New avenues in cancer research

Dr Gang Zheng is working tirelessly to synthesise novel nanoparticles which could open up new possibilities in the fight against cancer. Here, he talks in detail about his current project

DR GANG ZHENG

The main objective of your research is to develop clinically translatable technology platforms to combat cancer. Why have you chosen to focus this project on interventions for vascular and lung diseases?

Image-guided interventions are already standard practice in both the catheter-based treatment of vascular occlusive disease and in early-stage lung cancer biopsy procedures. Developing minimally invasive techniques to assess the status of peripheral lung cancer lesions or vulnerable atherosclerosis plaque *in situ*, and enable the destruction of these lesions on the spot, will fulfil critical clinical needs. Clinically translatable nanotechnology, with its powerful capability to combine imaging, drug delivery and *in situ* therapeutic delivery, could have high potential to facilitate timely clinical interventions to mitigate patient risk and disease progression, with fewer side effects than conventional approaches.

What is particularly noteworthy about this research project?

This project is particularly exciting not only because of the potential enabling role of nanotechnology, but because of our unique window of opportunity to transfer nanoparticle-based technologies into clinical trials through NanoMedFab – the Canada Foundation for Innovation-supported first Good Manufacturing Practice facility in Canada for clinical nanoparticle fabrication. We have established our multidisciplinary programme with the support of Canadian Institutes of Health Research (CIHR), and as part of the process we are strategically integrating the expertise of leaders in the fields of nanomaterials, bioconjugation chemistry, bioimaging science, biomedical engineering and clinical trials for cardiac and lung applications.

Could you provide an overview of your recent discovery of porphysomes, the first all-organic nanoparticle with intrinsically multimodal biophotonic properties?

Porphyrins are the endogenous chromophores of nature, such as hemes in red blood cells and chlorophylls in green plants. Porphyrins and porphyrin-like molecules are well known photosensitisers for photodynamic therapy and fluorescence imaging. During the course of examining porphyrin self-quenching in liposomes to explore their potential use as activatable photosensitisers, we discovered 'porphysomes', the first all-organic nanoparticles with intrinsic multimodal photonic properties. These are self-assembled from porphyrin-lipid building blocks to form liposome-like bilayer vesicles. The very high porphyrin packing density results in both 'super'-absorption and structure-dependent 'super'-quenching, which in turn converts light energy to heat with extremely high efficiency, giving them ideal photothermal and photoacoustic properties that are unprecedented for organic nanoparticles.

How might porphysomes be applied to improve fluorescence imaging and drug delivery?

Upon porphysome nanostructure dissociation, fluorescence of free porphyrins is restored to enable low background fluorescence imaging. As a result of their organic nature, we found porphysomes to be biodegradable *in vivo*; they also induced minimal acute toxicity in mice with high intravenous doses. In a similar manner to liposomes, porphysomes can be easily scaled up via commercial extrusion techniques, and the large aqueous core of porphysomes could be passively or actively loaded with drugs, opening up a new avenue for image-guided drug delivery.

By changing the way porphyrin-lipid assembles, we developed ultra-small porphyrin nanodiscs, large porphyrin shell microbubbles and giant porphyrin vesicles, expanding the purview of porphyrin nanophotonics.

How does the simple yet intrinsic multimodal nature of porphysomes represent a novel principle for designing multifunctional nanoparticles?

Traditional 'all-in-one' nanoparticles contain many functional modules, such as layer-by-layer fabrication or stacking multiple building blocks. However, the simple yet 'one-for-all' nature of porphysomes – which are self-assembled from a single, low molecular weight building block but are inherently multimodal – represents a novel approach to the design of multifunctional nanoparticles and confers high potential for clinical translation.

As porphyrins are natural high-affinity metal chelators, your research group has employed porphysomes as positron emission tomography (PET) and magnetic resonance imaging (MRI) probes. How does porphysome nanotechnology efficiently ablate tumours?

