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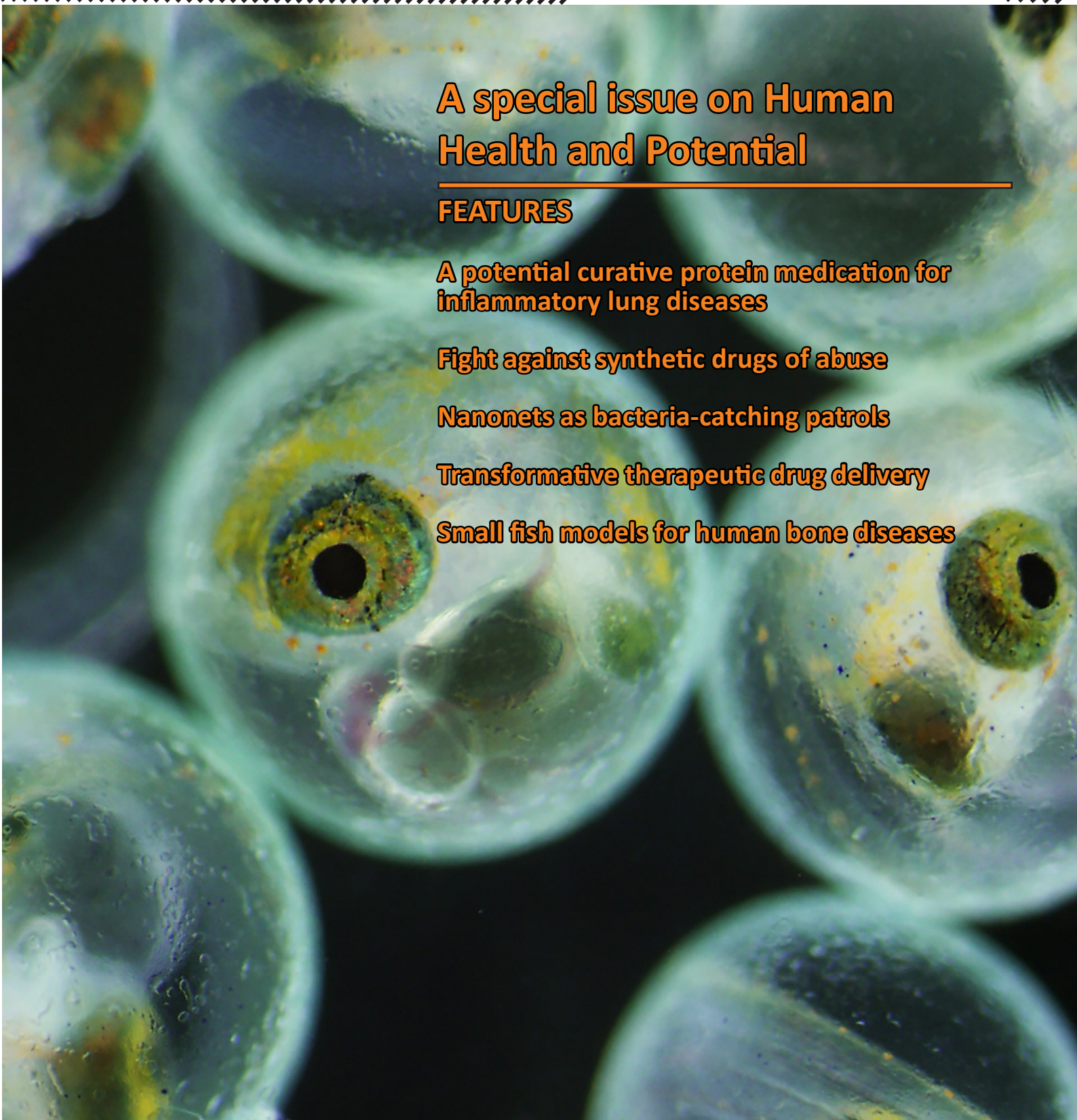

A special issue on Human Health and Potential

FEATURES

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Advances in Science

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On the cover: The visual shows medaka fish embryos. Medaka fish are a valuable and versatile model for studying bone development and diseases. Their transparency and similarities to human bones make them an ideal system for studying mechanisms of bone formation, understanding the causes of bone diseases and exploring potential new therapies. [Image credit: Kyoshi NARUSE]

A potential curative protein medication for inflammatory lung diseases

The anti-inflammatory lung-resident protein ISM1 has emerged as a potential first-in-class curative medication for chronic obstructive pulmonary disease (COPD)

Introduction

Inflammatory lung diseases encompass a range of serious disorders from chronic diseases such as chronic obstructive pulmonary disease (COPD), asthma and pulmonary fibrosis, to acute ailments such as acute lung injury or its severe form of acute respiratory distress syndrome (ALI/ARDS). The common pathophysiological factors for these diseases are lung and airway inflammation.

Among these pulmonary diseases, COPD affects more than 300 million patients worldwide, thus representing a significant global health challenge. It is characterised by irreversible lung tissue damage (emphysema) and chronic small airway inflammation (bronchitis). The symptoms associated with established COPD are chronic shortness of breath on exertion, chronic cough, and flare-ups of respiratory symptoms called exacerbations, often caused by respiratory viral or bacterial infections. COPD occurs as a result of damage and subsequent repair to the airways and lungs, which are exposed to various external inhaled irritants including cigarette smoke, air pollutants and infections. While specific statistics may vary in different countries, COPD poses a significant socioeconomic burden to public health in both developed and developing countries.

COPD: Urgent need for curative therapeutics

The treatment of COPD currently remains suboptimal due to the lack of curative therapies. Available treatments primarily consist of bronchodilators to alleviate symptoms of breathlessness and exercise intolerance. However, none of the existing treatments can halt, reverse or cure the disease. Compounded by the typical late diagnosis of COPD and the absence of assessment tools for grading its severity, treatment of COPD remains elusive.

Over the past 50 years, significant advancements in molecular cell biology, biochemistry and genetics have shed light on the pathophysiology of COPD at the molecular and cellular levels. This has led to improved medical diagnosis and public awareness of this disease. Nevertheless, while several medications have been developed for the symptomatic relief of COPD in the past decades, none can effectively suppress lung tissue damage (emphysema) or slow down disease progression. Thus, there remains an unmet medical need for curative medications capable of halting the progression of COPD and restoring lung function. Gaining further insights into the disease's pathophysiology is essential for the development of novel, mechanism-based medications.

rISM1: A potential first-in-class protein therapeutic for COPD

In our efforts to understand the biological functions of novel proteins, we discovered a lung resident protein known as Isthmin-1 (ISM1). Using murine experimental models, we found that ISM1 plays a critical role in maintaining healthy lungs by eliminating inflammation and protecting the lung from inflammation-induced damage. Our research revealed that ISM1 achieves this by selectively targeting the inflammation-promoting immune cells, such as the pro-inflammatory subfraction of alveolar macrophages, and trigger the demise of these cells in a harmless manner via a cell surface receptor called GRP78 [1, 2]. Based on these findings, we produced a large amount of recombinant ISM1 protein (rISM1) and administered it via the respiratory airway to the lungs of COPD-afflicted murine models. This effectively suppressed cigarette smoke-induced COPD pathology and restored damaged lung function to nearly normal levels (see Figure 1).

