Medulloblastoma remains the most common form of brain tumour in children. What are some of the problems with current treatment approaches?

These include surgery, radiation and chemotherapy, and have achieved five-year survival rates of 70-80 per cent in patients with standard-risk disease. Despite recent advances in imaging and neurosurgery, complete resection is not usually feasible due to the location and diffuse nature of the tumour. In addition, a common feature of medulloblastoma is its propensity to metastasise, with approximately 30 per cent of children demonstrating cerebrospinal fluid metastasis at diagnosis. Therefore, the incorporation of radiation and cytotoxic chemotherapy is integral to achieve good clinical outcomes.

Although this approach has improved survival rates, the high doses of cytotoxic chemotherapy required often give rise to side effects including cognitive impairment, deafness, psychiatric disorders and growth retardation, which compromise quality of life from an early age. Furthermore, there is a subset of high-risk patients who have an extremely poor prognosis. Therefore, the identification of synergistic drug combinations to allow dose reductions and reduce adverse effects, while maintaining clinical efficacy, would be an attractive approach for patients with medulloblastoma, and may improve outcomes in high-risk patients.

What role does Wee1 kinase play in targeting medulloblastoma? Has your team developed inhibitors of the kinase?

Many conventional anticancer treatments damage DNA as part of their mechanism of action. When DNA is damaged, cells can arrest the cell cycle temporarily to allow DNA to be repaired. This cell cycle checkpoint can protect normal cells from damage, but reduces the effectiveness of chemotherapy on tumour cells. One approach to circumvent this drug resistance mechanism is to worsen the dose of chemotherapy, but this can increase the severity of side effects. An alternative approach is to selectively target key proteins involved in the checkpoint mechanism in tumour cells, which may improve the efficacy of DNA-damaging agents.

Wee1 phosphorylates and inactivates cyclin-dependent kinase, which is involved in G2-M checkpoint regulation. Since the tumour suppressor p53 is a key regulator in the G1 checkpoint, p53-deficient tumours rely only on the G2 checkpoint after DNA damage. Although Wee1 inhibition compromises both p53 wild-type and p53-deficient tumours, the sensitivity of p53-deficient tumours to DNA-damaging agents should be enhanced by Wee1 inhibition.

One goal of your research is to create an inhibitor that targets Wee1, while mitigating cellular toxicity. What progress have you made so far?

We have designed and chemically synthesised five novel nanomolar inhibitors of Wee1 and taken them forward for evaluation in cell-based systems. Their effect on cell viability has been examined using the MTS assay and xCELLigence system for label-free and real-time monitoring. These assays have demonstrated that the commercially available Wee1 inhibitor MK1775 as a single agent is more toxic than our Wee1 inhibitors. In addition, some of our Wee1 inhibitors act synergistically with cisplatin at lower concentrations than MK1775. We are currently repeating these assays using a panel of medulloblastoma cells including p53 wild-type and deficient, and high and low Myc-expressing, cell lines.

Will you be looking to apply your research findings to other diseases?

We have already started to work with other groups to assess Wee1 inhibition as a chemosensitising strategy for other cancer types. We have published our research examining the effect of Wee1 inhibition in acute myelogenous leukaemia (AML) with Dr Chris Porter at the Department of Pediatrics, University of Colorado. In these studies, we found synergistic inhibition of cell proliferation with the Wee1 inhibitor MK1775 and the antimetabolite cytarabine in AML cell lines, independent of p53 functionality.
From chemist to clinic

Medulloblastoma is a highly malignant brain tumour that affects children 10 times more than adults. A cross-disciplinary team from the University of Colorado is developing novel treatments that improve survival rates while minimising side effects.

MEDULLOBLASTOMA IS THE most common primary brain tumour in children, with an estimated 540 new cases every year in the US alone. Current treatment options include surgical resection, when feasible to remove the bulk of the tumour, followed by radiation (in children greater than three years old) and chemotherapy. While this approach has improved survival rates, drug resistance is common and high doses of cytotoxic chemotherapy are given in an attempt to circumvent resistance mechanisms. Although this approach is often successful at eradicating the tumour, it can result in lifelong problems in growth, deafness and, paradoxically, can even result in secondary tumours. Moreover, while standard-risk patients have almost 80 per cent survival rates, a subset of high-risk patients with an amplification in a gene called Myc still have an extremely poor prognosis, with over 70 per cent dying within five years of diagnosis.

Dr Philip Reigan, a chemist at the University of Colorado Skaggs School of Pharmacy and Pharmaceutical Sciences, has recognised these flaws in treatment and is working to develop a new and improved standard. He aims to advance the specificity and efficacy of chemotherapy such that doses can be reduced, along with the associated side effects. In addition, he hopes that a better molecular level understanding of medulloblastoma could facilitate more targeted therapeutics, improving outcomes for those unfortunate high-risk patients.

