Moderating metastasis

Pioneering cancer researchers **Professor Philip Rudland** and **Dr Roger Barraclough** share insights into their latest work towards combating metastatic cancer, and discuss the people and institutions they collaborate with in the pursuit of this goal

Your main research endeavour is the discovery of genes and proteins that induce the most life-threatening aspect of cancer: metastasis. How did your scientific backgrounds lead you to this interest?

PR: I completed my PhD on the mechanism of initiation of protein synthesis at the Medical Research Council (MRC) Centre, Cambridge, in 1970 under the supervision of FHC Crick and S Brenner, and went on to co-discover fibroblast growth factors (FGFs) in the early 1970s. I then joined the Imperial Cancer Research Fund and used FGFs to isolate the first breast stem cell line. Before joining the University of Liverpool as Professor of Biochemistry in 1986, I headed the Department of Cell and Molecular Biology at the Ludwig Institute for Cancer Research in Surrey, where I was successful in isolating human and rodent versions of benign and malignant breast cell lines.

RB: I completed my PhD on nuclear protein phosphorylation in 1973. Three years later, I received a postdoctoral research fellowship at the University of Warwick, where I discovered the chloroplast protein chaperone. I joined the Ludwig Institute for Cancer Research in 1979. Since 1987 I have been Lecturer, Senior Lecturer and Reader at the University of Liverpool.

Your group has identified four novel metastasis-inducing proteins (MIPs). What is the importance of these MIPs and how do they relate to findings from your current research endeavours?

PR & RB: The importance of the discovery of the MIPs was that it was the first time that proteins inducing the life-threatening aspect of cancer were identified systematically. The present programme has identified several of these MIPs as being useful for predicting patients who are liable to die prematurely from metastatic breast cancer. Therefore, these at risk patients can be treated extensively with current chemotherapies and other patients can be spared from the debilitating side effects of such treatments. Another MIP, which appears in the early premalignant stages of breast cancer, may be utilised as a diagnostic marker in blood for ovarian cancer.

You have backed up your investigations with more than 20 years of research, including an array of groundbreaking pilot studies funded by the Cancer and Polio Research Fund (CPRF). Speaking generally, is there enough support for early-stage research?

PR & RB: Unfortunately, in the UK, there is very little research money available for pilot studies. We have been particularly fortunate in that the CPRF has enabled us to conduct pilot studies into fresh areas on a long-term, five-year basis. Their support has given us the chance to operate from the start in a virgin territory such as metastasis, which the standard governmental agencies and major cancer charities have been reluctant to fund at a basic scientific level in the past.

Are you collaborating with other laboratories in the course of your investigations?

PR & RB: We conduct collaborations with many other laboratories. In Liverpool, for example, high throughput, massively parallel DNA sequencing has been undertaken with Professor R Sibson of the Centre for Genomic Research and the Beijing Genomics Institute, Hong Kong, to identify mutations in the DNA within and surrounding our MIPs in breast cancer cell lines. Additionally, former postdoc Professor Y Ke discovered a prostate cancer MIP, which is hypothesised to work by stimulating the production of factors for new blood vessels. Outside Liverpool, another new MIP has been discovered by Dr M El-Tanani, who is also a former postdoc and now at Queen's University Belfast.

Work on our existing MIPs continues with Dr S Gross in Aston University, Birmingham, using an automated system to measure cell migration. It also continues with Dr N J Jones here at the University of Liverpool and Dr B Davies at Astra Zeneca, Manchester, in attempting to block the remaining (nonhomologous) double strand DNA repair process in order to destroy the cancer cell selectively. External collaborations are now starting to support our model for the MIPs and suggest that our MIPs are multifunctional proteins with more than one target in a cell.