Our work revealed a novel mechanism of action in COPD pathophysiology and identified cell surface GRP78 as a promising target for COPD. ISM1, being a natural high affinity ligand of GRP78, is a potential first-in-class curative medication for COPD patients.

Mechanism of action: Targeting pro-inflammatory alveolar macrophages for COPD

Alveolar macrophages (AMs) are specialised immune cells residing in the airway of the alveolus (plural alveoli), the small air sac within the lungs, where pulmonary gas exchange occurs through the air-blood barrier on the alveolar wall. AMs constitute the innate immune system's first line of defense against invading pathogens or toxic materials from the air. They protect the lung from environmental damage by constantly capturing and removing these invaders. However, environmental pathogens or harmful particulates from cigarette smoke or air pollution can change some AMs into a pro-inflammatory state. These pro-inflammatory AMs release various pro-inflammatory factors and proteinases, leading to lung inflammation. Chronic (long-term) inflammation can result in tissue damage to the surrounding alveolar wall, compromising gas exchange and reducing lung function, leading to COPD.

ISM1 selectively targets the pro-inflammatory AMs and induces cell death without affecting the normally anti-inflammatory AM population. It targets and modulates specific inflammatory pathways responsible for exacerbating lung diseases. ISM1's model of action is illustrated in Figure 2:

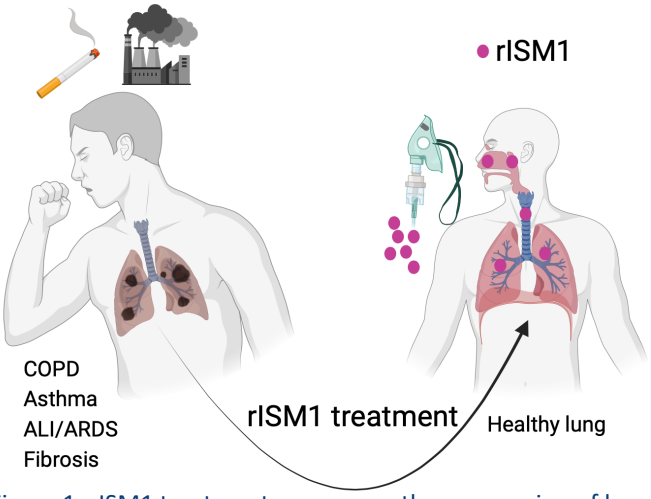

Figure 1: rISM1 treatment suppresses the progression of lung inflammatory diseases including COPD, asthma, ALI/ARDS and fibrosis.

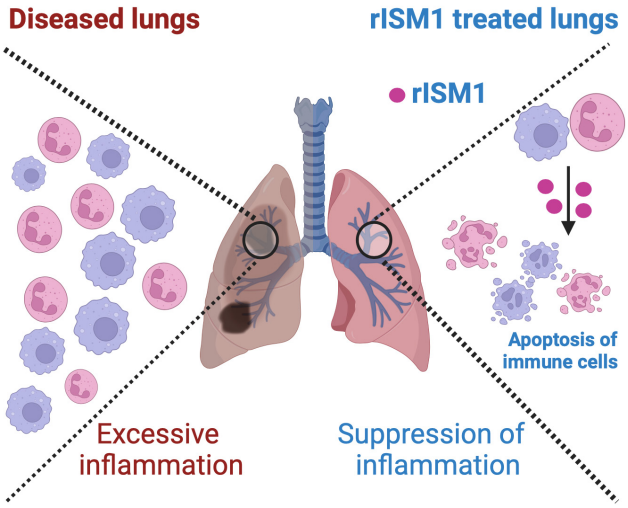

Figure 2: rISM1 treatment suppresses the excessive inflammation in the lungs of patients with lung inflammatory diseases by inducing apoptosis of immune cells.

1. Precision targeting: ISM1 operates like a guided missile, specifically homing in on GRP78 receptors on the cell surface of the inflammation-driving AMs. This precision targeting minimises the risk of side effects often associated with broader treatments.

2. Inhibiting of inflammatory AMs: When ISM1 reaches its target cell, it effectively triggers cell death and eliminates the source of inflammation. By removing the drivers of inflammation, ISM1 halts further lung tissue damage.

3. Facilitating recovery: With inflammation under control, the lung can focus on repairing the damaged tissue and restoring normal function.

To the best of our knowledge, there is currently no medication in development that targets a subpopulation of AMs through the GRP78 pathway. Hence, ISM1 represents a first-in-class therapeutic approach for COPD.

A promising future: What's next?

While this putative medication is still in the preclinical stage, ISM1 is poised to move further down the translation path towards becoming a novel therapeutic for COPD. Our work demonstrated that ISM1 also suppressed inflammation in several other inflammatory lung diseases. The foundational studies in our laboratory have paved the way for further research and development for a curative medication for COPD and other inflammatory lung diseases.

The journey ahead remains lengthy and fraught with challenges. It involves several crucial phases:

1. Preclinical pharmacological investigations: These investigations are important for assessing medication safety and stability using animal models.

2. Manufacturing and delivery technology development: This involves developing methods for the efficient scale-up production, storage and delivery of rISM1 to reach deep airway regions in patients.

3. Clinical trials and regulatory approval: rISM1 has to undergo stringent safety and efficacy clinical evaluations through multiple phases of clinical trials before it can be approved by regulatory agencies.

Conclusion

We aim to complete preclinical pharmacological investigations and advance rISM1 into clinical trials for COPD and/or other inflammatory lung diseases. Our aspiration is to witness the successful transition of rISM1 from our laboratory bench to the bedside of patients.

