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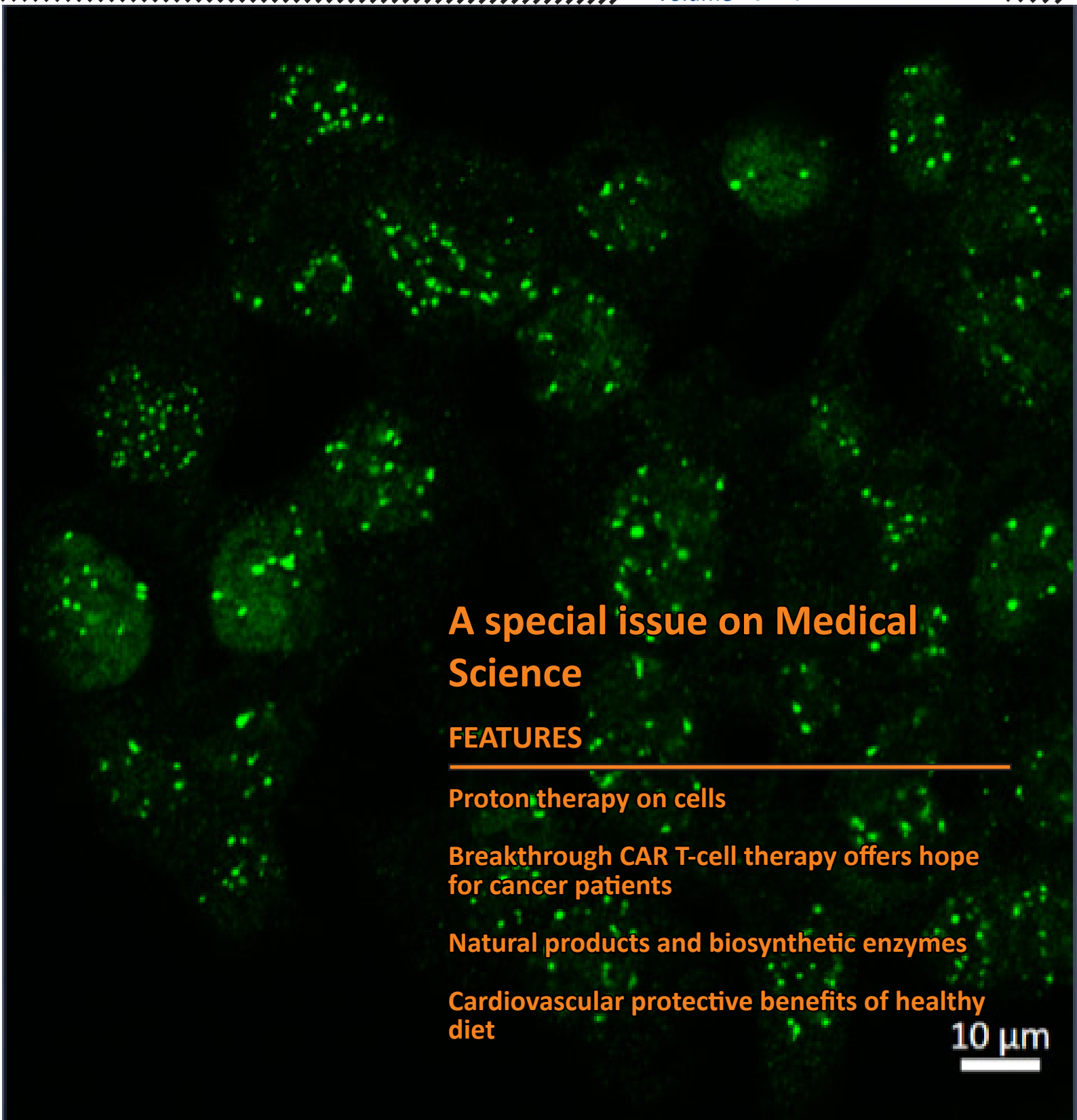

A special issue on Medical Science

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On the cover: 2 MeV protons causing DNA double strand breaks in HepG2 cells. The dose delivered to the cell is 2.5 Gy. [Credit: Prof Andrew Bettiol]

Proton therapy on cells

Radiobiological studies of proton irradiated cancer cells

Introduction

Particle therapy is a form of radiation therapy that uses high energy particles (usually protons or carbon ions) to treat cancer. Around the world, facilities for particle therapy have been increasing in number in recent years. Currently there are over 100 facilities worldwide, with more than 30 under construction and another 30 in the planning stage*. Singapore has three proton therapy facilities that are currently under construction, the main one being located at the New National Cancer Centre, Singapore (NCCS) Outram campus. At the Centre for Ion Beam Applications (CIBA), NUS Physics Department, an active research programme devoted to studies of radiation effects on cells for cancer treatment, has been running for the past five years. This programme is conducted in collaboration with several Principal Investigators from NCCS and Duke-NUS Medical School. The main facility for radiobiology in CIBA is the focused beam live cell irradiation beamline. This was constructed over several years with funding provided by Academic Medicine Philanthropic Funds, Duke-NUS. An additional beamline at CIBA that uses a broad beam to irradiate large numbers of live cells (many millions at a time) was later included in the research programme and now provides external collaborators and CIBA researchers with proton irradiated cells under various conditions on a weekly basis.

At CIBA, we are interested in fundamental studies of radiation effects on living cells. Many of our programmes are devoted to understanding, at the cellular and sub-cellular level, the differences between proton and x-ray damage and repair of Deoxyribonucleic Acid (DNA). In addition to these irradiation beamlines, at CIBA we have developed

