A promising target

Dr Suzanne Peyrottes discusses how her research is fuelling the design of potential therapeutic agents to treat infections and cancers, and she outlines the development of new synthetic methodologies related to nucleic acid components.

Can you introduce the possible correlation between cytosolic 5’-nucleotidase (cN-II) and cancer?

Clinical and preclinical observations have led to the hypothesis that cN-II could constitute a therapeutic target in oncology, either per se or to increase the activity of cytotoxic nucleoside analogues. The expression level of cN-II is of crucial interest for patients treated with nucleoside analogue-based chemotherapy. Indeed, a high level of expression of cN-II mRNA in leukaemic blasts is predictive of a worse outcome in patients who are receiving cytarabine-based regimens – that is, a well-known nucleoside analogue used to treat acute myeloid leukaemia. In addition, the inhibition of cN-II expression by short hairpin RNA has been associated with the induction of apoptosis in human astrocytoma cells, suggesting that cN-II could be a therapeutic target in brain tumours. Finally, recent reports show that in relapsed children with acute lymphoblastic leukaemia, who are treated with the anti-metabolite 6-mercaptopurine, there is a correlation between poor survival rate and expression of an hyperactive mutated cN-II.

What led you to launch your research project on the description and characterisation of cN-II?

In light of the various structural, functional and regulatory properties of cN-II, we embarked on a project devoted to the description and characterisation of this important regulatory protein as a potential therapeutic target.

This has led us to develop different types of inhibitors – which target either catalytic or effector sites on the protein – able to interfere with protein function or regulation.

How did you go about developing your ligand identification approach?

It was developed as a result of three medicinal chemistry approaches: firstly, the rational design and synthesis of substrate analogues – that is, nucleotide analogues, secondly, the virtual screening of freely available chemical databases (approximately 13,500 compounds) and thirdly, nuclear magnetic resonance-based fragment-based drug design (NMR-FBDD) and molecular docking approaches.

Could you briefly outline the methods you used to test the potential inhibitors of cN-II?

All potential inhibitors were tested in vitro using enzymatic assays with recombinant cN-II. Following this initial step, the selected compounds were tested in soaking experiments with crystals of truncated cN-II. They were also evaluated in biological models – including cell culture experiments, on ex vivo and in vivo models – either alone or in combination with cytotoxic nucleoside analogues.

What have been some of your most exciting discoveries?

Thus far, our findings have led us to propose that three families of small molecules are able to inhibit cN-II in vitro and in vivo. For instance, within the ribonucleoside phosphate analogues, the purine-containing analogues were the most potent competitive inhibitors identified in vitro. However, due to their anionic charges, these derivatives have limited cell penetration, and the development of their corresponding prodrugs is ongoing. In addition, using virtual screening we identified that an anthraquinone derivative blocks the enzyme activity. Thus crystallographic data – derived from soaking experiments performed with truncated cN-II and obtained at a resolution of 2.9 Å – indicated interaction with the amino acids situated in one of the effector sites. Interestingly, these compounds exhibited different levels of cytotoxicity in vitro on several cancer cell lines and increased the induction of apoptosis when co-incubated with some cytotoxic nucleosides. Finally, our NMR-FBDD approach led to the discovery of small-size heterocycles – designed for an oral delivery – that can interfere with the cN-II enzymatic function and sensitize the anti-proliferative effects of some cancer drugs using preclinical models.

Collaboration is an essential part of the project. Together, we have developed a tightly integrated consortium that includes a wide variety of different scientists, including synthetic and analytical chemists, enzymologists, structuralists, cancer cell biologists and pharmacologists. This diverse skillset is crucial for informing our aims and objectives and for enabling us to carry out our experiments. It is as a result of this multidisciplinary expertise that we are seeking to drive drug design and development.

In what ways has the project benefited from the respective technical expertise and theoretical knowledge of the research partners involved?

Left: Rebuilt model of the protein, shown as a tetramer and including the natural substrate (IMP), the cofactors and the magnesium ions.

Right: View of one of the substrate analogues belonging to the pyrimidine ribonucleoside phosphate series docked into the catalytic site of the enzyme.
Enzyme inhibition

Operating at the forefront of advances in bioorganic chemistry, scientists in the Nucleosides and Phosphorylated Effectors Team based at the Institute of Biomolecules Max Mousseron in Montpellier, France, are designing innovative therapeutic agents and identifying new targets for chemotherapy.

WITH THE DISCOVERY of new therapeutic targets and agents, recent years have witnessed significant advances in antiviral and anticancer treatments. For instance, compounds known as nucleoside and nucleotide analogues are widely used as therapeutics today because of their ability to mimic physiological metabolites and interfere with the processes implicated in viral particle production or the proliferation of cancer cells. However, in order to be effective, the phosphorylated derivatives – namely, the activated forms – of these analogues must compete with endogenous nucleotides. Surprisingly, despite the intensive studies performed on nucleoside transport and nucleotide metabolism, relatively little is known about the regulation of nucleotide pools in mammalian cells and its possible implication in drug resistance.

One group of researchers seeking to address this knowledge gap is based in the Nucleosides and Phosphorylated Effectors lab at the University of Montpellier in France. Headed by Dr Suzanne Peyrottes, this team – in collaboration with several partners – is building on previous studies that have examined the synthesis of nucleic acid components and their structurally related derivatives. Using an integrated approach that blends organic chemistry, biochemistry, structural biology, cellular pharmacology and biological evaluations, the researchers are drawing on a range of cutting-edge tools and techniques to make new insights into drug agents and targets. Funded by the French National Research Agency, Peyrottes’ current project is specifically focusing on exploring the potential of cytosolic S’-nucleotidase II (cN-II) as an innovative new drug target.

