From mechanism to management

Dr Branko Stefanovic gives an insight into his studies on potential antifibrotic drugs, proposing some of the applications of this work and identifying his goals for future research

Could you discuss your latest research endeavour, citing its main goals and vision? From what context did this project emerge?

Fibrosis, as the primary disease, can affect many organs, but the liver is the most common. The end stage of liver fibrosis is cirrhosis. The mechanism of excessive synthesis of type I collagen in fibrosis, as the driver of pathogenesis of fibrosis, is similar in all organs, so the knowledge we obtain studying liver fibrosis can easily be translated to fibrosis of other organs. This will make a whole spectrum of fibroproliferative disorders amenable to therapy. The project emerged from the concept that the initial event that triggered fibrosis is not the main culprit of the pathological changes, instead it is an excessive synthesis of type I collagen in response to this event. For example, it is not the presence of the Hepatitis C virus in the liver that kills patients, it is the fibrosis which develops as a liver cell response to the virus. So, the vision is to directly target collagen biosynthesis by drugs.

In theory, how might an antifibrotic drug work? Have there been any previous trials of such drugs?

There are no approved antifibrotic drugs. In theory, an antifibrotic drug should decrease biosynthesis of type I collagen in fibrotic

Fighting fibrosis

Researchers at the College of Medicine, **Florida State University**, USA have identified and tested a candidate compound capable of inhibiting the synthesis of type I collagen. If approved, this drug would represent the world's first antifibrotic compound and would be relevant to the treatment of millions of patients worldwide

AUTOPSY STATISTICS SUGGEST that as much as 50 per cent of the world's population will experience fibrosis during their lifetime. Fibrosis is a general term given to the build-up of scar tissue in organs. If that scar tissue becomes extensive, it is manifested as fibroproliferative disease. Of the fibroproliferative diseases, the most common is hepatic fibrosis – the build-up of scar tissue in the liver. There are several known causes of hepatic fibrosis, such as the hepatitis B and C viruses, alcoholism and obesity. Despite the diversity of cause, the mechanism of scarring remains the same; the body over-produces type I collagen, which is deposited as insoluble fibrils. The resilience and insolubility of these fibrils make treatment extremely difficult and healthcare strategies are limited only to managing symptoms. In the case of hepatic fibrosis, the only known cure for advanced stage disease (cirrhosis) is liver transplantation.

In light of the prevalence and challenges of managing fibroproliferative diseases, it is clear that any new breakthroughs in treatment and/or prevention would be profoundly valuable to patients, healthcare providers and society in general. To this end, Dr Branko Stefanovic and his colleagues from the College of Medicine, Florida State University, USA have spent the last 20 years attempting to elucidate the biochemical mechanisms facilitating the process of internal scarring. That work has now began to pay dividends in dramatic fashion, through the discovery of a compound with the potential to inhibit fibrosis and as such have huge social and medical benefits.

ELUCIDATING THE MECHANISM

Stefanovic's research goal for the last two decades has been ambitious yet focused: "The ultimate objective of the research is to find a cure for fibrosis (excessive scarring)," he explains. The mechanism that causes fibrosis is known to be similar across most tissues

tissues and not affect normal tissues. By decreasing collagen synthesis the progression of fibrosis should be retarded or completely prevented. An alternative approach would be to increase elimination of type I collagen from the tissues, but this is difficult, because the protein is insoluble and, once deposited, it can only be removed by special enzymes. These enzymes cannot be used in therapy.

What are the difficulties of this work?

Strong basic research in the biology of fibrosis must first be established. Then, the work has to focus on relevant targets for drug development. The difficulties are that a successful antifibrotic drug must target only collagen synthesis in fibrotic tissues and not in normal tissues, and it must be specific, effective, nontoxic and easily available and affordable. This is a lot to ask. Development of a chemical compound with such properties requires integration of basic molecular biology research to the drug development process. This is a lengthy process, requiring careful and critical verification of the results, repeated trials and coordinated effort of specialists.

How far along is your research and what are the next steps?