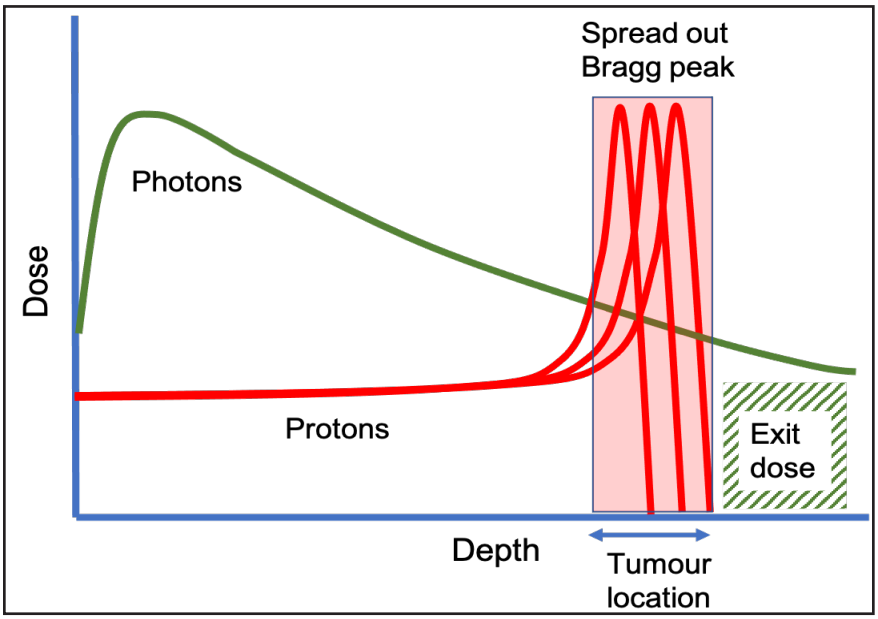

Figure 1: Schematic diagram showing the difference between protons and photons in delivering a radiation dose to a tumour.

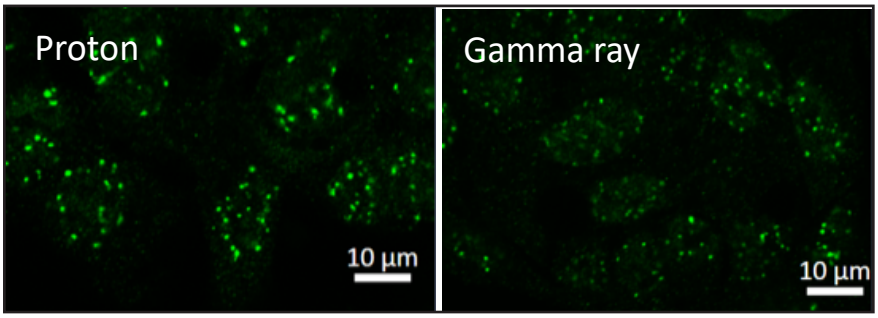

Figure 2: Comparison between 2 MeV protons and gamma rays in causing DNA double strand breaks in HepG2 cells. The dose delivered to the cells for both irradiations is 2.5 Gy.

many state-of-the-art techniques for imaging whole single cells. These techniques allow us to quantitatively image sub-cellular structures and visualise nano-particles such as gold, lanthanides and nanodiamonds that have been introduced into cells [1, 2]. One of the applications of introducing nanoparticles into cells is to enhance the DNA damage process, thus potentially improving the effectiveness of proton therapy (Radio-sensitisation).

Protons *versus* Photons

The way in which protons interact with human tissue is very different from photons (x-rays, gamma-rays). Most of the photon energy absorbed by tissue occurs at the surface. The amount absorbed then drops off exponentially as it enters the body. As a result, one reason the dose delivered to a patient is fractionated is to reduce the amount of the dose delivered to tissue at the surface. Unfortunately, some radiation is also deposited beyond the tumour (Exit dose), which is unavoidable. Protons, on the other hand, deposit most of their energy in a region where they come to a stop. This region is called the Bragg peak. The Bragg peak

* Source: Particle Therapy Co-Operative Group website (www.ptcog.ch)

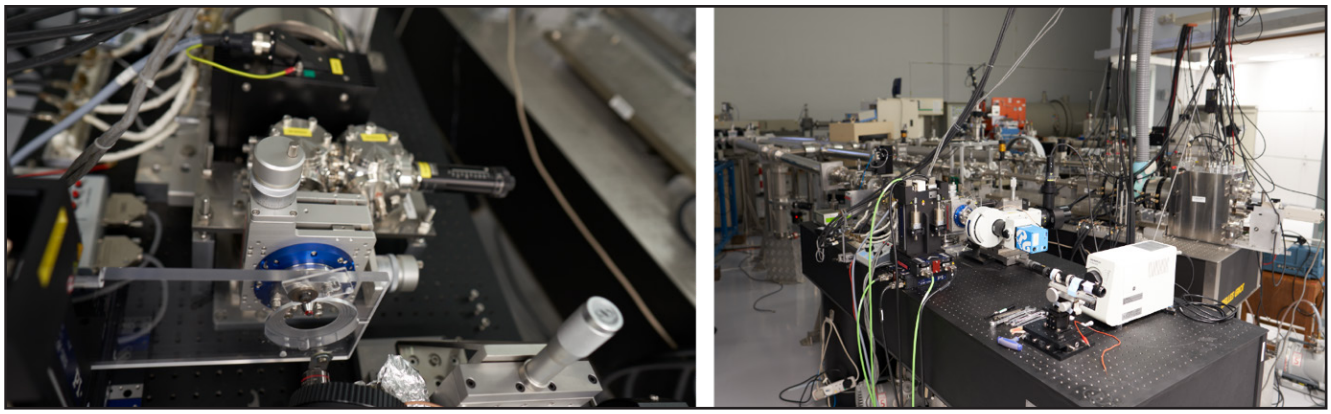

Figure 3: The CIBA radiobiology beamline. The left image shows a closeup of the end station and the Perspex cell dish holder. The right image shows an overview of the beamline.

for a single particle energy occurs at a well-defined depth, so in order to irradiate the whole tumour, a range of particle energies is employed to produce a *spread out Bragg peak*. Figure 1 illustrates the difference in the dose delivered by protons and photons.

Visualising DNA damage

In many of the radiobiological studies performed at CIBA, quantifying the radiation damage to cells is important. This is typically achieved by imaging techniques where we use a scanning confocal microscope to visualise cells that have been irradiated by protons and gamma-rays. The confocal imaging also allows us to study the morphology of cells that have been irradiated, and the subsequent death of the cells. The main mechanism for cell death in irradiated cells is by breaking the DNA strands by the ionizing radiation. If a single strand of DNA is damaged by radiation, a cell can usually repair the damage. Two strand breaks within close proximity, known as double strand breaks (DSB), are much harder for cells to repair and usually result in cell death. Two biomarkers that are commonly used to detect the presence of DNA damage or DSBs are γ-H2AX and 53BP1 proteins. Immunofluorescence staining allows us to tag the proteins of interest with a fluorophore, which make them visible in confocal microscopy.