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Fight against synthetic drugs of abuse

Wet laboratory experiments using human liver systems have proven effective in establishing urinary biomarkers for detecting abuse of novel illicit drugs

Introduction

New psychoactive substances (NPS) are chemical compounds designed to mimic the action of existing illicit recreational drugs. Originally designed as potential medicinal candidates, NPS have subsequently become widely available to drug abusers via subtle modifications of their chemical structures to evade laws on controlled substances. The demand for NPS has increased as these drugs could achieve similar or even more potent effects compared to other illicit recreational drugs. Additionally, the abuse of NPS can often go undetected by standard drug screening tests and toxicology screens, since many current tests lack the ability to detect all possible variations of NPS. This growing demand, together with the emergence of clandestine laboratories synthesizing newer analogues of NPS using published synthetic methods, contribute to the prevalence of NPS abuse.

Due to limited information on the acute and chronic toxicities of NPS, these illicit drugs have been recognised as a severe "public health problem" around the world. NPS are broadly classified into four main categories: stimulants, depressants, hallucinogens and synthetic cannabinoids (SCs), which mimic the effects of cannabis. Some SCs are more potent than natural cannabis and can cause severe adverse effects including death. Consequently, it is essential for forensic laboratories to be able to confirm the abuse of SCs to relate the adverse effect or fatality to the specific toxicant.

Challenges associated with surveillance of SCs

SCs have been synthesized with various structural modifications to evade detection by forensic drug testing laboratories. This renders the detection of novel SCs challenging. Additionally, SCs are extensively broken down or metabolised by enzymes in our liver into unknown products or metabolites, resulting in nearly undetectable derivatives in urine samples. From both the forensic and toxicological perspectives, it is therefore important to study how the human body processes SCs to establish the urinary biomarkers for both parent SCs and their metabolites. Ethical and safety concerns have limited human clinical studies on SCs. Consequently, such important information is unavailable for scientists to develop urinary biomarkers for detecting the abuse of SCs.

Although animal models have been adopted for investigating how mammalian systems process SCs, discrepancies in the metabolites have been observed among different species due to inter-species variations. Moreover, cost and ethical considerations regarding animal experiments cannot be disregarded.

Beyond these challenges, SCs were often detected in mixtures containing counterfeit prescription pills and other psychoactive substances (e.g. heroin, alcohol, stimulants and hallucinogens). This suggests the potential for clinically significant drug-drug interactions in scenarios involving polysubstance abuse. Without knowledge on how SCs are processed by the human body and their interactions with human enzymes, their potential implications in drug-drug interactions remain unknown.

Our proposed two-pronged solution

With the continuous emergence of novel SCs to the extent that they have become a globally recognised major public health concern, it is important to be able to detect and identify the specific SCs that have been consumed. This is essential to support both forensic and clinical decision-making. Drug Metabolism and Pharmacokinetic (DMPK) research, comprising a series of comprehensive wet laboratory experiments, plays a longstanding and critical role in pharmaceutical drug discovery and development. It characterises the absorption, metabolism, distribution and excretion (ADME) of drugs in humans. Similarly, DMPK research can be applied to the study of other foreign substances, such as illicit drugs.

To address the conundrum of detecting novel SCs with limited knowledge on their identities, our laboratory has devised a DMPK-guided approach that is elaborated below for the early prediction of urinary biomarkers of novel SCs. Using human-relevant enzyme systems, such as human liver cells or laboratory-cloned human liver enzymes, our laboratory is able to address the following questions:

- How fast can a novel SC be broken down in the liver?
- What are the products or metabolites being derived from the SC?
- How quickly are the metabolites being broken down sequentially in the liver?
- What are the specific members of a family of enzymes that are responsible for breaking down the parent SC?
- Are the metabolites excreted into the urine?
- Do the SC and metabolites inhibit the activities of specific enzymes?

By applying our expertise in DMPK research to address these pertinent

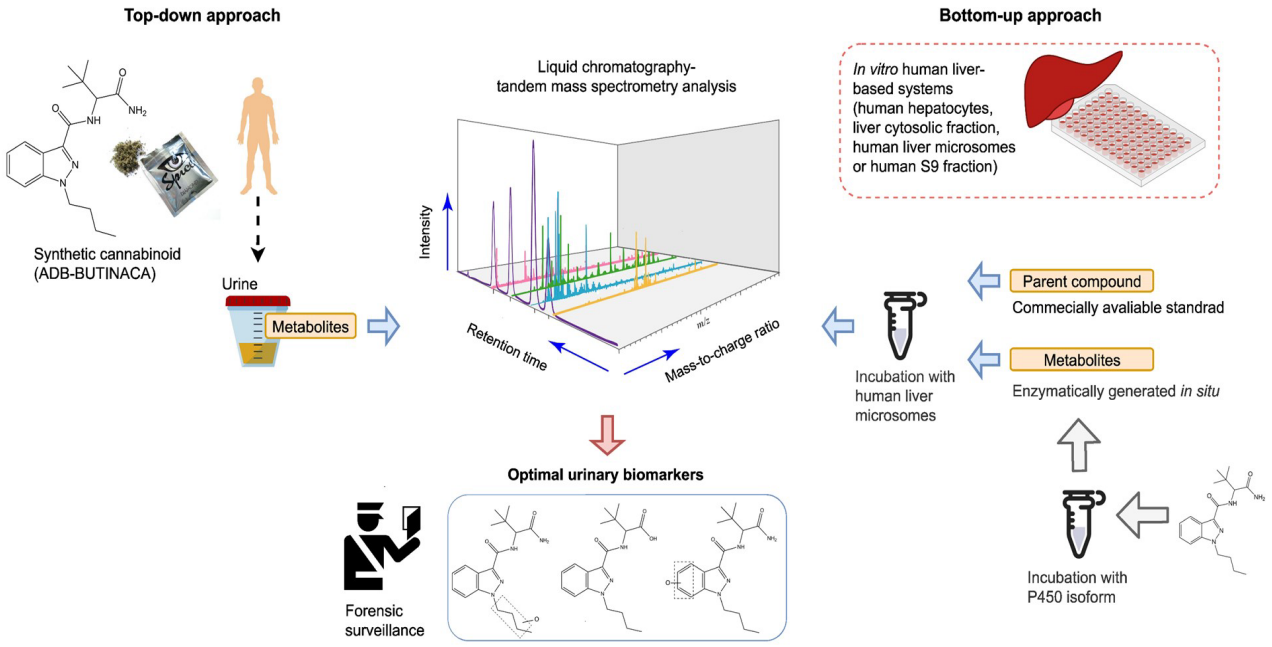

Figure 1: An illustration of the two-pronged approach for discovering urinary biomarkers to detect the abuse of novel synthetic cannabinoids (SCs). "Top-down" refers to the analysis of actual human urine samples to identify key metabolites derived from novel SCs, while "bottom-up" refers to conducting laboratory experiments to understand how extensively our human body breaks down the novel SCs.

questions, we can predict the urinary metabolites of each novel SC as potential biomarkers for detection. However, due to the limited availability of seized urine samples collected from individuals who have consumed novel SCs, comprehensive analysis of unknown urinary metabolites becomes challenging.