A KEY ENZYME
cN-II is a member of a family of enzymes that plays a role in catalysing the hydrolysis of deoxyribo- and ribo-nucleoside S’-monophosphates into corresponding nucleosides and inorganic phosphate. Additionally, this family of enzymes is thought to affect the phosphorylation level and pharmacological activity of nucleoside analogue-based drugs. Yet the researchers’ specific interest in cN-II arose from the observation that high levels of expression of cN-II mRNA in blasts correlates with worse outcomes among patients treated with cytarabine-based chemotherapy – a common treatment for individuals with acute myeloid leukaemia.

The researchers are drawing on a range of cutting-edge tools and techniques in order to make new insights into drug agents and targets.

A significant body of evidence demonstrates that cN-II interferes with anticancer treatment and is instrumental in dictating the results of the final outcome for patients. Indeed, some studies even suggest that cN-II triggers the inactivation of nucleoside analogue-based chemotherapy, thus leading to drug resistance. In response, Peyrottes’ team and her partners are scrutinising the resistance mechanisms related to cN-II and cytotoxic nucleoside analogues with the long-term goal of improving the efficacy of these derivatives in cancer therapy. The hope is that cN-II will prove to be a viable target for the development of different types of inhibitors. “Altering the enzymatic function of cN-II has an indirect effect on nucleotide pools and is supposed to enhance the therapeutic efficiency of cytotoxic nucleosides,” she elucidates.

CHARTING THE JOURNEY

At the start of their project, Peyrottes and her colleagues conducted a study on the
A KEY ENZYME IN NUCLEIC ACID BIOSYNTHESIS: 5'-CYTOSOLIC NUCLEOTIDASE (CN-ll) AS A NEW DRUG TARGET

OBJECTIVE
To design potential therapeutic agents to treat cancer and infection with a focus on cytosolic 5'-nucleotidase II.

KEY COLLABORATORS
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DR SUZANNE PERYOTTES graduated in Chemistry from Montpellier University in France and completed her postdoctoral study at the Medical Research Council, UK. She occupies a position at the National Centre of Science Research, France, working on the design of potential therapeutic agents to treat infections and cancers, as well as new synthetic methodologies related to nucleic acid components.

structure-activity relationship of pyrimidine-containing derivatives. This was an important initial step because the actions of cN-II as a resistance mechanism were first observed in patients receiving cytarabine-based courses of treatment – that is, nucleoside analogues that contain pyrimidine as a nucleobase.

Following this, the researchers then analysed the structure-activity relationship of purine-containing derivatives – a similarly important step as cN-II plays a crucial role in purine cellular metabolism. This enabled them to investigate chemical diversity based on the ribonucleoside phosphate skeleton: “Using these sets of compounds, we were able to characterise the first competitive inhibitor, which was the starting point for further structural optimisation,” Peyrottes explains. “Excitingly, by improving our synthetic approaches, we have eventually succeeded in obtaining purine-containing analogues – and identified one compound, which to date, is the most potent cN-II inhibitor.”

Moreover, a further aspect of their project involved screening ribonucleoside phosphonates in silico in order to explore their function as potential cN-II inhibitors. Here, the team prepared a library of non-hydrolysable substrate analogues and predicted their theoretical binding affinities through molecular docking. After this, the researchers ranked the compounds according to their respective docking scores and selected the most promising candidates for synthesis. To test their compounds, they used two different assays: a colorimetric assay based on the dosage of inorganic phosphate produced as a result of the enzymatic reaction and a kinetic assay that allowed them to characterise the inhibition mode and constant.

FUTURE IMPLICATIONS
Ultimately, Peyrottes’ project is furthering basic scientific knowledge about the regulation of nucleotide pools in mammalian cells and helping to pinpoint compounds with therapeutic potential. To date, its results demonstrate that the inhibition of cN-II in human cancer cells has the capacity to either directly induce the death of harmful cells or sensitize these cells to the activity of nucleoside analogues. With the pivotal role of nucleoside analogues in the treatment of deadly haematological diseases, an enzyme inhibitor that restores and improves their function has enormous social and economic implications that benefit both patients and health systems.

FINDING SOLUTIONS

In addition to her current project on the identification of inhibitors to cN-II, Peyrottes is also active in the design of therapeutic agents to treat infectious diseases and the development of antimalarial drugs. As the following case studies demonstrate, she is deeply committed to pursuing solutions to a number of pressing challenges in biomedical research.

CASE STUDY 1
The issue: Although nucleotides play a pivotal role in multiple biological processes and their corresponding phosphorylated analogues are highly useful biological tools, their synthesis in classical solution-furnished complicated crude mixtures requires drawn-out purification methods, accordingly leading to decreased yields.

The response: Peyrottes and her colleagues are attempting to develop innovative methodologies for the supported synthesis of nucleoside phosphorylated forms. They are currently exploring the use of polyethylene glycol as a means of soluble support.

CASE STUDY 2
The issue: The mechanisms of phosphoantigen recognition and activation by a non-conventional T-cell subset (namely, γδ T-cells) are not clearly understood.

The response: Peyrottes’ team is using the supported chemistry methodology to obtain nucleotide analogues to investigate these mechanisms at the molecular and cellular levels – using the knowledge they generate to analyse the potential of these lymphocytes in the context of the immune response to viral infections.

CASE STUDY 3
The issue: There is a need for targeted delivery of nanoprobe that can be used in nanomedicine techniques.

The response: The researchers in Peyrottes’ lab are developing different types of targeting ligands for use in functionalised nanopores, thus pushing the boundaries of nanobiomedicine.