

Mike Price Fellowship: Sotiris Missailidis

A report from Sotiris Missailidis, winner of the Mike Price Fellowship 2008. His fellowship award supported two visits to Professor Sotiropoulou's laboratories at the University of Patra and focused on the expression of the two target proteins, in Pichia pastoris.

During the first visit in Prof. Sotiropoulou's laboratories at the Department of Pharmacy, University of Patras, facilitated by the support of EACR and ECCO through the 2008 Mike Price Fellowship, I had the opportunity to familiarise myself with the laboratories of Prof. Sotiropoulou and learn some of the basic techniques of protein expression in *Pichia pastoris* expression systems, and the expression of the target proteins in particular.



Sotiris Missailidis

As it was previously agreed between the two labs and approved by the EACR, the fellowship would be taken in two stages. In the first stage, apart from establishing the foundations of this collaboration and planning in detail the various experiments, I focused my work on the expression of the two target proteins, in *Pichia pastoris*. Briefly, cells were plated and a single colony was subsequently used to grow 10ml of starting culture. These were grown for 24hrs at 30°C before being transferred to a liter 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 in our laboratories in the UK, where appropriate setup is available for the selection of aptamers.

It is expected that our screening should result in aptamers with affinity for the target protein by the end of January/beginning of February, which will be used in my main visit to Prof. Sotiropoulou's labs from February onwards, to ascertain the functionality of these aptamers as binders and inhibitors of the two target proteins.

This has been a very interesting experience, which has allowed me to foster new collaborations with members of the Pharmacy Department in Patra, establish the potential for other national and international grant applications and the pursuit of a variety of other

projects for the treatment of cancer.

I am grateful for being given this opportunity to pursue novel areas of cancer research.



The
Mike Price
Fellowship
is Sponsored
by EACR
and ECCO -
The European
CanCer
Organisation