One unique feature of porphysomes is that metal ions, such as radioactive copper-64, can be directly incorporated into the porphyrin building blocks of the preformed porphysomes. This enables faithful and quantitative tracking of the fate of porphysome *in vivo*. Porphysome efficiently ablates tumours by reaching thermal ablation temperatures of over 55°C in order to induce tumour necrosis.

Rat and mice models were chosen to assess the efficacy of porphysomes for cancer treatment. What conclusions were you able to draw from your trials?

We have performed porphysome efficacy studies on both rodent and rabbit models, and these experiments demonstrated that porphysomes are fast, safe and effective in ablating tumours. In order to take our work forward, we now need to complete the clinical grade porphysome production and preclinical toxicity studies. This will enable us to perform first-in-human studies.

Developing clinically transferrable **nanotechnology**

Cutting-edge research at the **Princess Margaret Cancer Centre** and **Techna Institute** in Toronto, Canada, is generating a range of technology platforms that could improve the diagnosis and treatment of cancer

DUE TO THE complex and aggressive nature of many forms of cancer, there is often a significant number of obstacles to negotiate during the course of their diagnosis, demarcation and treatment. Ongoing research around the world is attempting to tackle these problems in a variety of ways in the hope that prognoses for the millions of individuals suffering from cancer can be improved.

Pioneering research being carried out at the Princess Margaret Cancer Centre is at the forefront of this work. Led by Senior Scientist Dr Gang Zheng, researchers at the Princess Margaret have, within the last few years, succeeded in synthesising a novel nanoparticle that combines a number of highly desirable properties. Known as porphysomes, the non-toxic nanoparticles are capable of serving a number of therapeutic and imaging roles. As such, there is a great deal of optimism that Zheng's work could unlock new doors in the battle against multiple forms of cancer.

COMBATING PROSTATE CANCER

Since their inception, porphysomes have been established as a multifunctional agent with capabilities for combined imaging, drug delivery and *in situ* therapeutic delivery. They have also proven to be innocuous due to their organic nature. It has recently emerged that they could play a key role in targeting and identifying prostate cancer.

At present, prostatectomy is the main course of treatment for the disease, after watchful waiting and active surveillance through biopsies and tests. Considered to be a radical procedure, prostatectomies involve the removal of the entire prostate gland which, whilst stopping the cancer from spreading, can result in a number of undesirable risks and side-effects. Subsequently, focal therapy is considered a preferable method for treating prostate cancer and involves the ablation of only the cancerous region. In many cases, however, accurately demarcating the tumour in order to successfully perform focal treatment has until now proved difficult.

With this in mind, Zheng and his colleagues at the Princess Margaret Cancer Centre pursued the possibility of using porphysomes and positron emission tomography (PET) for the real-time delineation of orthotopic prostate tumours and micrometastases as small as 2 mm. "PET allows for real-time delineation, with porphysome-enabled PET imaging facilitating more precise thermal focal ablation of low and intermediate risk prostate cancers," Zheng enthuses. The technique has the potential to enable doctors to pinpoint and characterise prostate tumours in a non-invasive way, and to accurately locate the edges of the tumour. Additionally, the innovative porphysomes are capable of identifying cancerous cells that have migrated into the bone, which could help health professionals to more effectively treat aggressive cancers that have spread beyond the prostate. "However, whilst focal therapy is an alternative to watchful waiting or radical therapies, we have to be absolutely sure it is safe and effective for it to become a truly viable clinical modality, and we are not at this stage yet," Zheng warns.

MULTIFUNCTIONAL NANOCARRIERS

Alongside the ongoing research into porphysomes, Zheng and his team have also been striving to develop a novel class of biocompatible, biodegradable and multifunctional nanoparticles similar to lipoproteins. The studies into the nanocarriers have been underway for around a decade and Zheng identifies a number of promising properties displayed by the nanoparticles as being the key behind the longevity of the

ZHENG LAB

OBJECTIVES

To develop clinically-translatable technology platforms to combat cancer. The lab is investigating porphysome nanotechnology, lipoprotein-like nanoparticles and photodynamic molecular beacons. The ultimate goal is to engineer experimental and intelligent molecules that hopefully will translate into clinical benefits in the future.

