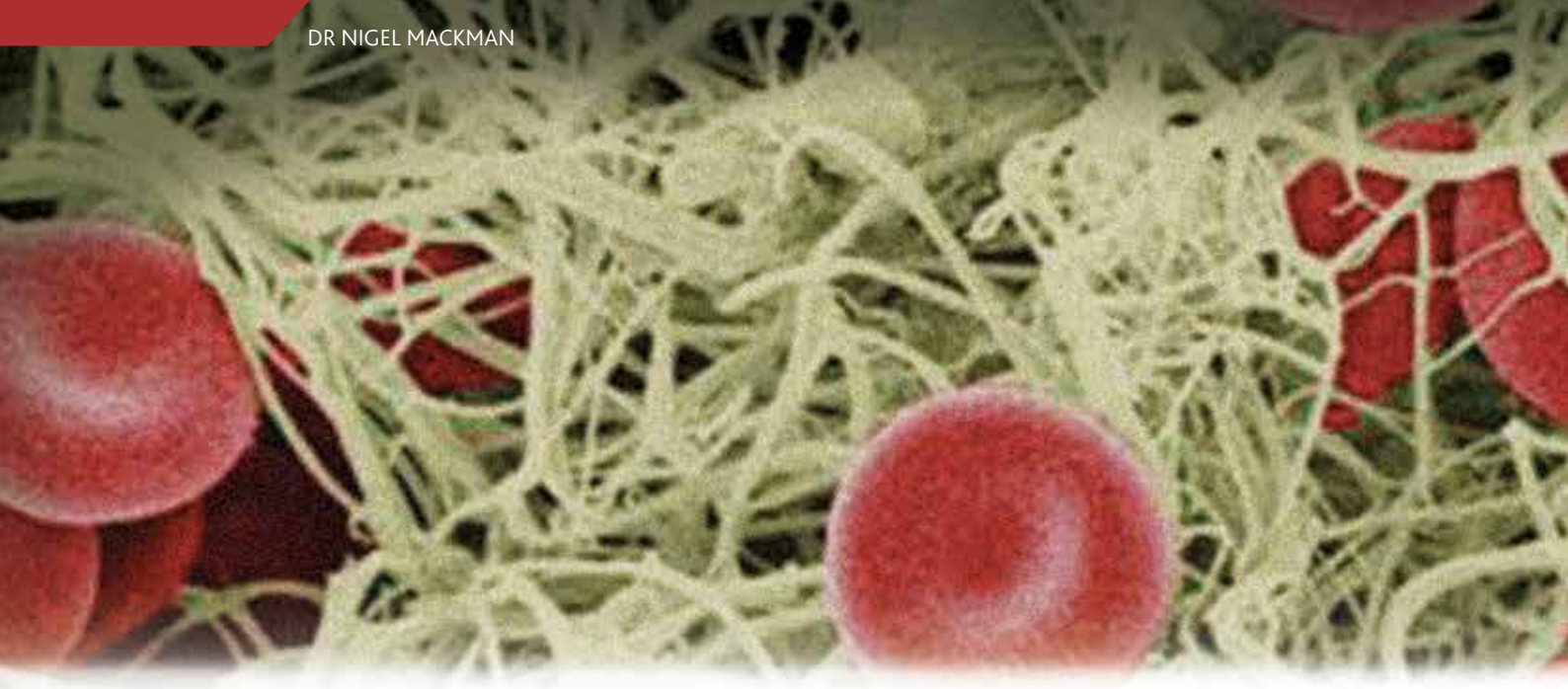

Pathways of thrombosis

Researchers from the Mackman laboratory at the **University of North Carolina at Chapel Hill** are studying an essential gene in search of new and better anticoagulant therapies to reduce the incidence of vein thrombosis

VENOUS THROMBOEMBOLISM (VTE) encompasses deep vein thrombosis (DVT) and pulmonary embolism (PE). DVT most often occurs in the leg. If a portion of the clot breaks off (embolises) it travels to the lung and can cause a PE. Blood clots can be asymptomatic or symptomatic (leg swelling). Anticoagulants are used to prevent and treat VTE, but they are not without their risks. The major side-effect of current anticoagulant drugs is bleeding.

Risk factors for VTE include ageing, obesity, cancer, pregnancy, hormone-based contraceptives, inactivity (bedrest), acute infection, surgery and smoking. There are also genetic risk factors, such as blood type and a mutation of a blood coagulation protein (factor V) which occurs in about five per cent of the Caucasian population. Having more than one risk factor significantly increases the chances of developing VTE. The condition is also a leading cause of death or disability among cancer patients, as both the cancer itself and therapies used to treat it can exacerbate the risk of blood clotting.

"The Holy Grail is generating effective anticoagulant drugs that do not have the side-effect of increasing the risk of bleeding," asserts Nigel Mackman, the John C Parker Distinguished Professor of Medicine at the University of North Carolina at Chapel Hill (UNC). Mackman is Director of the UNC McAllister Heart Institute there and his laboratory focuses on tissue factor (TF) – the primary cellular initiator of blood coagulation.

TF is expressed in the skin and around blood vessels to stop bleeding when body surfaces and vessels sustain damage. Increased levels of TF likely trigger thrombosis in a wide range of diseases, so the scope of Mackman's research ranges from cancer to atherosclerosis and viral infections.