Figure 2 shows an example of HepG2 cells that have been irradiated with 2.5 Gy of 2 MeV protons and gamma-rays from a Co-60 source. The green spots are called foci and indicate regions within the nucleus of the cell where DNA DSB have occurred. Our preliminary studies show that foci produced by protons for the same dose are brighter and hence the DNA damage is clustered. This is consistent with protons generating tracks of DNA damage as they traverse through cells.

The CIBA radiobiology beamline

The radiobiology beamline in CIBA was purposely designed and built to perform radiobiological experiments on live cells in air [3]. It is the first line in CIBA that allows the beam to penetrate from high vacuum into air. This is important because live cells cannot exist in a high vacuum environment where samples are usually mounted for ion beam analysis. To extract proton beams in air, a custom nozzle was designed and mounted at the end of the beamline, which has a very thin membrane of silicon nitride mounted on the end. This membrane is thin enough (one-thousandth of a single strand of hair) for the MeV proton beam to penetrate while maintaining a vacuum seal. As is the case for several other CIBA beamlines, the radiobiology beamline has a set of four high performance magnetic quadrupole lenses that allow for the beam to be focused to a sub-micron (less than 500 nm) spot in air, and scanned over an area of approximately 1 mm x 1 mm. The high resolution beam and the ability to scan allow us to target specific regions within the cell with controlled numbers of protons. We are currently in the process of installing particle detectors that can count every single proton that impinges on a cell. This allows us to perform precise dosimetry at the sub-cellular level. A fluorescence microscope and a precision stage are also used to visualise the cells during experiments. This facility can help advance proton therapy in cancer treatment.

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Breakthrough CAR T-cell therapy offers hope for cancer patients

Engineering immune cells to treat cancers

Introduction

Cancer is a deadly disease, and scientists and medical doctors are working concertedly to develop new ways to combat it. We hear of new breakthroughs in cancer treatments almost every year, but many of them are not effective. CAR- T cell therapy is, however, believed to offer hope after many other cancer treatments have failed.

CAR T-cell therapy

The "CAR" in CAR T-cell therapy represents "chimeric antigen receptor". The term "chimeric" means that different biological sources are used to assemble the T cell receptor. The CAR-T cell comprises a part to recognise cancer cells derived from a cancer cell-specific antibody, and an activation part derived from T lymphocytes. Thus, CAR-T cell therapy is a combination of gene or cell therapy, plus antibody therapy.

With CAR-T cell therapy, T lymphocytes, a type of white blood cells, are collected from patients or healthy donors and genetically engineered to express a new type of receptor (CAR) which is capable of recognising cancer cells and simulating T cell potency. The newly modified T-cells are grown in an external environment until millions of them are produced. They are then re-infused into patients, where these CAR-T cells can recognise, target and attack the cancer cells (see Figure 1).

Clinical trials of CAR-T cell therapy began in the mid-1990s and accelerated rapidly in the early 2010s. These trials led to the first approvals of two CAR-T cell products by the US Food and Drug Administration Agency (FDA) in 2017 and the European Medicines Agency (EMA) in 2018 to treat blood cancers (including leukaemia and lymphoma).

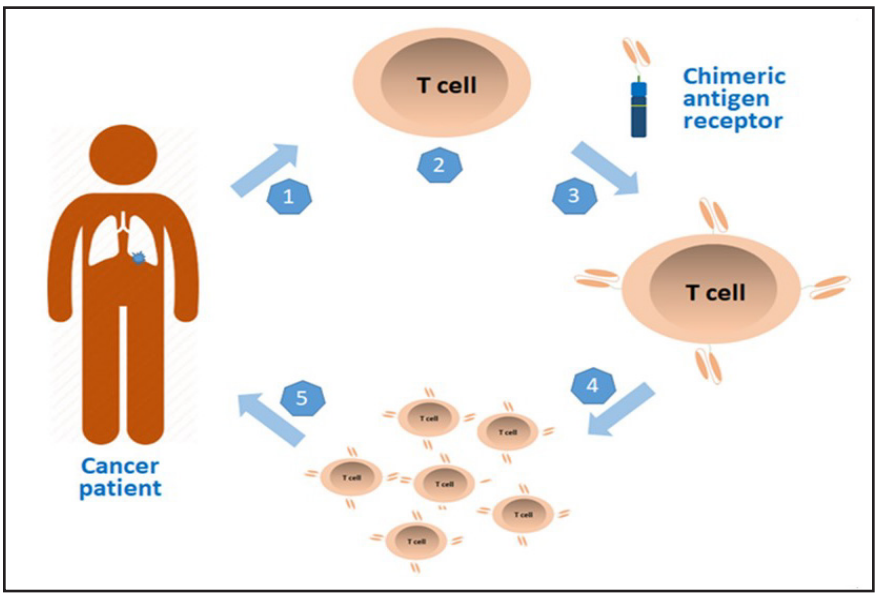


Figure 1: Steps involved in CAR-T cell therapy. Step 1: Blood collection; Step 2: T cell activation; Step 3: CAR gene transfer to generate CAR-T cells; Step 4: CAR-T cell expansion; Step 5: CAR-T cell infusion back to the patient.

Currently, FDA has approved five CAR-T cell therapies, two of which were approved in the early 2021, and there are approximately more than 800 completed and ongoing CAR-T cell clinical trials registered globally.

Why are scientists so keen on developing and testing CAR-T cell therapies? Simply because these new therapies have cured blood cancer patients for which nothing else had worked. In some types of blood cancer, CAR-T cells have achieved unprecedented therapeutic efficacy, with up to a 90% complete remission rate in patients who do not respond to other treatments or have relapsed two or more times after previous treatments. In many of these patients, all traces of cancer disappeared. The impressive efficacy is due to a totally new mechanism underlying CAR-T cell action. CAR-T cells act as a "living drug" against cancer cells: the infused CAR-T cells multiply during an attack against cancer cells, then move on to kill other cancer cells acting as serial killers, and can persist in the patient's body for many years, potentially lasting for a lifetime. Although only certain types of blood cancers can be cured by CAR-T cells at this point, T cells can potentially be modified by CARs to target virtually any type of cancer.