To efficiently and accurately discover urinary SC metabolites as biomarkers, we have introduced a two-pronged approach (see Figure 1). The "top-down" analysis of limited urine samples is performed in parallel and iteratively with the "bottom-up" wet laboratory experiments to establish a panel of optimal urinary biomarkers for novel SCs. In this context, "top-down" refers to the analysis of actual human urine samples. This process allows us to effectively scout for, and identify the key metabolites that emerge from the consumption of novel SCs. Conversely, "bottom-up" refers to laboratory experiments aimed at understanding how extensively our bodies metabolise and break down these novel SCs. These experiments provide invaluable insights into the intricate pathways and transformations these substances undergo within the human system.

Conclusion

The increasing prevalence of SC abuse has created a serious global health problem, leading many countries to enforce strict legal controls over SCs. This, in turn, drives abusers and chemists to explore novel structural analogues of SCs to evade both detection and legal liability. As legislation pertaining to novel SCs is expected to be expedited in more countries, increasingly complex or uncommon structures of SCs may continue to emerge in the recreational drugs market. Considering all these trends, there is a strong impetus for forensic toxicologists to efficiently and effectively discover urinary biomarkers to tackle this ongoing SC epidemic. Our proposed two-pronged approach is a validated strategy to address future outbreaks of novel SCs [1, 2].

For more details, please visit: https://pharmacy.nus.edu.sg/team/prof-chan-chun-yong-eric/

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Nanonets as bacteria-catching patrols

Synthetic attempt at applying a lesser known immune defence strategy for treating human infections

Introduction

While the COVID-19 viral pandemic has dominated news headlines over the past few years, it is important not to overlook the "silent" pandemic caused by bacterial pathogens. Bacteria, increasingly resistant to last-line antibiotics, are projected to cause 10 million deaths annually by 2050, which far exceed cancer-related deaths. Urgent efforts by researchers are underway to explore different strategies for developing new antibiotics to tackle this global healthcare issue. A significant emphasis is placed on medications with novel ways of combatting bacteria resistance and maintaining efficacy over time. One commonly explored avenue is the use of antimicrobial peptides (AMPs), which draw inspiration from host defence peptides found in nature. However, progress in producing clinically useful AMPs that can be applied to treat human infections has been hampered by a number of challenges. The primary obstacle lies in the vulnerability of these peptides to degradation by enzymes found in the human body.

Nanonets formation – a unique way the human body combats infections

In nature, the trap-and-kill is an immune defence mechanism employed by various species, including humans, as an effective strategy to combat infections. In response to the presence of pathogens, peptides are released from human cells. These peptides readily self-assemble in solution to form an interlinked network of very thin fibrils, together known as "nanonets". These nanonets are effective at trapping the bacteria cells and make them easier to kill by the immune defence system. Compared to soluble peptides, the peptide molecules in their nanonets form exhibit a generally stronger structure and display enhanced resistance to the action of human enzymes. This characteristic is particularly appealing since it addresses a major challenge of AMPs development.

Recognising the potential of peptide nanonets, several research groups have tried to design synthetic versions as a promising avenue for addressing the global healthcare challenge of antibiotic resistance. Unfortunately, the field is still in its early stages of development. Notably, current prominent works have only resulted in the development of disjoint short nanofibrils which unfortunately are less capable of capturing the bacteria compared to expansive nanonets. Besides, these synthetic designs lack control over the nanonets formation process. This means that their peptides indiscriminately form nanofibrils even when no bacteria cells were present in the mixture.

Learn from the best, then take the next step

In the history of antibiotic development, scientists have continuously learned from, and attempted to mimic immune defence mechanism observed in nature. This approach has a proven track record of leading to the discovery of clinically useful medications. Noticing shortcomings in both naturally occurring peptide nanonets and existing synthetic designs, our research group aimed to apply prior knowledge to design our in-house synthetic nanonets, with improved attributes and greater suitability for use in human treatments.

We succeeded in designing short β-hairpin peptides, comprising of 15 to 16 amino acids that are capable of self-assembling into nanonets selectively in response to endotoxins, which are integral membrane components unique to bacteria (see Figure 1). These synthetic peptide nanonets displayed a dual functionality, combining effective trapping and antimicrobial killing mechanisms, hence presenting direct improvements when compared to the "trap-only" nanonets found in nature. The unique combination of achieving both functions and a specificity towards bacteria distinguishes our synthetic peptides from their counterparts in the field.

Furthermore, we demonstrated that both the degree of killing and trapping activities could be finetuned by simply changing one or two amino acids at the hairpin turn region of the sequence. Of interest, our peptides were effective at killing bacteria that are even resistant to last-line antibiotics, affirming their clinical relevance. This attribute of functional tunability is a testament to the broad applicability of our research findings.

After showing that the peptides were effective against pathogens in laboratory settings, we tested them in animal models, in a collaborative effort with Associate Professor Rajamani LAKSHMINARAYANAN from the Department of Pharmacy, NUS. The results yielded promising results. Our synthetic peptide nanonets were effective at reducing bacteria burden from infections in the abdominal region, hence limiting their spread to the bloodstream. Importantly, the peptides demonstrated an absence of toxic effects in the murine models, as determined from meticulous observation of their behaviour and analysis of their blood and urine components.

In instances where bacterial endotoxins enter the bloodstream in high quantities during infections, excessive reactions from the human immune defence can