CRITICAL COLLABORATION

Critical to Reigan’s work are close links with clinicians, who have first-hand experience of medulloblastoma. One particularly important collaborator is Dr Rajeev Vibhakar at the Department of Pediatrics at the University. When they first started working together, Vibhakar was looking to understand the pathogenesis of medulloblastoma, searching for factors critical to its development. An integrated genomic analysis of medulloblastoma cells and tissue conducted by Vibhakar’s group using pathway analysis of gene expression revealed Wee1 kinase as a potential molecular target.

Wee1 is an important kinase in the cell cycle. Cancer cells often have changes in the pathways that regulate this cycle and DNA damage repair, and many anti-tumour agents (such as cisplatin) work by damaging DNA in cancer cells. However, this causes the cell cycle to stop in order for DNA to be repaired. This mechanism helps protect healthy cells, but reduces the efficacy of chemotherapies in the context of cancer.

Wee1 is an inhibitory regulator of the G2-M checkpoint that enables entry into mitosis (cell division). In other words, Wee1 prevents cell division in the presence of DNA damage, thus contributing to drug resistance. Inhibiting Wee1, thereby permitting entry into mitosis, might allow chemotherapy agents that cause DNA damage to become more active.

A high-throughput screen (HTS) of a chemical compound library identified MK1775 (Merck Research Laboratories) as a potent small-molecule inhibitor of the Wee-1 kinase. “Dr Vibhakar initially approached me to synthesise MK1775 as he wanted to examine its effect in combination with cisplatin, a common cytotoxic chemotherapeutic agent used to treat medulloblastoma,” Reigan explains. Reigan’s group did synthesise the compound, allowing Vibhakar’s group to study its effect alone, and in combination with cisplatin, in medulloblastoma cell-based systems. Vibhakar’s group found that MK1775 did in fact sensitise medulloblastoma cells to cisplatin, working in synergy to reduce medulloblastoma cell proliferation.

TOXIC HURDLE

However, MK1775 was not designed to target Wee1 and is known to target other kinases in the nanomolar range. Therefore, Reigan’s group set out to synthesise new inhibitors that were more selective for Wee1, beginning by determining the structural requirements for the binding of a small molecule to Wee1.

Reigan’s laboratory used the crystal structure of Wee1, and docked MK1775 to its ATP binding site using computational-based molecular modelling to examine its binding interactions. Based on this structural insight, they were able to identify important interactions in the binding site and design a series of inhibitors exhibiting optimised interactions with the binding cavity. These inhibitors were chemically synthesised and evaluated alongside MK1775 in a TR-FRET assay established at the HTS Facility at the Skaggs School of Pharmacy using recombinant Wee1 protein to test the activity of the compounds.

A LESS TOXIC ALTERNATIVE

The results of these evaluations demonstrated that five of the 16 compounds the Reigan group had synthesised had a potency in the same range as MK1775. On further investigation, they found that two of their Wee1 inhibitors were actually less toxic than MK1775. This was exactly what they were looking for – compounds that would potently inhibit Wee1, sensitising the tumour to chemotherapy, without adding to the existing toxicity of treatment. Combining their inhibitors with cisplatin in medulloblastoma cells, the team found that their compounds had synergy with cisplatin at concentrations lower than those observed with MK1775.

Based on these extremely promising findings, Reigan now plans to transition these two compounds into in vivo model systems. Using animal models, he aims to determine the tissue distribution and pharmacokinetics of the two inhibitors compared with MK1775, as well as their impact on tumour growth alone and combined with cisplatin.

A TALE OF INGENUITY

Recognising the common lifelong problems associated with medulloblastoma treatment, Reigan and Vibhakar’s teams identified a target – Wee1 – and developed novel small molecule
compounds to inhibit its activity. Despite the challenges along the way, they have developed two encouraging small molecule inhibitors of Wee1, currently being extensively evaluated in cell-based systems. Upcoming in vivo studies will confirm their use as chemosensitisers for cisplatin, paving the way for human clinical trials.

Using expertise from both ends of the drug development spectrum, Reigan looks set to successfully translate these findings to the bedside. The clinical potential is vast, chemosensitising medulloblastoma will enable a lower dose of cytotoxic chemotherapy, reducing side effects while maintaining – and perhaps even increasing – clinical efficacy. This work could also extend to other cancers where cisplatin or other DNA damaging agents are used as the standard treatment. In fact, the team is already exploring leukaemia and other childhood cancers where this strategy could be effective.