Treating tumours by stealth

Most targeted treatments for cancer seek to disrupt tumour growth signalling. Dr Lali Medina-Kauwe and her team have developed an approach that targets, infiltrates and then kills tumour cells directly.

How have you modified synthetic penton base proteins to specifically home in on and penetrate certain tumour cells?

I have used genetic engineering to add an ‘address’ to the penton base protein by appending a specific tumour-recognising peptide sequence to the protein, thus directing the penton base to tumour cells that match that address. The peptide recognises specific features on the surface of certain tumour cells and fits these features like a key fits a lock. This lock and key interaction opens a ‘porthole’ in the cell, thus allowing the protein and any attached cargo to enter the cell.

Does your method of systemically delivering the anti-cancer chemotherapy drug doxorubicin to tumours improve on other targeted approaches?

Most tumour-targeting approaches, especially those currently used in the clinic, use antibodies or small molecules to inhibit signalling inside tumour cells that otherwise instruct the cells to proliferate. A longstanding problem with this approach is that the majority of tumours acquire the means to ignore or overcome signal inhibition. My approach presents an improvement in that the protein I have developed, HerPBK10, circumvents the need to modulate signalling: instead, it directly delivers a toxic molecule into the interior of the tumour cell.

What is the function of corrole molecules and how do they aid tumour detection and treatment?

Corroles are synthetic compounds that structurally mimic naturally-occurring chemicals in the body called porphyrins. A main structural feature of corroles is a chemical ring, the middle of which can be bound to a metal ion. Depending on the metal ion, corroles can possess different features such as fluorescence, toxicity, or antioxidant activity.

What are the therapeutic differences between HerDox, HerGa, HerSi and HerMn?

HerDox delivers the approved and commonly used chemotherapy agent doxorubicin. While its tumour toxicity is highly effective, doxorubicin also has adverse effects on non-tumour tissue, including the heart, if it is not targeted to tumour cells. The HerGa and HerMn particles deliver gallium and manganese corroles, respectively. Both particles are toxic to tumours. HerGa enables imaging via fluorescence, HerMn via magnetic resonance imaging (MRI). The HerSi particle delivers nucleic acid molecules called siRNA. These small nucleic acids can silence certain genes, depending on the nucleotide sequence of the molecule.

HerGa can emit an intense red fluorescence to track tumour targeting while selectively killing HER2+ tumours. What are the benefits of embodying tumour imaging and tumour killing in a single nanoparticle?

Currently, tumour imaging is a separate clinical procedure from treatment. We envisage being able to kill tumours with the agent that is used to detect them, while they are being detected. Using HerGa fluorescence for tumour detection could allow visualisation of the tumour to facilitate its surgical removal. Additionally, light-stimulation of HerGa augments its toxicity, thus enabling one to use a light source to kill off residual tumour cells containing HerGa. HerMn takes this concept a step further because it can be detected by MRI, which is a better imaging method than fluorescence imaging for the detection of deep tumours.

Certain cancer cells have become resistant to Herceptin®, is your ultimate aim to find a replacement therapy?

Herceptin® is an antibody that specifically binds HER2 to inhibit signalling. Recent studies show that elevation of HER2, which confers unregulated signalling for the tumour cell to proliferate uncontrollably, can occur through elevation of HER3. As HerPBK10 specifically binds HER3 to enter cells, Herceptin®-resistant cells make themselves targets for HerPBK10-mediated therapy. Additionally, as mentioned earlier, HerPBK10-mediated penetration into target cells allows delivery of a toxic molecule and killing of the tumour cell from within, rather than modulating signalling to which the cell is already non-responsive.

Have you made any significant discoveries recently?

Yes. One of our most significant discoveries is the finding that tumours that have become Herceptin®-resistant are targets for HerPBK10-mediated therapy through the elevation of HER3. Even more exciting is our recent observation showing that HerPBK10-targeted treatment indeed kills off Herceptin®-resistant tumour cells in culture.

What are the next steps for your research?