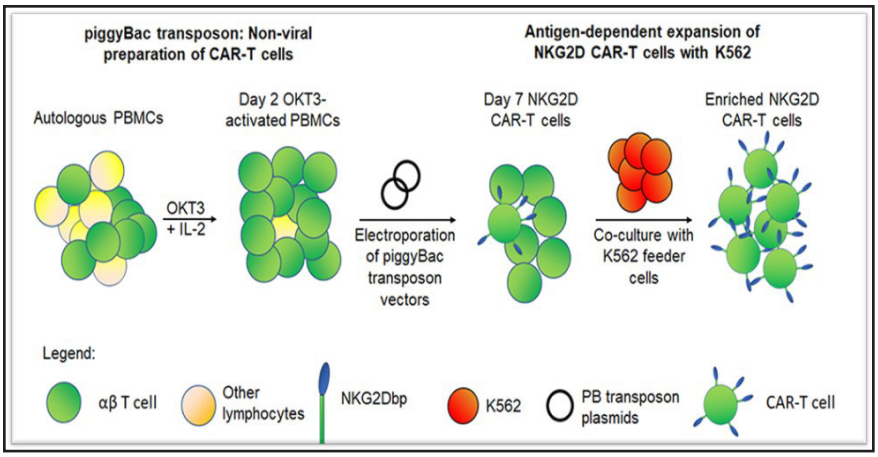
Cancer therapy using CAR T-cells

The research thrust of my laboratory has been to develop gene- and cell-based platform technologies applicable to cancer therapy. Our aim is to make a contribution to the progress of translational medicine, translating discoveries in functional genomics and basic immunology into therapeutic applications. We started cancer immunotherapy 10 years ago and published our first CAR-T cell therapy paper in 2016. In the CAR therapy area, we have been working towards building platform technologies that support the development of novel CAR-T therapeutics and also other types of CAR immune cells beyond commonly used T lymphocytes. Powerful immune cell expansion methods and genetic modification technologies suitable

for these cells have been established. Several new CAR therapeutics with potential for use against a broad range of cancers are currently in the testing stage. We are particularly interested in those that hold potential clinical value in providing safe, effective, and economic treatment.

However, while the effectiveness of CAR-T therapy in blood cancer treatment has been proven, it comes with related toxicities and safety concerns that were also observed in patients. One of the most prevalent side effects is known as the cytokine release syndrome (CRS), which may lead to multi-organ system failure or even patient death. CRS is typically caused by massive in-vivo CAR-T cell activation and the associated excessive secretion of the inflammatory cytokines. These secretions include interleukin (IL)-6, IL-2, interferon (IFN)-γ, tumour necrosis factor (TNF)-α by CAR-T cells or other immune cells that are activated during the treatment. However, some of the cytokines also play an important role in the CAR-T cell activation process and the cytotoxic effects on malignancy. Therefore, finetuning the CAR-T cell signalling to balance the cytokine secretion is vital to establish a CAR-T cell therapeutic window that exerts effective anti-cancer functionality without the excessive release of the cytokines.

We have designed a new CAR construct that allows for such a finetuning mechanism [1]. In animal studies, we have also demonstrated the efficiency of the CAR-T cells to eradicate tumours effectively while yet secrete significantly lower levels of IL-2, IFN-γ and TNF-α. Thus, our CAR design may possibly provide a potential clinical advantage in mitigating the risk of CRS.



Credit: Molecular therapy methods & Clinical development

Figure 2: CAR-T cells can be prepared in large numbers for clinical application through a non-viral platform.

Generation of CAR-T cells

To generate CAR-T cells, almost all laboratories utilise viruses to integrate the CAR gene into the T cell genome for persistent expression. Using viruses has been popular for decades among clinicians working on gene therapy, and the method is well-established. Viral methods are, however, associated with issues such as high manufacturing costs, cumbersome processes, and risks of residual viral elements. Clinical applications of the virus method are highly expensive because each step of the clinical-grade viral manufacturing must comply with good manufacturing practices (GMP). This is the main reason why CAR-T cell therapy in the United States can cost as much as US$375,000 for a one-time treatment.

To overcome the limitations associated with the use of viruses and to provide a cost-efficient technology for CAR-T cell manufacturing, we have developed a DNA-based non-viral method [2]. The DNA, called piggyBac (PB) transposon, was originally isolated from the cabbage looper (a type of moth) and can mediate gene integration in human cells, including T cells. Using our method, CAR-T cells with a purity of 89.2 ± 10.2% can be expanded to between 50,000 to 60,000 folds within four weeks to produce enough CAR-T cells for clinical applications, which may need 109 cells per patient (see Figure 2). The advantages of our PB method include high cargo capacity to deliver a large piece of DNA fragment (for the delivery of multiple CAR genes), significantly lower manufacturing costs as compared to virus methods, and potentially less regulatory restrictions in clinical translations as it does not make use of viruses.

Following the landmark FDA approval of two CAR-T cell therapies in 2017, the field has gained increasing recognition and momentum. The cancer world is coming to the view that, besides the mainstream treatments of surgery, chemotherapy, radiotherapy and targeted therapy, there is now concrete hope that CAR-T cell therapy can be an effective method for the treatment of some cancers.

WANG Shu is a Professor in the Department of Biological Sciences, NUS. He obtained his Ph.D. from University of Gothenburg, Sweden. The research focus of his laboratory is on the development of biological therapy approaches applicable to cancer immunotherapy. In recent years, he has been working towards building platform technologies that support the development of novel immune cell-based therapeutics.

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Natural products and biosynthetic enzymes

A spotlight on natural products for drug discovery and fighting infectious diseases

Introduction

Natural products (NPs) are secondary metabolites derived from plants, animals and microorganisms and they play a key role in drug discovery and development. The majority of small-molecule drugs used in medicine are either the NPs themselves or their derivatives. Host organisms use NPs to confer advantages in their local environment for various functions including communication, attraction, signalling, and defense. We can repurpose these molecules as pharmaceuticals for the treatment of infectious diseases and cancer. These molecules have evolved over millions of years and often have extremely potent and selective activities that cannot be matched by synthetic compounds. The chemical structures of NPs cover a much broader range of chemical space compared to their synthetic counterparts. This diversity increases NPs' versatility in drug properties and the ability to target diseases by different modes of action. Our group focuses on revealing the chemical diversity of natural products found in bacteria and utilising their enzymes to create next-generation therapeutics.

Genome mining as a contemporary approach to identify new and bioactive NPs

Historically, the discovery of novel NPs has been based on bioactivity-guided fractionation and isolation from extracts of various organisms. Over time, a significant number of studies resulted in the rediscovery of known compounds, and it was believed that the resources for new NPs had been exhausted. However, technological advances in genome sequencing and analysis have renewed interest in the discovery of new NPs. The bacterial genome sequences in public databases revealed that only a small percentage of gene clusters, which encode NPs can be linked to an isolated and characterised NP. There remains a significant repository of uncharacterised biosynthetic gene clusters (BGCs) and their associated NPs for small-molecule discovery. As the genes encoding NPs are "clustered" in bacteria, they can be readily identified. The pursuit of new NPs based on genome analysis is termed "genome mining". With genome mining, NPs that were previously hidden from traditional discovery methods, such as NPs without bioactivity or those that are not produced naturally (termed silent), can be discovered along with their biosynthetic pathways. This opens a new dimension in the field of NP discovery (see Figure 1).