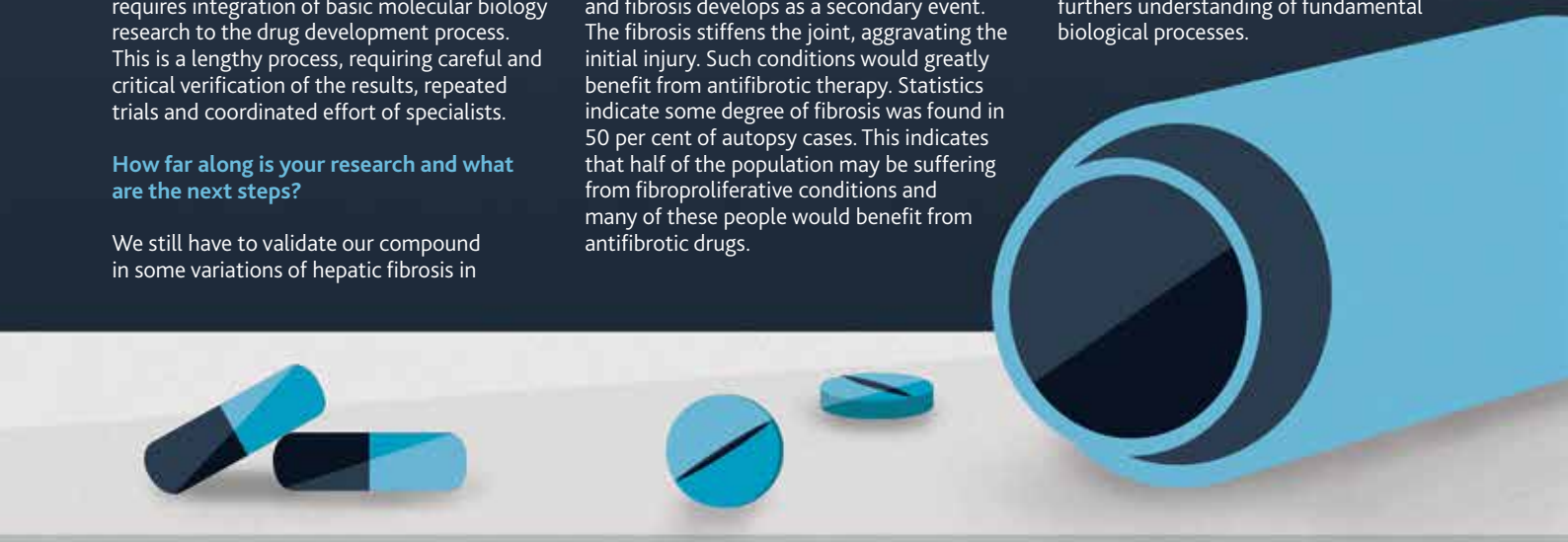
We still have to validate our compound in some variations of hepatic fibrosis in experimental animals. There is also a possibility that its potency can be further enhanced, so chemical modifications of its structure and additional testing of the derivatives will be performed. The compound will also be tested in animal models of other fibroproliferative diseases, like scleroderma, pulmonary fibrosis or heart fibrosis. We are fully committed to accelerate the process and clinical trials will follow as soon as possible.

Are there any broader applications of this latest research in fibrosis?

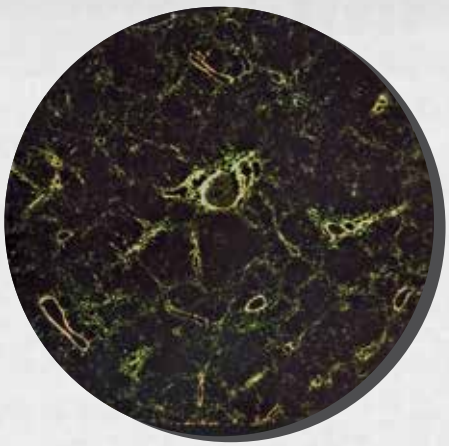
Subclinical fibrosis is very common. Fibrosis does not have to be a primary disease, it can be associated with other diseases, representing a co-morbidity. For example, osteoarthritis, one of the most common conditions in humans, is caused by loss of cartilage in joints. The resulting mechanic injury to the bone promotes inflammation and fibrosis develops as a secondary event. The fibrosis stiffens the joint, aggravating the initial injury. Such conditions would greatly benefit from antifibrotic therapy. Statistics indicate some degree of fibrosis was found in 50 per cent of autopsy cases. This indicates that half of the population may be suffering from fibroproliferative conditions and many of these people would benefit from antifibrotic drugs.