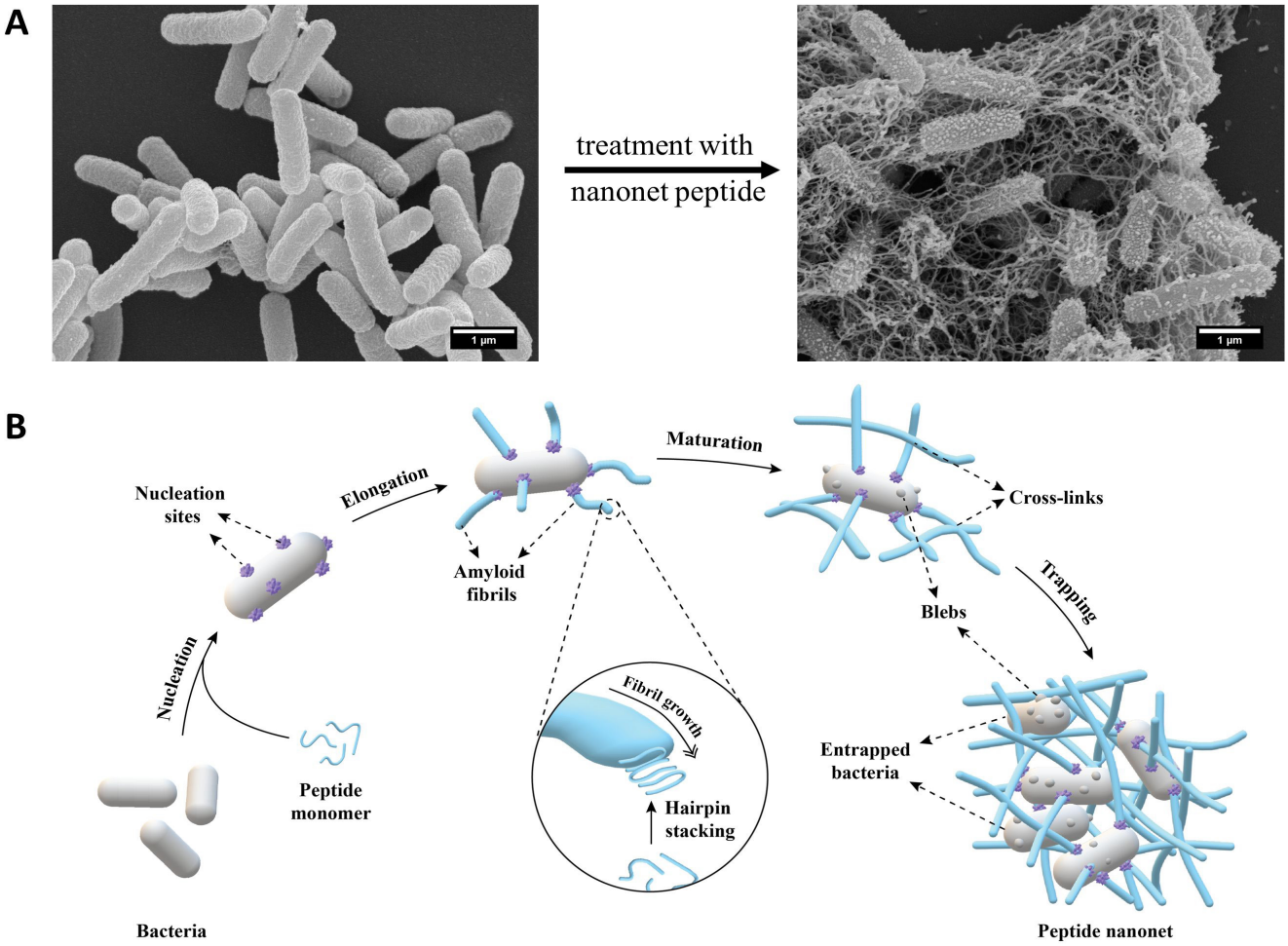

Figure 1: (A) Scanning electron microscopy images of Escherichia coli bacteria before and after treatment with the nanonet-forming peptide. (B) Schematic representation of the nanonets formation process. [Credit: Advanced Functional Materials]

cause serious self-inflicted damages, and in some cases, fatal outcomes. In a follow-up work in collaboration with Professor Fred WONG from the Department of Pharmacology, NUS, our exploration extended to investigating an additional useful function of the peptides in mitigating such undesirable inflammatory responses from the human body.

Capitalising on the high positive charges shared among our peptides, we hypothesised that they would be able to selectively capture bacterial endotoxins and pro-inflammatory mediators in the human body, which are mostly negatively charged. This concept of charge-based interactions, still relatively underexplored in anti-inflammatory treatments, offers performance advantages over the traditional strategy of targeting specific mediators. Our research successfully showed the anti-inflammatory activity of our peptide nanonets in diverse settings, including laboratory environments, mammalian cell lines and, notably, in an acute lung inflammation murine model.

With the potential to address antibiotic resistance and mitigate inflammatory responses, the promise of our synthetic nanonets brings us one step closer to a future where infectious diseases are met with resilience and precision inspired by the intricate wisdom of nature.

For more details, please visit:
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Transformative therapeutic drug delivery

Bridging the gap from bench to bedside to improve healthcare outcomes and address unmet medical needs

Introduction

In a rapidly evolving healthcare landscape, translating research findings from the bench to the bedside is at the core of unlocking transformative change. Nanomedicine, a field that has been at the forefront of scientific research for more than three decades, offers the potential for delivering impactful therapies that go beyond academic recognition, as demonstrated during the COVID-19 pandemic.

To bring about such a significant transformation, additional advancements are necessary to elevate this singular occurrence — a global pandemic coinciding with a market-ready delivery strategy — into novel platform technologies that streamline the entry of new therapies into the market. While computational methods have been instrumental in facilitating such transformations in other areas, their application to the diverse and interdisciplinary field of nanomedicine is still in its early stages [1].

Our research explores the interface of nanotechnology-enabled drug delivery and data science, marking not only a pathway for streamlining the development of new medicines using bioprediction but also facilitating easier access to follow-on products and generic medications. By forging connections between nanomedicine and computational advancements, we strive to unlock new frontiers to enhance the efficiency and accessibility of therapies.

In vitro release testing: A fingerprint of drug delivery systems

For the development of medications, *in vitro* release testing assumes a critical role in the evaluation and selection of drug formulations, guiding their progression into clinical trials. It involves studying how a nanomedicine is released from its delivery system in a controlled laboratory environment, simulating various aspects of its behaviour. This technique generates unique profiles, reminiscent of fingerprints, that capture the interactions among different elements within a dosage form and reflect the inherent mechanics of the delivery system.

For instance, this method is commonly used to compare generic medications with their brand-name counterparts, showcasing their similarities. Generic medications, which are designed to mirror brand-name medications in composition and functionality, often experience a streamlined approval process. In certain cases, computational methods aided by *in vitro* release testing have enabled virtual bioequivalence assessment for generic medications, reducing the need for extensive preclinical and clinical testing. This translates to significant cost savings in the healthcare sector and has attracted the attention of pharmaceutical manufacturers worldwide.

The inherent advantages of utilising *in vitro* release testing as a tool in the development of medications stem from their biopredictive capabilities. By leveraging an expanding knowledge base, these methods have the ability to pinpoint promising formulations for clinical testing by emulating important aspects of the human physiology.

Bioprediction: Accelerating nanomedicine development with data science

One key challenge in nanomedicine development lies in the time-intensive process of testing them, progressing from animal models first and then in clinical trials. Bioprediction emerges as a potent strategy to address this issue. It combines data science with *in vitro* release testing, yielding valuable insights into nanomedicine behaviour. Through the integration of data analytics and computational models, we can forge meaningful predictions about nanomedicines in real-world scenarios and forecast the impact of their properties on clinical performance [2]. This innovative approach not only expedites the development of nanomedicine but also accelerates the creation of life-saving therapies for the wider population.