Ribosomal peptides are attractive for discovery of small molecules and enzymes

Natural products comprise several NP families based on their structure and biosynthetic process. They include alkaloids, terpenes, polyketides, peptides, fatty acids, etc. Among the NP families, ribosomally synthesised and post-translationally modified peptides (RiPPs) have emerged as attractive model systems for research studies due to their diversity in structures, bioactivities and biosynthetic enzymes. In contrast to other pathways, the gene clusters required for the biosynthesis of ribosomal NPs are relatively small and easy to manipulate for efficient production. Many RiPPs have been shown to mediate microbial interactions and often contain antibiotic activity. The gene cluster for a RiPP NP is composed of a substrate precursor peptide, post-translational modifying enzymes which change the precursor, and protease and transport proteins to cleave and export the peptide NP out of the cell (see Figure 2). In RiPPs, the diversity in chemical structures generated by the post-translational modifying enzymes can introduce an astonishing range of peptide modifications important for proteolytic stability, rigidity and bioactivity. Collectively, these attributes can lead to the production of peptide therapeutics or drugs. One of the characteristic and enigmatic features of RiPP pathways is the inability of genome mining programmes or algorithms to identify them. This means that we are unable to predict the prevalence of these systems in nature and they remain an open-ended resource for NP and enzyme discovery.

Ribosomally synthesized peptides modified by radical SAM enzymes

The sea of genome sequences makes targeting specific gene clusters for NPs a daunting task. One strategy for interrogating unknown sequence function space is to target new enzymes within an enzyme superfamily. We have particular interest in post-translational modifying enzymes from the radical S-adenosylmethionine (rSAM) superfamily. Our interest in rSAM enzymes began during the search for the genes responsible for the biosynthesis of a family of giant peptide toxins, named polytheonamides [1]. The polytheonamides are highly cytotoxic, pore-forming, β-helical peptides originally isolated from a marine sponge (*Theonella swinhoei*) but biosynthesised by an uncultivated bacterial symbiont (*Candidatus Entotheonella* factor) of the sponge. Using a metagenomic approach, the biosynthetic genes were identified and it is shown that polytheonamides were of ribosomal origin. We were particularly intrigued by the enzymes of the rSAM superfamily that catalyse radical mediated C-methylation and L- to D- epimerization. Both of these are challenging chemical reactions. Research on the rSAM enzymes in the polytheonamide pathway led to

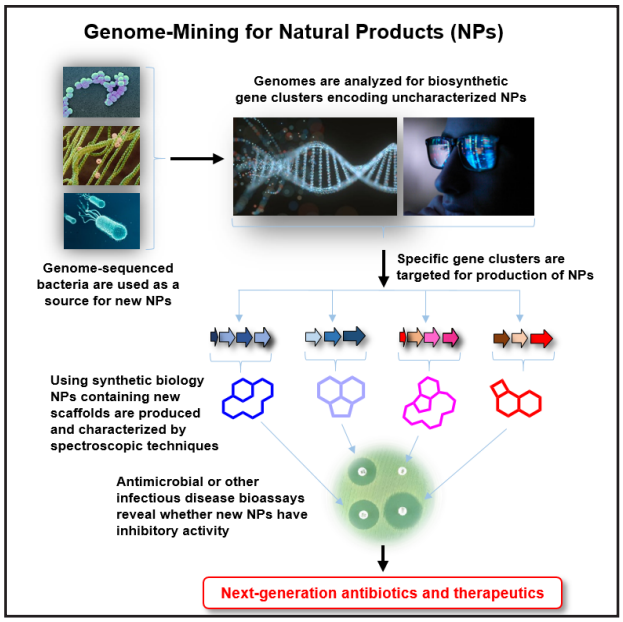

Figure 1: Genome mining from bacteria as a source for new therapeutics. The process starts from bacteria for which the genome sequence is known. The genome sequences are analysed for biosynthetic gene clusters that contain the potential to create new natural products. These natural products are produced by native or engineered strains of bacteria using synthetic biology. The molecules are characterised by analytical chemistry and spectroscopy. The new compounds are tested against a range of assays relevant to infectious diseases. The end goal is to identify new NPs that can kill antibiotic-resistant pathogens, viruses, and other infectious diseases.

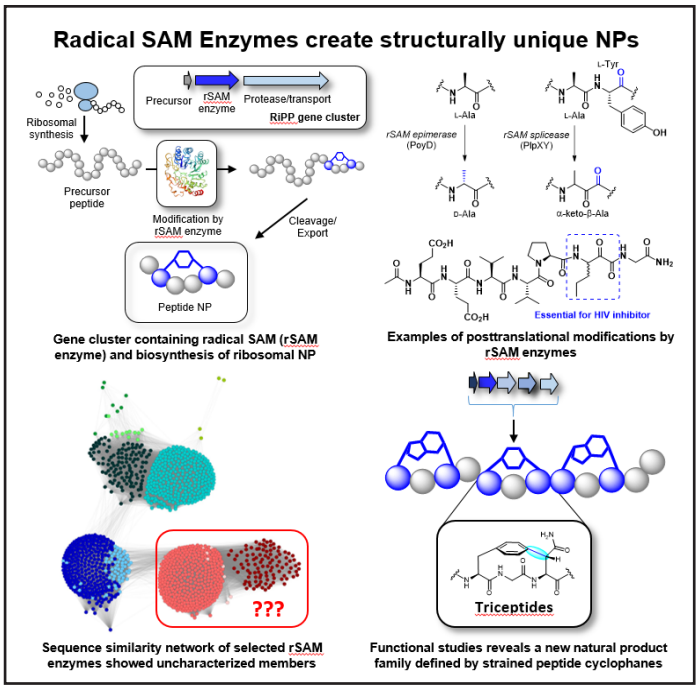

Figure 2: Radical SAM enzymes in the biosynthesis of ribosomally synthesized and post-translationally modified peptide (RiPP) natural products.

both genome-mining and functional validation workflows for identifying and characterising rSAM post-translational modifying enzymes found in bacterial gene clusters.