Can you elaborate on your contributions to the understanding of biological processes?

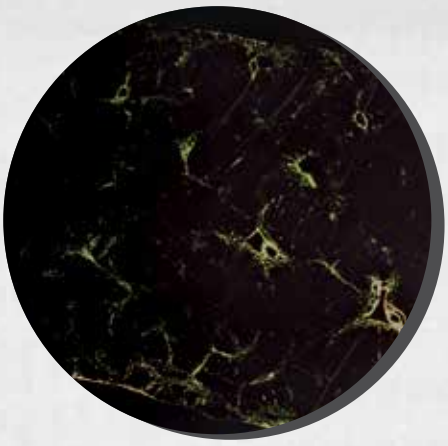
My research has not only resulted in an original approach to find antifibrotic drugs, but has also contributed to the basic understanding of biosynthesis of complex proteins. Vertebrate organisms have larger body sizes than lower organisms mainly due to their ability to synthesise type I collagen. Incidentally, the 5' stem-loop structure is found only in vertebrate collagen mRNAs, while invertebrate collagen mRNAs lack this structure. It has always been assumed that mRNA carries only the coding information for a protein. The collagen example indicates that mRNA also carries the instruction on how to make a protein. Namely, how, where and when to recruit the synthetic machinery so that the protein is synthesised in the right place, at the right time and in a proper manner. Thus, studying such mechanisms furthers understanding of fundamental biological processes.



(regardless of the agent of cause), and for this reason the team saw an opportunity to tackle a wide range of conditions by targeting one pathway. During the early stages of their work the researchers attempted to elucidate the key players in the mechanism of type I collagen synthesis. Many of the steps in this process are generic and shared amongst many pathways, making them unsuitable for drug development, as any compound inhibiting these processes would also deter other untargeted pathways. As such, the aim of the group was to find highly specialised and unique steps in the fibrosis pathway that could be targeted with high specificity. This was provided by the existence of a highly specialised mRNA – protein interaction, as Stefanovic expands: "The collagen mRNA, which is the molecule that carries the genetic instruction to make collagen protein, contains a specific structure, which we termed the 5' stem-loop". Fortuitously, this structure is not found in any other mRNA, making it unique to collagen synthesis. Subsequently, the protein tasked with binding to the 5' stem-loop, is similarly specialised. This protein, called LARP6 and its partner mRNA, provided the team with a suitable interaction for drug development.



Advanced liver fibrosis in non-treated liver.



Reduced fibrosis in liver treated with our drug.

A BESPOKE ASSAY

Empowered with the identification of this targetable interaction, Stefanovic and his colleagues began to consider the screening of potential compounds for the inhibition

INTELLIGENCE

REGULATION OF TYPE I COLLAGEN IN HEPATIC FIBROSIS

OBJECTIVES

To elucidate the unique mechanism of collagen synthesis in hepatic stellate cells (HSCs) and identify the regulatory roles of the factors involved, as potential targets for development of antifibrotic drugs.

PARTNERS

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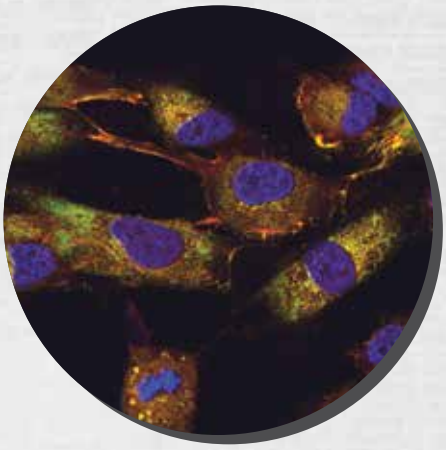
BRANKO STEFANOVIC received his BSc in Medical Biochemistry from the University of Zagreb, Croatia in 1978 and his PhD in Molecular Biophysics from the Florida State University (FSU), Tallahassee, USA in 1991. After earning his doctorate in molecular biophysics at FSU in 1991, he undertook postdoctoral training at the University of Bern, Switzerland.

