Mike Price Fellowship: Sotiris Missaillidis

Sotiris Missaillidis brings us up to date with the work he undertook at the University of Patras with support provided through the Mike Price Fellowship sponsored by EACR and ECCO: The European Cancer Organisation

The Mike Price Fellowship is a unique and prestigious opportunity. It brings together researchers across Europe on novel and interesting projects against cancer that can potentially increase the impact of European cancer research. My project was defined by the selection of aptamers as novel diagnostic and therapeutic modalities against human kallikreins, and in particular human kallikrein 5 and 6 as enzymes of particular relevance to prostate, ovarian and breast cancer, and it was hosted by the University of Patras in Greece. There, I was fortunate to work under the direction of Prof. Sotiropoulou, a leading authority in the field of proteases and kallikreins in particular, and the group that first produced, isolated and published the crystal structure of the enzyme human kallikrein 6. The nature of the project was such that it offered the opportunity to bring together researchers and subsequently groups working on different areas, to work on a common goal. Thus, it allowed my expertise on aptamers, short oligonucleotide with exceptional binding capabilities against a variety of targets, and with broad diagnostic, imaging and therapeutic applications, to be coupled with Prof. Sotiropoulou's expertise on kallikreins, to facilitate the best chances of success of such a project.

Over the duration of the work, KLK5 and KLK6 was produced in pichia pastoris systems, based on previously established methodologies. Briefly, cells were plated and a single colony was subsequently used to grow 10ml of starting culture. These were grown for 24hrs at 30oC before

being transferred to a litter of BMGY growth medium. Cells were grown for a further 2 days and were harvested through centrifugation at 3,000 rpm for 10min. Cells were resuspended in 200ml of BMMY growth medium for the methanol induction of the protein product in the supernatant and cultured for two additional days. Cells were again centrifuged and discarded and the supernatant was found rich in protein product, as judged by gel electrophoresis. Protein was purified following a number of steps, including an initial purification step by hydrophobic interaction chromatography on a Butyl-Toyopearl 650M column matrix, elution of the protein with a decreasing gradient of ammonium sulphate, monitoring by UV and SDS-PAGE, dialysis of the salt in large volumes of 10mM Tris buffer pH 8.0 and a final stage of anion exchange FPLC purification step. Protein expression resulted in 3-4 mg of protein, which would form the basis for the initial aptamer library screening.

Aptamer selection was performed originally in Top yield PCR tubes and subsequently in ELISA plates, according to the following protocol. The target enzyme was immobilised on the plate overnight and was subsequently subjected to the aptamer library, which had been initially PCR amplified in two processes, a double stranded amplification followed by a single stranded amplification. This increased the presence of each individual aptamer species and enhanced selection stringency through competitive binding. Following incubation of the aptamer library with the enzyme for an hour at 37°C, aptamers were subjected to wash steps to remove non-binding species or non-specifically bound species and elution of bound aptamers was achieved using a step gradient from 300mM to 1.5M NaCl. Aptamers eluted from the 1.5M fraction were isolated, desalted and amplified for further selection rounds and ultimately for cloning. Selected aptamer were cloned, identifying positive closes that were subsequently sequenced and used in a variety of assays including fluorescence quenching, ELISA, quartz crystal microbalance and enzyme inhibition assays. Three main aptamer families were identified, bearing significant sequence homology, which also showed good predicted structures with high forming propensity, based on hairpin structures with conserved-sequence loop formations. These aptamers showed high-affinity binding, with affinities in the nanomolar range, and selectivity for their target enzyme, as characterised

View of the bridge from the University of Patras.

by fluorescence quenching and ELISA assays. Enzyme inhibition assays demonstrated the capability of the aptamers to successfully inhibit their cognate enzymes in direct assays against the enzyme in the presence of its substrate, as assessed by changes in absorbance or fluorescence, indicating a potential value as enzyme inhibitors in in vivo studies. Furthermore, immobilised aptamers were able to bind to the enzymes in quartz crystal microbalance and ELISA assays, demonstrating the diagnostic potential of the aptamers in identifying the presence of secreted enzyme. Following small modifications, these aptamers also demonstrated stability in serum and urine, exhibiting potential for in vivo use as inhibitors, or for analysis of biological material in diagnostic assays, in immunohistochemistry or as recognition agents in sensors. Our experiments demonstrated that such aptamers could be potentially valuable as alternative to antibodies against kallikreins, offering greater affinity, specificity and temperature stability, no immunogenicity, and great flexibility in a variety of modifications and uses.

The opportunity provided by the Mike Price fellowship has offered additional opportunities in developing long term links with the University of Patras. This has led in the successful continuation of the project, with members of the Patras group developing further this research into cancer, and a PhD student at the Open University currently looking at the applications of such aptamers both in cancer and in Alzheimer's disease, where the human kallikrein 6 has been particularly implicated. I am grateful to have been given this opportunity and it has been an invaluable boost to my research and career. We now hope to have the opportunity to present these results at the forthcoming EACR conference in Norway, in 2010.

Dr Sotiris Missailidis
Lecturer / EACR Fellow
Department of Chemistry and Analytical Sciences, The Open University, Walton Hall, Milton Keynes, MK7 6AA, UK

The Winner of the 2010 Mike Price Fellowship Award: Gerben Borst

Sponsored by EACR and ECCO: The European Cancer Organisation

Goal of the study:

This in vitro study will study radiosensitization by Chk1 inhibition and explore the possible mechanisms.

A report of the study will appear in the 2011 edition of the EACR Year Book

Gerben Borst
Department of Radiation Oncology,
Antoni van Leeuwenhoek Hospital
Netherlands Cancer Institute (AvL-NKI),
Plesmanlaan 121, 1066 CX Amsterdam,

The research will be conducted at
The Institute of Cancer Research, Royal Cancer Hospital, London.
Section of Cancer Therapeutics (including the Cancer Research UK Centre for Cancer Therapeutics and Section of Cell and Molecular Biology Research)