In a subsequent study, a similar approach was used to identify another subfamily of rSAM enzymes from cyanobacteria that catalyse protein splicing to introduce β-amino acids into ribosomally synthesised peptides [2]. The net result of the protein splicing reaction is the excision of a tyramine from the protein backbone, which yields an α-keto-β-amino acid residue. The α-keto-β-amino residue can be utilised for developing novel therapeutics. For example, this functional group is an essential feature in peptides that are used to treat hepatitis C. The rSAM epimerase and splicease enzymes represent methods to install D-configured and β-amino acids into ribosomally synthesised peptides, which changes the paradigm that only L-configured, α-amino acids can be installed ribosomally. Both transformations can be utilised in preparing therapeutic peptides because they add advantageous structural features and prevent peptides from being broken down by digestive enzymes.

Our current research is focused on a new subfamily of rSAM enzymes that catalyse peptide cyclisation. These efforts were initiated to expand the post-translational modifications by rSAM enzymes. We targeted several subfamilies which were annotated in the TIGRFAM database. A series of functional studies and characterisation of products revealed a new natural product family defined by the formation of three-residue peptide cyclophanes [3]. This transformation is broadly distributed in bacteria but mainly found in Actinobacteria, Cyanobacteria, and Proteobacteria. The diverse range of products formed by these enzymes has potential use against infectious diseases such as antibiotic resistant pathogens and viruses.

Brandon I MORINAKA is an Assistant Professor with the Department of Pharmacy, NUS. He was trained in marine natural products chemistry and obtained his B.S. in Chemistry from UC Santa Cruz (Phil Crews) and Ph.D. in Chemistry from UC San Diego (Ted Molinski). He then carried out postdoctoral research in genome mining of natural products at the University of Bonn and ETH Zurich (Jörn Piel).

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Cardiovascular protective benefits of a healthy diet

Exploring the health promoting benefits of dietary strategies

Introduction

Cardiovascular disease (CVD) is one of the world's leading causes of death and it accounts for nearly 30% of deaths in Singapore. Many significant risk factors associated with CVD have been identified. Endothelial dysfunction due to oxidative stress and inflammation in the artery is one of the major risk factors contributing to the development of CVD. Mounting evidence points towards the importance of measuring clinically relevant biomarkers associated with endothelial dysfunction when conducting CVD-related research. This goes beyond assessing conventional blood lipid profiles.

Reports from several health organisations indicate that individuals with a higher risk of CVD should modify their lifestyles, including altering their diet, to improve chronic diseases-associated risk factors. In particular, the American Heart Association recommends having a healthy diet to combat CVD. Dietary strategies such as following a healthy dietary pattern or regularly consuming food rich in bioactive compounds are gaining significant attention due to their cardiovascular protective effects. Healthy dietary patterns associated with a decreased risk of CVD are characterised by regular consumption of fruits, vegetables, whole grains, low-fat dairy and fish, and the relatively low consumption of processed meat and sugar sweetened food and drinks. A food item rich in bioactive compounds that is widely consumed in Singapore is the wolfberry or goji berry (*Lycium barbarum*). This is a well-known traditional Chinese medicinal fruit which is becoming increasingly popular in many countries due to its health promoting properties. The biological activities and potential health benefits of wolfberries derive from their bioactive constituents which include carotenoids, polysaccharides and polyphenols.

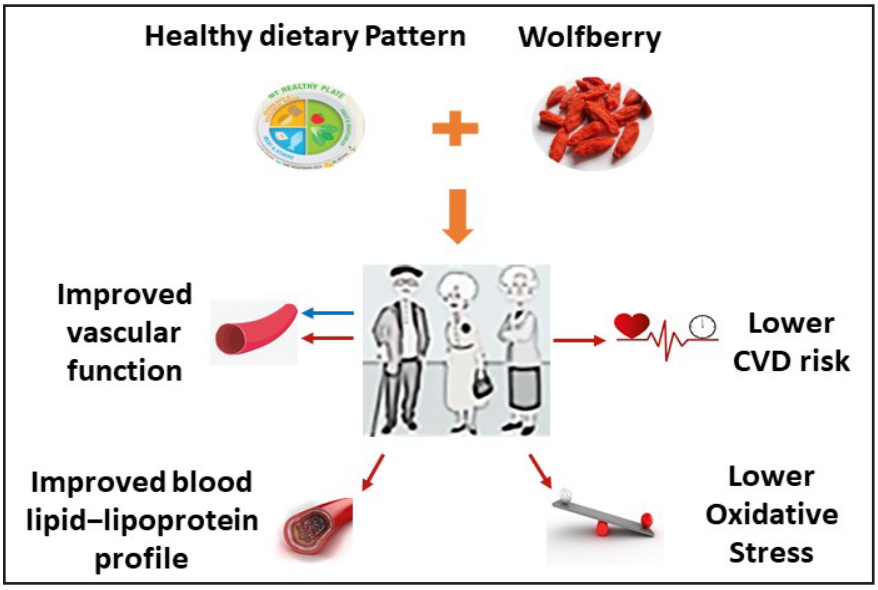

Figure 1: Enhancing the cardiovascular protective effects of a healthy dietary pattern with wolfberry (*Lycium barbarum*). Blue arrow indicates changes observed after following a healthy dietary pattern. Red arrows indicate changes observed after incorporating wolfberry to the healthy dietary pattern.

Although the importance of having a healthy diet is well-known, there still exists a knowledge gap in the local setting due to the lack of: 1) well-designed randomised control trials to validate the effects; 2) involvement of middle-aged and older adults with higher risk of CVD; and 3) local studies involving a more comprehensive spectrum of vascular health-related biomarkers.

My laboratory seeks to find ways to develop and validate dietary strategies that effectively protect against age-associated morbidities. Recently, we investigated the impact of wolfberry consumption as part of a healthy dietary pattern on vascular health-related outcomes and classical CVD risk factors in middle-aged and older adults living in Singapore.

Enhancing the cardiovascular protective effects of a healthy dietary pattern with wolfberry

The research involved a 16 week parallel design, randomised controlled trial. All participants received dietary recommendations to follow a healthy dietary pattern. A separate group (wolfberry group) was given additional instructions to prepare and consume 15 g/day of whole, dried wolfberry with their main meals. Biomarkers associated with vascular function, vascular structure and vascular regeneration, as well as blood lipid–lipoproteins and blood pressure were assessed.