The latest generation of drug molecules, such as peptides, proteins or nucleic acids, exhibit increased susceptibility to degradation within the human body. These compounds could benefit from the protective shield offered by a nanodelivery system. Consequently, it becomes even more important to provide the appropriate toolkit for swiftly translating these therapeutics from the laboratory bench to the patient's bedside.

Understanding *in vitro-in vivo* correlations (IVIVCs)

Establishing robust *in vitro-in vivo* correlations (IVIVCs) is of paramount importance to validate the predictive capacity of *in vitro* assays and ensure clinical success. IVIVCs serve as bridges connecting *in vitro* data obtained through laboratory experiments with the *in vivo* performance observed during clinical trials. These correlations provide crucial insights into the behaviour of nanomedicines within the human body, bridging the gap between preclinical findings and effective clinical treatments.

Investigating nanomedicine dynamics to overcome brain delivery challenges

A sophisticated simulation of physiological reality, which involves

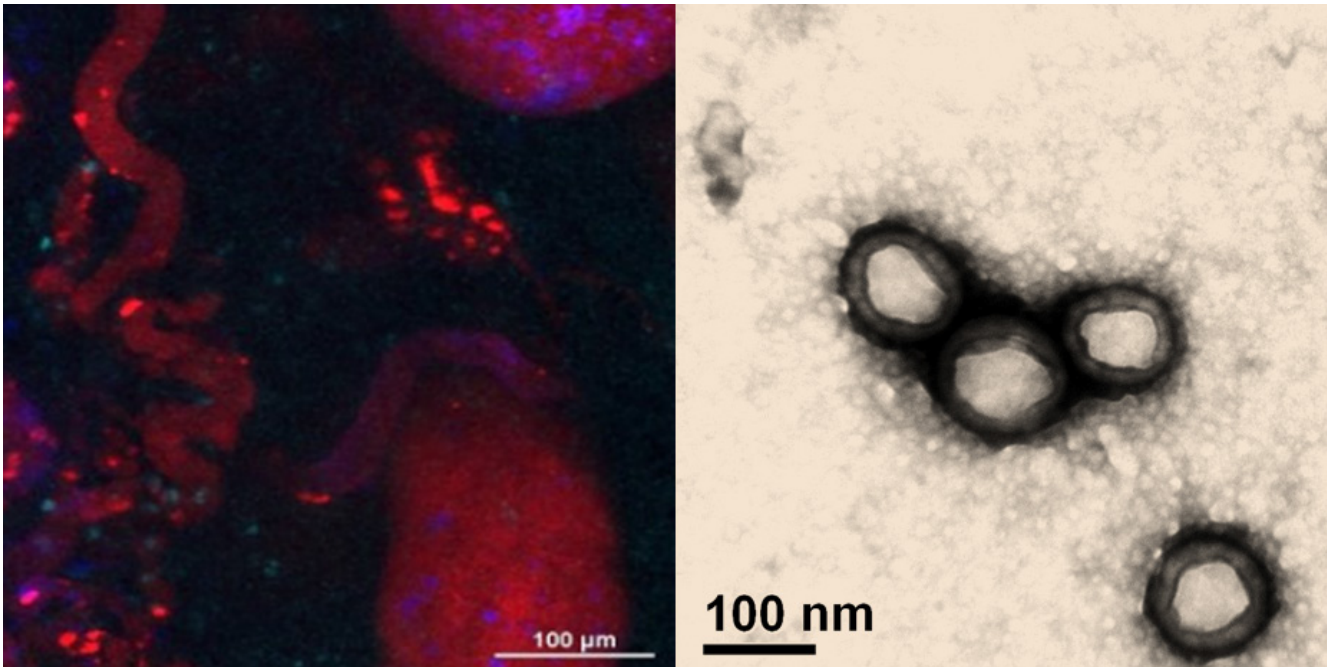

Figure 1:Figure 1: Visualisation of nanoparticle delivery: (Left) Fluorescent overlay of Doxorubicin-releasing nanoparticles in the blood vessel of murine models [Credit: Julia MALINOVSKAYA]; (Right) Transmission electron micrograph of polylactide co-glycolide nanoparticles post-preparation.

integrating multiple data sources, is essential for comprehending and replicating the dynamics of nanomedicines within the body. In our research endeavours, we leverage various data sources, such as real-time images captured within animal blood vessels, alongside measurements of drug concentrations in diverse tissues.

While imaging methods provide a detailed view of the dynamics within blood vessels and enable a clearer understanding of the targeted delivery, pharmacokinetic studies and related model estimations allow for the quantitative analysis of accumulation in various tissues at a high resolution [3]. To obtain such live imaging data, intravital microscopy in the tumour area is a suitable tool. It provides the intensity signals of the drug and the carrier in the blood vessels, which can be combined in the computer simulation. The result is a holistic view of the accumulation, elimination and release behaviour in these blood vessels [4].

These resources facilitate the exploration of the time-sensitive and location-specific behaviour of drug carriers. Notably, our recent investigations have concentrated on the challenge of delivering medications to the brain, a common issue in drug discovery.

The brain is safeguarded by a barrier that restricts the entry of medications, thereby limiting the effectiveness of numerous therapeutic agents. Through meticulous and comprehensive analysis, we unveil critical insights into novel pathways for delivering medications to the brain, potentially revolutionising the treatment landscape for life-threatening conditions like stroke, Alzheimer's disease and brain cancer.

Conclusion

In summary, our research at the nexus of nanotechnology-enabled drug delivery and data science highlights the immense transformative potential inherent in the field of nanomedicine. Looking ahead, the convergence of nanomedicine and computational advancements holds the key to unlocking novel frontiers, poised to catalyse a paradigm shift in healthcare solutions.

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Small fish models for human bone diseases

Studies in laboratory fish discover new cell signalling pathways and therapeutic drugs to control bone health

Introduction

Bones are very dynamic organs that undergo constant remodelling to preserve their rigidity. In healthy individuals, an aged and less rigid bone matrix is continuously removed by bone-resorbing cells, named osteoclasts, and immediately replaced by new bone matrix that is produced by bone-forming cells, called osteoblasts (see Figure 1). In our bodies, this process of bone remodelling is tightly controlled by signalling pathways that bone cells use to communicate with each other. While our understanding of these pathways has improved tremendously over the past years, there are still many questions that remain open.