EXPERIMENTAL APPROACH

"One of the challenges with studying venous thrombosis is that there are many risk factors that together conspire to induce a clot," Mackman outlines. "Another challenge is that we lack good animal models." His laboratory predominantly uses mice for *in vivo* experiments, alongside *in vitro* methods. VTE in humans most often occurs in valve pockets in large veins, but because of their size, the equivalent is difficult to model in mice.

Mackman and collaborators found that mice lacking TF did not survive. Therefore, he instead engineered mice with very low levels of TF to study its role in haemostasis (arrest of bleeding). He also generated mice with the TF gene deleted in various cell types. The modified mice have provided new insights into the role of TF, leading Mackman and his team to propose a model in which the extrinsic (ie. TF) and intrinsic pathways regulate bleeding in different tissues: "Importantly, individuals with haemophilia (who have a defect in the intrinsic pathway) bleed into joints and muscle, which express very low levels of TF. This clinical observation supports our proposal," he underlines.

The Mackman laboratory has additionally used mouse models of various diseases and conditions, including endotoxaemia and ischaemia-reperfusion injury, to analyse how the coagulation cascade enhances inflammation. Key connections between the two pathways are protease-activated receptors (PAR) that are activated by coagulation proteases. From such analyses, Mackman has proposed that inhibition of PAR-1 represents a new approach for treating cardiac hypertrophy and heart failure.

INVESTIGATING CANCER

His team also examines the role of TF in tumour growth, metastasis and thrombosis. The investigators have found that some cancer patients have elevated levels of TF circulating in their blood, which they suggest may contribute to increased incidence of VTE. Some of the highest levels of circulating TF are found in pancreatic cancer patients.

Tumour cells release large amounts of microvesicles (MVs) into the blood. MVs are small membrane vesicles formed from

Mackman hypothesises that the release of TF-positive MVs into the blood by tumour cells raises levels of circulating TF further, increasing the possibility of VTE

fragments of cellular membrane which enhance clotting. Since a blood clot in a cancer patient usually appears at a different site from that of their cancer, Mackman hypothesises that the release of TF-positive MVs into the blood by tumour cells raises levels of circulating TF further, increasing the possibility of VTE. Pancreatic tumour cells, in particular, release very high levels of TF-positive MVs into the blood, and it has been shown that there is a correlation between this and VTE.

LINKING CANCER AND THROMBOSIS

In a recent four-year project examining TF in cancer, Mackman and Nigel Key, the Harold R Roberts Distinguished Professor of Hematology and Oncology at the UNC, applied a dual approach: mouse models of cancer and two trials with human cancer patients. Their collaborators in this study included medical specialists at other universities and health centres in The Netherlands, Canada and Austria, as well as the US.

The human trials examined adult patients with either advanced pancreatic or colon cancer, as the chemotherapy procedures for these cancers are different. In addition to basic medical data and assessment of asymptomatic DVT via compression ultrasound, the trials scrutinised the composition of the patients' blood, drawn eight times over three months before, during and after their chemotherapy treatment. The levels of TF activity in isolated MPs were also analysed to determine whether they were associated with asymptomatic and/or symptomatic VTE. Another strand of the project examined whether anti-cancer drugs lead to the development of blood clots. So far, no clear association has been found between chemotherapy drugs and an increase in the amount of TF-positive MVs in the blood.

ADVANCED ASSAY

A major breakthrough for Mackman has been his laboratory's development of a highly sensitive assay to measure the level of TF-positive MVs in plasma. This assay has been used to determine whether elevated levels of TF in the blood are predictive of VTE occurring in pancreatic cancer patients: "If we find evidence to support the hypothesis, these patients could be treated with anticoagulant drugs to prevent the formation of a clot," explains Mackman. They have indeed found a strong correlation between MV TF activity and VTE in these patients, and so MV TF activity may become a useful biomarker to identify which patients are at risk of thrombosis. However, further work is required to refine the assay before it is used clinically.

Mackman intends to continue to explore the role of the clotting cascade in haemostasis, thrombosis and inflammation, including whether different types of MVs have different effects: "This is a very exciting time to be studying blood coagulation, because the number of approved drugs to prevent and treat thrombosis has doubled in the last few years," he enthuses. "New drugs are in the pipeline and I hope to contribute to their development and approval for use in patients."

INTELLIGENCE

MECHANISMS OF VENOUS THROMBOEMBOLISM IN CANCER

OBJECTIVES

To determine whether an association exists between tissue factor activity in circulating microvesicles and venous thrombosis in patients with pancreatic or colon cancer, and in tumour-bearing mice.

KEY COLLABORATORS

Professor Nigel Key; **Assistant Professor Raj Kasthuri**, University of North Carolina at Chapel Hill (UNC), North Carolina, USA

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Dr Alok Khorana, University of Rochester, New York, USA

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DR NIGEL MACKMAN received a PhD in Biological Sciences from the University of Leicester, UK in 1985, where he continued his postgraduate training. Next, he moved to the Scripps Research Institute in La Jolla, California, USA for a second postdoctoral fellowship. Mackman then rose up the ranks at Scripps to Associate Professor with tenure. In 2007, he moved to the University of North Carolina at Chapel Hill as the John C Parker Distinguished Professor of Medicine and co-Director of the Thrombosis and Hemostasis Program. Mackman took on the role of Director of the UNC McAllister Heart Institute in 2011.

UNC MCALLISTER HEART INSTITUTE mhi