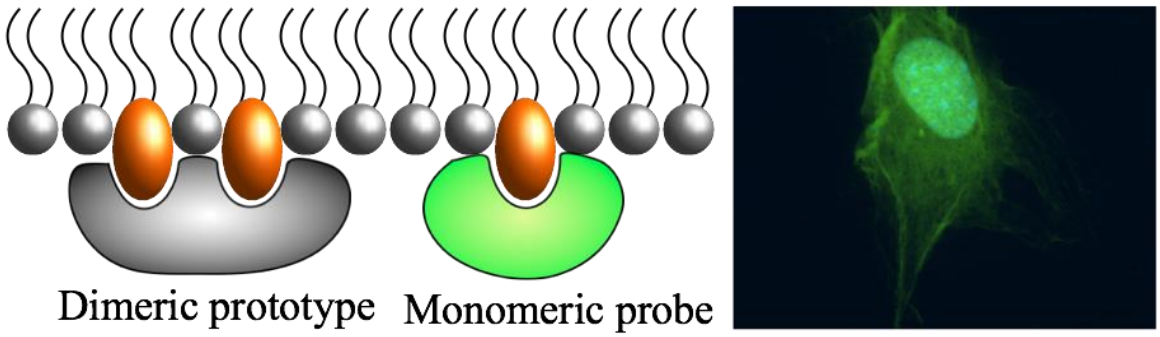

Figure 2. The dimeric prototype was used to design a monomeric PIP2 recognition probe capable of revealing celluar distribution of PIP2 lipids in cells. Left: dimeric versus monomeric recognition of PIP2 (orange). Right: Cellular staining for PIP2 by the monomeric probe (green).

compounds were evaluated with the goal of translating the achievements into a possible diagnostic tool or technique capable of measuring PIP2 levels in biopsies. In collaboration with chemists at Imperial College London chemical compounds were screened in order to identify new lead compounds that could target OCRL itself, which at the time had not any known inhibitors. This particular area of research was important, since the availability of OCRL selective inhibitors would aid enormously future drug discovery programmes. These inhibitors would be able to mimic the disease model in biological systems used to screen for drug efficiency and safety. While we pursued this work new 5-phosphatase inhibitors were discovered, yet these seemed to be chemically very distinct to the ones we discovered. The widening of the

chemical leads for both lipid and enzyme targets has in its own right advanced our knowledge, aiding the advance to the final goal of a chemical drug for future treatment. The data generated by the latest funding from the LST have:

(i) Expanded significantly the chemical molecules capable of targeting inositol lipids, with some of them showing new interesting characteristics and providing essential guidance for further drug development,
(ii) added new chemical structures of potential lead compounds for OCRL selective inhibitors,
(iii) established new lipid extraction methods that could be translated into biopsy sampling.

In summary, the work undertaken since the founding of the LST has had great impact on the wider global research community, as well as directly influencing the research agenda of many relevant research groups through funding project in their respective groups. Our particular aim was to establish a chemical framework to explore possible drug discovery avenues for Low syndrome patients, a task that is normally undertaken by pharmaceutical companies. Here we show that the first steps towards such a goal have been taken successfully, providing hope and purpose for patients and researchers. The LST was and still is an essential catalyst in this effort, prompting awareness and fostering research thrusts in the UK and beyond. We are grateful for the support delivered so far and hope that the current growth in research activity will continue to widen our knowledgebase, which ultimately underpins the hunt for a cure for all Lowe Syndrome patients.

Research paper: American Society for Cell Biology 2015

OCRL1 engages with the F-BAR protein pacsin 2 to promote biogenesis of membrane-trafficking intermediates

https://spiral.imperial.ac.uk:8443/handle/10044/1/39416