One such question is how dysregulation of these signalling pathways leads to bone diseases, and whether therapeutic drugs can target these newly identified pathways to prevent or cure such conditions. Addressing such questions requires the use of animal models to investigate how newly identified factors function in bone health and disease. While most studies have been performed using murine models, the medaka and zebrafish, which are small laboratory fish have become more popular recently in bone research. They are used to model human bone diseases, characterise novel bone genes and screen for therapeutic drugs that promote bone health.

The skeletal structure looks very different in humans and fish. For example, fish lack long bones that are part of our limbs. Despite these differences, the genetic pathways controlling bone formation and maintenance are remarkably similar. This similarity offers unique opportunities, as fish embryos and larvae are translucent, enabling the analysis of dynamic bone cell behaviour by live imaging in intact specimen (see Figure 2). This transparency allows a detailed investigation into how bone cells interact with each other and respond to different disease-relevant conditions.

Additionally, fish offer practical advantages in research. They produce an abundance of embryos that can be genetically modified and are amenable to drug treatments simply by adding substances to the surrounding water, eliminating the need for injections or other invasive procedures. Over the past decade, research using fish models has led to a number of important discoveries that contributed to a better understanding of bone biology, not only in fish but also in mammals.

Our team at NUS has recently identified a novel bone cell communication pathway in medaka fish that is essential for controlling osteoclast formation under osteoporotic conditions.

Osteoporosis – a common human bone disorder that mostly affects the elderly

Osteoporosis is a prevalent bone disorder that is characterised by reduced bone mineral density and increased fracture risk. Worldwide, nearly one in three women and one in five men aged 50 and above are affected by osteoporosis. These individuals have an increased risk for fragility fractures, mostly in the hips, wrists and vertebrae. These fractures not only inflict considerable pain but also significantly diminish the quality of life, posing an immense burden on patients, their families and public healthcare systems.

The underlying cause of osteoporosis lies in an imbalance of bone cell activity, where bone-resorbing osteoclasts become more abundant and are more active than bone-forming osteoblasts. Consequently, bone resorption becomes dominant over bone formation, resulting in weakened bones. Once diagnosed, current therapies typically adopt a combinatorial approach of providing medications that stimulate bone formation, followed by those that prevent or reduce bone-resorbing activity. However, both categories of medications come with various side effects, most importantly an increased fracture risk after long-term use. Hence, there is an urgent need for improved and safer treatment options.

Studies in a medaka fish discover the chemokine CXCL9 that controls osteoporosis

The development of new osteoporosis treatments is hindered by a limited understanding of how bone cells communicate with one another to achieve balanced cell activities needed to maintain healthy bone. Osteoblasts have long been known to release a signalling factor named “receptor-activator of NF-kappa beta ligand” or RANKL, that is required to activate osteoclast precursor cells. However, the additional factors needed to initiate osteoclast recruitment and activation have remained unknown.

In our research at NUS, we induced osteoporosis in medaka experimentally by excessive RANKL activation, and used this model to identify novel factors that are important for osteoclast activation. We identified a small protein, the chemokine CXCL9, and found that it plays an indispensable role in recruiting osteoclasts to the bone matrix [1]. CXCL9 is produced and released from osteoblasts located on the surface of bone matrix. Under osteoporotic conditions, CXCL9 diffuses towards reservoirs that hold osteoclast precursors, the macrophages (see Figure 1). These osteoclast precursors produce a receptor, CXCR3, on their

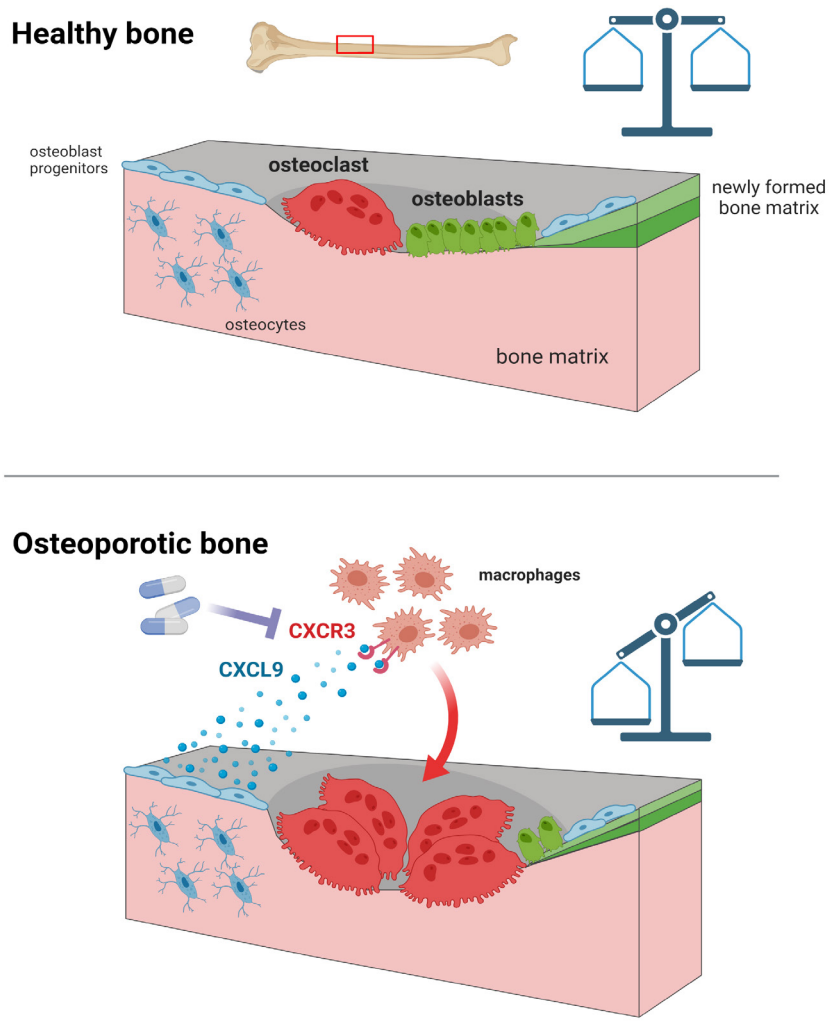


Figure1: In healthy bone (top), a balanced activity of bone-forming osteoblasts and bone-resorbing osteoclasts ensures controlled bone turnover, which is needed to maintain a healthy bone matrix. Under osteoporotic conditions (bottom), osteoblast progenitor cells release the small protein CXCL9, which binds to CXCR3 receptors on the surface of macrophages. These macrophages then get recruited to bone matrix, where they turn into activated osteoclasts. As a result, excessive bone matrix resorption could not be compensated by bone formation, leading to reduced bone mass and fracture-prone bone. The process of macrophage recruitment can be blocked by medications that prevent CXCR3 activity.

cell surface. Upon binding to CXCL9, the osteoclast precursors are mobilised and migrate long distances in a highly directed fashion towards the bone matrix, where they start maturing into fully activated osteoclasts responsible for bone resorption (see Figure 2).