KEY COLLABORATORS

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research. "Their natural biocompatibility, biodegradability and non-immunogenic properties set them apart," he elucidates, "as well as their long circulation half-life without the use of pegylation, and their ability to load varying types of imaging and therapeutic agents into different compartments of the particles."

In 2009, Zheng and his colleagues published the first ever paper on the high-density lipoprotein-like peptide-phospholipid scaffold particles which are able to deliver therapeutic molecules such as small interfering ribonucleic acids (siRNAs) to the cytosol of cancer cells, avoiding the common endosomal trapping for cancer therapeutics. "Interaction between the self-assembled network and the lipid monolayer, provides the desired structural control over nanoparticle size, monodispersity and stability, as well as its functional control over its direct cytosolic delivery mechanism," Zheng adds.

Following the considerable success of these studies, an Ontario-based start-up company called DLVR Therapeutics was launched in order to make this novel nanotechnology commercially available. Zheng and his collaborators are hopeful that, in light of DVLR Therapeutics' establishment, the nanocarriers they have developed will have the best possible chance of having a positive impact on cancer therapeutics.

PHOTODYNAMIC MOLECULAR BEACONS

In addition to their other work, the Princess Margaret Cancer Centre group has also been developing photodynamic molecular beacons (PMBs) – target-activatable probes that exploit the principle of fluorescence resonance energy transfer in order to regulate their fluorescence emission and photodynamic behaviour in response to specific biological stimuli. PMBs are essentially killer beacons and are normally made up of a disease-specific linker such as a peptide or an oligonucleotide, a photosensitiser such as porphyrin, and a quencher. Being thus constituted enables the photosensitiser's phototoxicity to be supressed until the linker-target interaction occurs. "In this way, the beacon can achieve a very high level of photodynamic therapy (PDT) selectivity by destroying only the targeted cancer cells, while leaving non-targeted cells unharmed," Zheng explains. As part of the first clinical utilities of PMBs, the investigators are focusing on breast cancer vertebral metastases because, in such cases, collateral damage to the normal tissues would have a deleterious impact on the spinal cord.

Because breast cancer often spreads to the spine, where it is capable of causing extreme pain and a variety of neurological complications, Zheng's group has highlighted the development of PMBs which exploit the overexpression of matrix metalloproteinases (MMPs) on the surface of migratory breast cancer cells as important. The PMBs are inactive at the point of being administered and only become photodynamically active when combined with MMP activity and the administration of lasers at selected sites. Indeed, a crucial part of their research revolved around the use of leporine and murine models of metastatic breast cancer, through which the investigators were able to show that the PMBs they had developed are capable of specifically targeting metastases of the vertebra. They can also be activated by breast cancer cells which express MMP, to destroy malignant cells while avoiding collateral neurologic damage.

The potential for PMPs does not end here, as Zheng concludes: "We have also shown that, when irradiated by light, the PMBs not only destroy spinal metastases but also bone cells responsible for the weakening of bones which express MMP, and can be seen in many patients with metastatic cancer." In this way, the researchers' 'smart' PMBs can potentially increase survival rates and improve the quality of life for patients who are affected by breast cancer vertebral metastases.

LOOKING AHEAD

Given the success of their work so far, Zheng and his team are discussing plans for the future of their research into nanotechnology intervention. "We are particularly focusing on translating porphysome nanotechnology to first-in-human studies, and our aim is to also explore all the different clinically-viable utilities of porphysome nanotechnology in highly clinically-relevant animal models," he asserts. There is much optimism amongst the investigators that, in pursuing this branch of their study, the use of porphysomes will one day become clinically available, with PMBs and nanocarriers not far behind. Should this materialise, the diagnosis and treatment of millions of individuals with cancer would be significantly improved.

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