Our next steps are to test our therapeutics in animal models of drug resistance, as well as testing targeted treatment to drug-resistant tumour cells obtained from patients, with the goal of moving into clinical trials in the near future. Additionally, we are creating new versions of HerPBK10 that are targeted to other types of tumours.
Smart anti-cancer strategies

Melding nanotechnology, genetic engineering and biomedical methods, the Cedars-Sinai Medical Center at the University of California, Los Angeles has developed an original means of introducing medication, imaging aids and gene modification to tumourous cells creating novel technologies that can improve medicine. Conceptualising an idea and then realising this idea into a feasible device is the most exciting aspect of my work," affirms Medina-Kauwe.

THE RESPIRATORY VIRUS known as adenovirus consists of strands of deoxyribonucleic acid (DNA) enclosed by a shell, or capsid, formed by three types of protein. The capsid gives the virus its shape and protects it; some viruses are further protected by a lipid bilayer envelope. One of the types of proteins that forms the capsid is the penton base: sited at each vertex on the capsid surface, it mediates the early processes of viral infection by penetrating the cell membrane and aiding the transfer of the viral genome to the inside of the target cell.

The Human Epidermal Growth Factor Receptor (HER) comprises two proteins, HER2 and HER3, found on the surface of tumour cells. In the establishment and progression of a more aggressive and treatment-resistant form of breast cancer, termed HER2-positive breast cancer, HER2 has been established as an important biomarker and is the target of the most-used treatment for the disease, a monoclonal antibody marketed as Herceptin®. Herceptin® suppresses the signalling mechanisms of HER2, but HER3 has been found to aid and abet HER2 signalling despite this, enabling tumour cells to proliferate. In addition, HER2 signal-blocking antibodies bind to tissue with normal HER2 levels, especially in the heart, resulting in cardiac damage due to the inhibition of heart maintenance signals. Raised HER2 expression is also found in ovarian, prostate, brain and colon tumours.

Another drug used in treating cancer is doxorubicin. Delivered intravenously, it is unselective, being toxic to tumorous and healthy cells alike. It is particularly known to damage heart cells and strategies to limit such damage have succeeded only to an extent.

Developed by Dr Lali K Medina-Kauwe’s laboratory in the Department of Biomedical Sciences at the Cedars-Sinai Medical Center, an ingenious and innovative approach to management of HER2 cancers features a viral capsid replica that constitutes a delivery mechanism for a range of substances to identify and treat tumour cells: "The main driver for my research is innovation: creating novel technologies that can improve medicine. Conceptualising an idea and then realising this idea into a feasible device is the most exciting aspect of my work," affirms Medina-Kauwe.

THE MEDINA-KAUWE LABORATORY

The Medina-Lauwe laboratory selected the capsid of adenovirus, the largest known non-enveloped virus, as the basis for the new delivery mechanism: "Targeting drugs to tumour cells requires that the drug be transported by a carrier molecule that delivers the drug into the cell," asserts Medina-Kauwe. "By studying how viruses and other pathogens accomplish the transfer of pathogenic substances, improvements can be made to make drug delivery more efficient and enhance therapeutic efficacy and safety."

TROJAN HORSE

Amongst her innovations, Medina-Kauwe has genetically engineered a Trojan Horse, a
nanoparticle replica of the adenovirus capsid, to introduce substances into tumour cells, mimicking the pattern of early viral infection and exploiting the role of the penton base. Her synthetic penton base proteins can self-assemble with nucleic acid and she has modified them so that they selectively penetrate tumour cells. One synthetic protein, HerPBK10, is designed to specifically single out and penetrate HER2-positive tumour cells. HerPBK10 achieves this via a receptor-ligand interaction at the cell surface upon cell binding. The interaction triggers a cellular response to consume the protein, thus providing access to the cell interior and penetration via the penton base. “The ligand, a peptide derived from a protein called heregulin, recognises and binds to a specific region of the HER. It is important to note that this interaction differs from that of clinically-used antibodies,” states Medina-Kauwe. “The ligand triggers its own rapid entry into the tumour cell upon receptor binding, whereas antibodies do not.”

**HERDOX: TARGETED CHEMOTHERAPY**

In the form of a nanoparticle called HerDOX, Medina-Kauwe’s laboratory has exploited the targeting and unlocking ability of the HerPBK10 vehicle to deliver doxorubicin to tumours. In studies with mice, intravenous delivery of HerDOx killed HER2-positive tumours at less than a tenth of the normally-required dose, without negative effects on the heart. HerDOx releases the drug after HerPBK10 entry into tumour cells, in much the same way that viruses deliver and release their DNA: “When fully assembled, HerDOx resembles a spherical or virus-like particle that protects the drug inside the sphere during its transit to the cell target. The penton base facilitates this assembly. The mechanism of drug release after delivery into the cell is not entirely clear – it is possible that it occurs similarly to how viruses disassemble after cell entry to release their cargo only once they are inside,” explains Medina-Kauwe.