Figure 1 illustrates our findings. Participants following the healthy dietary pattern experienced improvements in vascular tone. Those with daily consumption of whole dried wolfberries had more prominent improvements in their HDL cholesterol level, oxidative stress level and overall long-term CVD risk. These findings support the use of whole wolfberry as

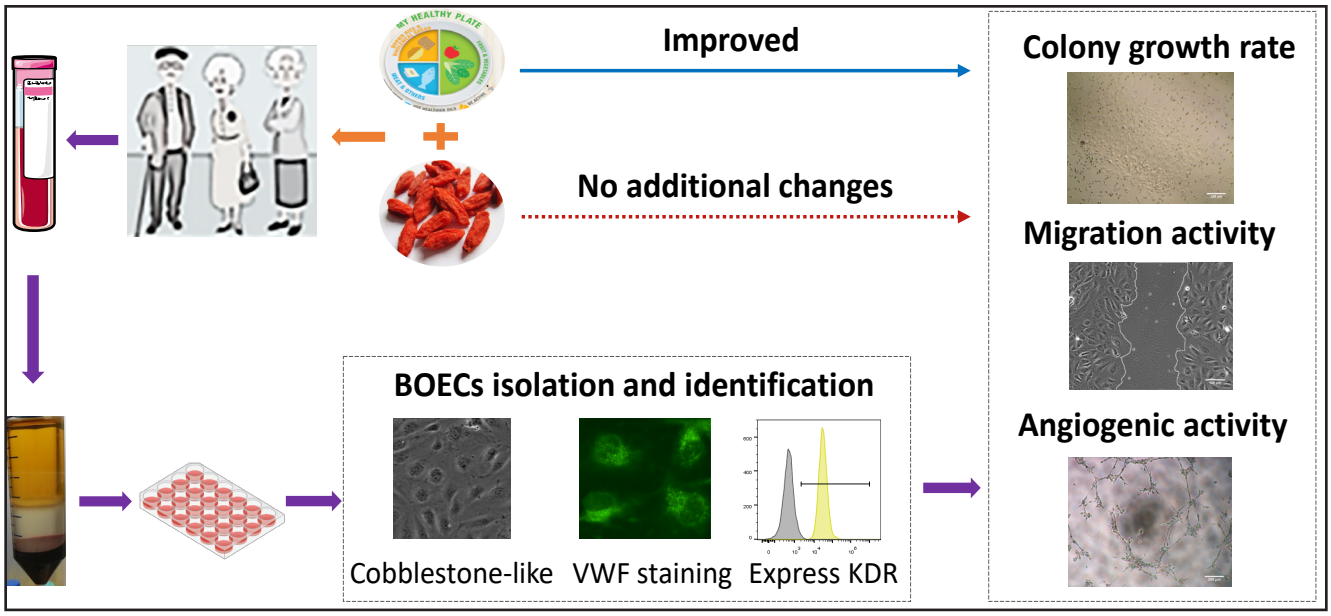

Figure 2: Healthy dietary pattern improves function of blood outgrowth endothelial cells (BOECs).

a potential dietary strategy to augment the cardiovascular protective benefits of a healthy dietary pattern for the local middle-aged and older adult population.

Impact of a healthy dietary pattern with co-consumption of wolfberry on endothelial progenitor cells

Circulating endothelial progenitor cells (EPC) have the capacity to migrate, proliferate and differentiate into mature endothelial cells and they play crucial roles in the regeneration of the endothelial lining of blood vessels. An accumulation of evidence has demonstrated that there is a pathophysiological link between circulating levels of EPCs and CVD risk factors and endothelial dysfunction, suggesting a potential role of EPCs in the progression of CVD. Among EPCs, blood outgrowth endothelial cells (BOECs) are considered the only cell type with the characteristics of a "true" EPC, given that these cells are self-renewing, clonogenic and able to integrate into functional blood vessels.

We have assessed the changes in the quantity and function of BOECs in collaboration with the National University Heart Centre, Singapore (NUHCS), Cardiovascular Research Institute (CVRI) and Yong Loo Lin School of Medicine, to explore the complex cardiovascular pathological states after incorporating wolfberry into a healthy dietary pattern.

Figure 2 summarises our findings. Observing a healthy dietary pattern improved the BOEC colony's growth rate. In addition, these BOECs showed improved tube formation capacity and migration activity. However, no further improvement was observed for participants who had also consumed wolfberries.

Conclusion

In conclusion, our findings suggest that adherence to the healthy dietary pattern contributes to improvements in BOECs function and vascular tone. Additionally, daily consumption of whole, dried wolfberry further increases HDL cholesterol while decreases oxidative stress level as well as overall long-term CVD risk amongst middle-aged and older adults.

These studies explored the evaluation of a broad spectrum of biomarkers associated with different areas of vascular health and provide researchers with a more comprehensive evaluation, and a mechanistic dissection of the cardiovascular protective effects received from the intervention. The focus of healthy dietary pattern on broader food groups permits greater flexibility, self-oriented choices and ease of compliance in a culturally diverse community such as in Singapore. Additionally, the consumption of dried, whole wolfberry may serve as a dietary strategy that can be adapted and easily incorporated into the diet.

Jung Eun KIM is an Assistant Professor with the Department of Food Science and Technology, NUS. As a nutrition scientist with advanced training in dietetics and human clinical research, her long-term research goal is to develop and validate dietary strategies and recommendations that effectively protect against age-associated morbidities and promote public health with human clinical trials.