Stefanovic is a tenured professor who conducts research in the area of molecular mechanisms of liver fibrosis. He also teaches a class on 'the molecular mechanism of common human diseases for graduate students' and facilitates small groups in pharmacology, biochemistry and physiology for medical students. He joined the faculty of the FSU College of Medicine in August 2002 after serving for eight years as a research assistant professor in the Division of Digestive Diseases and Nutrition in the Department of Medicine at the University of North Carolina – Chapel Hill.

By employing their new assay, the researchers were able to identify several compounds which exhibited the ability to inhibit the target interaction

of protein and mRNA binding. This in itself represented a significant challenge; no other antifibrotic drugs yet exist and, as such, no suitable assay has been developed to screen candidates. In order to meet this need, the Florida team developed a completely unique and bespoke assay, capable of screening each compound's ability to inhibit the binding of LARP6 to collagen mRNA 5' stem-loop. "The assay was critical to screen the large number of drug-like chemical compounds," highlights Stefanovic. "Without this assay the discovery of drugs with the ability to target LARP6/5' stem-loop binding would not have been possible."

By employing their new assay, the researchers were able to identify several compounds which exhibited the ability to inhibit the target interaction. Of this group, one particular compound stood out as an exciting candidate. The compound was able to inhibit the synthesis of type I collagen in both liver cells and other cells responsible for this process. Stefanovic and his colleagues then expanded their experiments: "We further tested the compound in liver organ culture and in animal models of liver fibrosis," explains Stefanovic. "In both experimental systems the compound showed remarkable potency." Alongside the impressive efficacy of this compound in animal models, it also seemed to exhibit little or no biological toxicity, with no side effects observed. In combination, the low toxicity and high efficacy of this potential drug in animal models make it an exciting candidate for early stage clinical trials.



Collagen synthesis by fibrotic cells (yellow).

CLINICAL TRIALS

Laboratory work in animal models is still required before clinical trials can begin, but Stefanovic is hopeful that this will be completed soon: "I hope to finish the pre-clinical evaluation of our drug within a year". Following this, trials will hopefully commence. Standardised clinical trial design is based on the 'phase 1, phase 2 and phase 3 (phase 4 post-release)' model and usually takes an average of 12 years to bring the drug to market and ultimately the patient. While this option remains open to the Florida team, Stefanovic believes that their compound may be suitable for the Food and Drug Administration (FDA)'s expedited development scheme, which offers a faster trial programme for drugs that promise significant improvement on current treatment for life-threatening disease or disease which is currently untreatable.

While work continues on their candidate compound, the researchers are keen to expand their investigation to elucidate more candidates and establish the impact that current and future drugs may have on a wider range of fibroproliferative conditions. In order to achieve the former of these goals, the group has returned its focus to the elucidation of the pathway concerning the synthesis of type I collagen: "LARP6 interacts with several accessory proteins and these interactions facilitate collagen synthesis. We are characterising and precisely mapping these interactions to discover points of contact between these proteins, as possible sites for drug intervention," explains Stefanovic. While this may seem unnecessary in the context of the promising results achieved with their current compound, the team consider diversity essential particularly as multiple drugs and targets can exhibit synergistic effects or different safety profiles.

The elucidation of the biochemical pathway facilitating the production of type I collagen represents a commendable scientific breakthrough. However, when combined with the identification and illustration of a compound capable of inhibiting this mechanism and thus treating fibroproliferative conditions, this development becomes profound medical and social progress – potentially relevant to the entire world. The long-term hope is that this compound will become the first-of-its-kind antifibrotic drug, offering hope to patients with fibroproliferative and related disease.