**HERMN AND HERGA: TUMOUR IMAGING AND KILLING**

In collaboration with chemists, Drs Harry Gray and Zeev Gross, and imaging expert, Dr Daniel Farkas, Medina-Kauwe has demonstrated that HerPBK10 can be combined with corrole molecules, forming nanoparticles capable of simultaneous tumour detection and treatment.

Detecting cancerous tumours that are deeply embedded is only feasible with magnetic resonance imaging (MRI). The Medina-Kauwe Laboratory attached a corrole containing manganese to the HerPBK10 delivery vehicle to facilitate imaging by MRI – the resulting particle, HerMn, renders contrast for MRI while being toxic to HER2-positive tumour cells.

By attaching a water-soluble corrole containing a gallium metal ion to HerPBK10, the Medina-Kauwe lab also created HerGa, which emits intense red fluorescence that allows tumour targeting to be tracked and also selectively kills HER2-positive tumour cells. Tumour cell killing by HerGa is further augmented by irradiation with light, as this excites the corrole molecule and releases free radicals that damage the target cell. The most recent studies in the Medina-Kauwe lab have also indicated that HerGa can kill tumour cells that have become resistant to Herceptin®, so holding promise for eradicating drug-resistant HER2-positive tumours.

**HERSI: TARGETED GENE EXPRESSION AND SILENCING**

Additionally, Medina-Kauwe has tested HerPBK10 as a means of delivering therapeutic nucleic acids to HER2-positive tumour cells. SiRNA molecules that can encode toxic products and small interfering RNA (siRNA) to silence cancer growth genes.

Gene delivery requires entry into the cell nucleus, thus necessitating the capability to penetrate several barriers within the tumour cell. To achieve this, Medina-Kauwe created a particle named H2PO, by attaching condensed DNA to HerPBK10, for delivering genes that encode toxic products.

For gene silencing, the Medina-Kauwe Laboratory created the HerSi particle by attaching siRNA to HerPBK10. HerSi can also bring about cell death by delivering modified siRNA molecules that stimulate immune-mediated toxicity against target tumour cells. It therefore allows the flexibility of strategically silencing certain pro-survival genes in tumour growth and drug resistance. Furthermore, the HerSi system has substantial potential for use in other applications: “As delivery is not dependent on the nucleotide sequence of the siRNA, this system is versatile in that there are nearly endless possibilities of combining different siRNA molecules with HerPBK10,” enthuses Medina-Kauwe.

There is a potential concern that the use of a protein derived from a virus might bring about unwanted effects in the treatment of cancer, but Medina-Kauwe is reassured that therapeutic levels of HerPBK10 have not evoked any immune reaction in mice. Importantly, HerDOx, HerGa, HerMn, HerSi and H2PO can all be formed by self assembly through the same natural, spontaneous intermolecular interactions that govern virus particle assembly, thus conserving drug potency during assembly, transport and release into target cells.

**INTELLIGENCE**

**MEDINA-KAUWE LAB**

**OBJECTIVES**

Examining mechanisms of tumour targeting and tumour cell penetration, and using this information to innovate improved cancer interventions are the key objectives of the Medina-Kauwe research group. Such information is being obtained from studying how certain pathogens invade cells and applying this to drug delivery.

**KEY COLLABORATORS**

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**DR. L. MEDINA-KAUWE** earned her doctorate in molecular biology from the University of California, Los Angeles (UCLA), studying disease-causing mutations in the neurotransmitter regulatory enzyme, GABA transaminase. These studies inspired her interest as a postdoc in targeting gene therapy at the University of Southern California Keck School of Medicine, leading to the development of drug targeting nanotechnologies. Dr Medina-Kauwe is now an Associate Professor at Cedars-Sinai Medical Center and the UCLA Geffen School of Medicine. She has been an invited speaker to several renowned research institutions as well as national and international conferences on gene and drug delivery, and therapeutics development.