References

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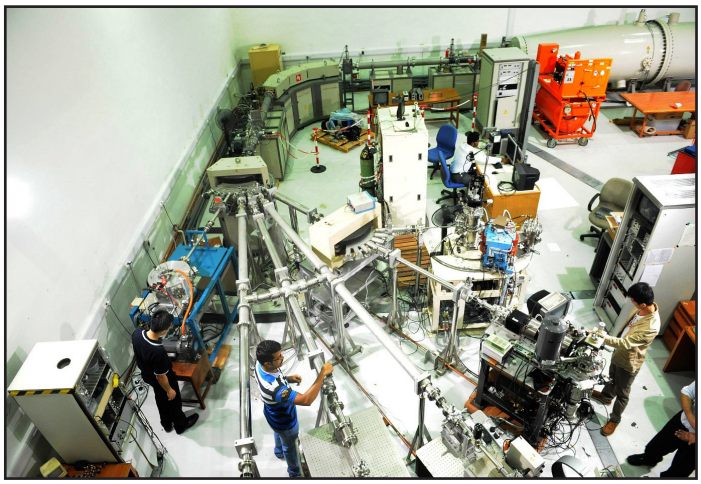
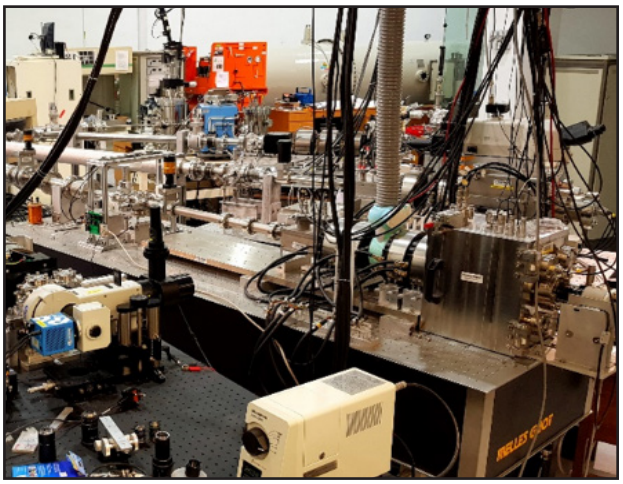
Centre for Ion Beam Applications (CIBA)

Top view (left) and close-up view (right) of the beamlines at the Centre for Ion Beam Applications.

The Centre for Ion Beam Applications (CIBA) is a multidisciplinary research centre at the Department of Physics, NUS. It has a 20-year track record in the field of Megaelectron volt (MeV) proton technology and applications, with the aim to develop new technologies based on fast protons and light ions (alpha particles, nitrogen and oxygen).

CIBA is led by a team comprising Prof Thomas OSIPOWICZ, Prof Andrew BETTIOL and Prof Jeroen van KAN. They are currently the only group globally with the capabilities to focus MeV protons to spot sizes below 20 nm, an advantage being used in a number of projects at CIBA.

Selected projects are highlighted below:

- Collaboration with the National Cancer Centre, Singapore on a large proton radiobiology research programme since 2017. This is motivated by the introduction of Proton Beam Therapy (PBT), the most advanced cancer radiotherapy technique, in Singapore. Three large-scale facilities are currently being built. As part of the study to improve the efficacy of PBT, CIBA currently operates two beamlines, one for *in vitro* single hit irradiation of sub-cellular structures, at resolutions below 500nm, and one for rapid large area irradiation of millions of cells in a monolayer configuration.

- Ion beam modification of materials, aiming at the creation of colour centres in wide band gap semiconductors, oxides and 2-D materials for applications in quantum sensing and single photon sources.

- Development of new imaging modalities for nuclear microscopy, such as Ion beam Fluorescence Lifetime Imaging Microscopy (IonoFLIM), for super-resolution imaging of single whole cells.

- Development of a new nano-structuring technology, known as Proton Beam Writing (PBW), which has unique characteristics in terms of the resolution, aspect ratios of structures and (near) absence of proximity effects.

- Building of a compact proton microscope, based around an in-house developed novel nano aperture electron impact ion source, with the potential for sub 10 nm beam spot performances in microscopy and nanofabrication applications.

- Use of PBW in single DNA molecule studies in nanofluidic devices. Nanofluidic devices have been fabricated using polymers with nanometric channels of tens to a few hundred nm in diameter. The response of single DNA molecules confined within such channels to the presence of proteins has been studied.

In the long run, protons may turn out more useful than electrons.

Thomas Osipowicz, Director, CIBA

In addition, dedicated beamlines for nuclear microscopy and high-resolution Rutherford backscattering spectroscopy are available for materials science studies.

The collaborative and cohesive structure of CIBA with domain specialists (physicists and biologists) working closely together allows for problems to be shared and solved collaboratively.

Website: https://www.physics.nus.edu.sg/ciba

Chemical, Molecular and Materials Analysis Centre (CMMAC)

Composite visual featuring a selection of instruments at the Chemical, Molecular and Materials Analysis Centre (CMMAC).

(Top row from left to right): Inductively coupled plasma-optical emission spectrometry (ICP-OES) system, Bruker D8 advance powder x-ray diffraction (XRD) system, Bruker D8 venture single crystal XRD system, AV NEO 500 nuclear magnetic resonance system.

(Bottom row from left to right): Matrix assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF) system, liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF) system, JEOL FA200 electron paramagnetic resonance (ESR) system, ultrawave microwave digestion system.

Quantitative and qualitative chemical analysis is essential in all areas of fundamental research and many industries covering manufacturing, petrochemicals, pharmaceuticals, environment, energy, water technology and materials science. Knowledge and skills about different modern methodologies for the separation, identification, and quantification of various analytes using various modern techniques are therefore crucial for the success and advancement of research and development (R&D) in these sectors and academia.

The Chemical, Molecular and Materials Analysis Centre (CMMAC) was established in 1998. It is a central instrumentation facility at the Department of Chemistry, NUS that provides quality professional analysis services to academia and industry in support of R&D and innovation.

Our mission and vision is to provide professional analytical solutions to all researchers and enterprises in the areas of (i) Chemical and elemental analysis, (ii) Molecular fingerprinting and mixture analysis, (iii) Materials studies and diffraction analysis, and (iv) Spectroscopic and microscopic studies.

To achieve this, the CMMAC consists of six core laboratories that provide analytical services using fundamentally diverse and state-of-the-art spectroscopic and spectrometric analytical instruments. These are the (i) Nuclear Magnetic Resonance (NMR) and Electron Spin Resonance (ESR) spectroscopy laboratory, the (ii) Mass spectrometry laboratory, the (iii) Elemental analysis laboratory, the (iv) X-Ray crystallography laboratory, the (v) Chromatography laboratory, and lastly the (vi) Electron microscopy laboratory. CMMAC has a fleet of more than 30 core instruments and smaller analytical equipment, and its combined capabilities essentially cover all major analytical techniques required for molecular and materials science.

All the laboratories are run by dedicated key personnel with expertise in the respective areas, who also contribute to teaching by providing hands-on training in analytical instrumentation and data processing for our students. Moreover, our CMMAC staff provide consultation and conduct user courses or workshops on current interest areas for clients. When possible, we also promote research interactions among scientists and industrial partners.

Website: https://chemistry.nus.edu.sg/facilities/chemical-molecular-and-materials-analysis-centre-cmmac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