While both CXCL9 and its receptor CXCR3 have been recognised to modulate the migration of immune cells to inflammation sites, for example in psoriasis and rheumatoid arthritis, their role in osteoporosis and recruitment of bone cells, however, is unknown. Interestingly, despite the limited success of chemical inhibitors targeting CXCR3 in clinical trials for psoriasis, our study showed that the small-molecule inhibitors AMG-487 and NBI-74330 were highly effective in blocking osteoclast recruitment and protecting bone from osteoporotic effects in the medaka model.

Elevated CXCL9 blood levels reliably predict the risk of osteoporotic hip fractures in men

In medaka fish, CXCL9 and its receptor CXCR3 are indispensable for osteoclast activation and blocking this signalling pathway efficiently prevented osteoporosis in the fish model. However, no study had analysed a possible link between CXCL9 and osteoporosis or fracture risk in human patients.

As a first step towards our goal of translating the model fish findings into a clinical context, we started a collaboration with Professor KOH Woon Puay from the School of Medicine, NUS. With her team, we conducted a matched case–control study and analysed CXCL9 levels in blood serum samples that were obtained from 55 men and 119 women who had experienced a hip fracture with an average of 6.3 years after their blood was collected [2]. The participants were matched individually to controls who did not develop hip fractures, but had identical age, weight and diabetic status.

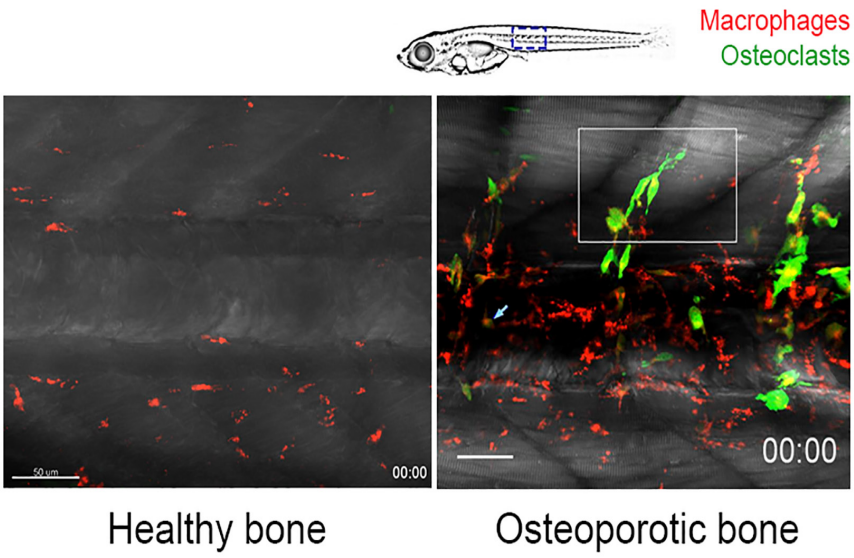
Importantly, we observed considerably higher blood levels of CXCL9 in the pre-fracture blood samples from men who later experienced hip fractures compared to their non-fracture controls. Unexpectedly, this difference was only seen in men but not for the participating women. This gender-based difference in the results may be explained by changes in sex hormone levels during ageing, potentially influencing the levels and effects of CXCL9 in older men and women.

Together, these studies demonstrated that blood levels of CXCL9 can reliably predict the risk of osteoporotic hip fractures in elderly men. Our findings also serve as a proof-of-principle that studies in a fish model can discover novel players in bone homeostasis that are relevant in humans. Moreover, our findings open the possibility for early interventions targeting CXCL9 or CXCL9-CXCR3 signalling to prevent hip fractures in older men.

Rare bone diseases that lead to abnormal bone formation in soft tissues

While common bone diseases, such as osteoporosis, have always attracted strong interest and research efforts from the pharmaceutical industry,

the same level of attention is often lacking for rare bone disorders. One notable exception is Fibrodysplasia Ossificans Progressiva (FOP). This is a skeletal disorder that mainly affects children and occurs with a frequency of 1 in 2,000,000 individuals. FOP is characterised by abnormal ossifications at heterotopic sites, occurring outside of bone in connective tissues such as muscle and tendons. These localised and painful bone formations often lead to severe mobility impairments and reduce the life expectancies of affected individuals. FOP-induced ossifications usually occur in episodes or "flare-ups" that are frequently linked to events of injury or inflammation.



Live imaging of macrophage recruitment to the vertebral bone of osteoporotic medaka fish. In healthy larvae (left), only few macrophages (red) are visible in the vertebral column, where they mostly perform immune functions. However, under osteoporotic conditions (right), macrophages are recruited to the vertebral column. Their numbers are strongly increased and they are activated to become bone-resorbing osteoclasts (green). This process of recruitment and activation is triggered by CXCL9. [Image credit: Phan Quang Tien]

Recently, much progress has been made for the development of therapeutic drugs to lower the incidence of flare-ups, with three of them (Saracatinib, Garetosmab, Palovarotene) currently in clinical trials or already approved for use. Despite this promising therapeutic progress, the underlying mechanisms leading to abnormal bone formation in FOP patients remain unclear. It is unknown which cells produce the heterotopic bone, their activation processes and the connection between abnormal ossification and local inflammation. A deeper understanding of these processes is likely to help in developing novel therapeutic strategies that can overcome the limitations of the current medications, which often exhibit considerable unwanted side effects.

FOP is caused by mutations in a single gene that encodes a cell signalling receptor, the Activin receptor ACVR1. Interestingly, medaka fish have a very similar receptor exhibiting not only an almost identical structure but also behaving in a similar manner to the human ACVR1 receptor in biochemical assays. This opens a unique opportunity to use the translucent medaka embryos and larvae to better understand the processes leading to FOP, including identifying the origin of cells that cause aberrant bone formation in soft tissues. Once identified, the molecular makeup of these cells can be characterised to investigate their response to local inflammation. Together, this will facilitate the screening for novel compounds that specifically target the newly identified cells, paving the way for the development of new drugs with less side effects than current approaches.

Conclusion

In conclusion, small fish models have been proven invaluable in identifying novel molecular pathways and small molecular compounds controlling processes needed to maintain healthy bone. Many of these processes are very similar in fish and humans, offering an opportunity for drug screening in fish to identify compounds that can be developed as therapeutic targets for human diseases.

For more details, please visit: https://www.dbs.nus.edu.sg/staffs/christoph-winkler/

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