The Biosciences Building

Professor Philip Rudland and Dr Roger Barraclough undertake much of their work in the Biosciences Building at the University of Liverpool. It is a unique location, that houses all the major equipment for biological research, including:

- Mass spectrometer-based proteomic facilities for protein identification (Professor R Beynon)
- High throughput gene sequencing and analysis in the MRC Centre for Genomic Research (Professor N Hall)
- Nuclear Magnetic Resonance for determination of atomic structures (Professor L-Y Lian and Dr I Barsukov)
- Bioinformatics for structure-based drug design (Dr D Rigden)
- Confocal cell imaging for observing the expression of genes in living cells (Dr D Bennett)
- Animal and *Drosophila* facilities for housing our rat and recombinant fly models of metastasis, respectively (Mr E Birnie and Dr D Bennett)

Putting secondary cancer first

Researchers at the University of Liverpool, supported by the Cancer and Polio Research Fund, have been making inroads in the fight against metastatic cancer, beginning with the identification of the proteins responsible

PRIMARY CANCER IS a nightmare diagnosis no patient hopes to receive from a doctor. The truth is that primary tumours themselves rarely kill people, as they can be removed with surgery. However, many of these primary cancer patients can become secondary cancer patients, if their cancer metastasises.

With secondary cancer, metastatic cancer cells break off from the original tumour mass and travel through the blood and lymphatic systems to colonise new parts of the body. Therefore, though the primary tumour has long ago been removed, its progeny can return to wreak havoc in another organ. Diagnosing these secondary, metastatic tumours is difficult. It currently calls for the use of imaging processes – which generally catch them too late – or the identification of cancer cells in the lymph nodes, which is unreliable. Neither method is a significant help to individual patients, as the timescale over which secondary tumours appear can be unpredictable, meaning that too often the disease gains the element of surprise even after its first strike.

THE TROUBLE WITH TREATMENT

Current treatment strategies are not much more effective than their diagnostic counterparts. Secondary cancer is often able to spread to numerous sites within the body before it is apprehended, at which point the surgical intervention, though so effective for the primary tumour, is no longer viable. Doctors are then forced to treat patients with cytotoxic chemicals, and these chemicals damage the rapidly multiplying cancer cells in addition to many other native cells. Chemotherapy is limited by its propensity for harming necessary bodily cells with 'friendly fire'. Therefore, it cannot kill the secondary cancer outright – severely limiting the healing capacity of current approaches. In the UK, the Cancer and Polio Research Fund (CPRF) was one of the first charities to recognise the danger secondary cancer poses, vowing to end its threat. To this end, the organisation has funded research into metastasis over the last 20 years.

Among the researchers undertaking these important projects are Professor Philip Rudland and Dr Roger Barraclough from the University of Liverpool, and their research is proving extremely promising for meeting the CPRF's secondary cancer-ending goal. In the early 1990s, Rudland and Barraclough were the first to discover metastasis-inducing proteins (MIPs), a group of proteins responsible for this most lethal form of cancer. The pair also identified the first four proteins to join the group: S100A4, S100P, osteopontin and AGR2. Since then, they have worked with a large pool of collaborators to help stop metastasis in its tracks – producing some highly interesting results in the process.

MIPS AND RAT MODELS

Identifying the first four MIPs was an important step towards improving treatment for cancer patients, as they have been shown to be strongly associated with early patient death from metastasis.

Rudland and Barraclough had to overcome many hurdles over many years of research in order to discover these MIPs. The major obstacle was developing a suitable stem cell line that could be manipulated in a cell culture *in vitro* and that would produce benign tumours that failed to metastasise when introduced into non-immunosuppressed animals. Though it took scientific investigations which spanned three decades, Rudland and Barraclough met this challenge. They produced a stem cell line from a benign rat breast tumour growing in an inbred colony of rats that only produced benign, non-metastasising tumours when reinserted into non-immunosuppressed rats. After a further 10 years of research, they isolated benign and malignant breast tumour cell lines in humans, testing the differentially expressed genes by inserting them into their unique benign nonmetastatic breast cell line. When proteins from these genes cause metastasis in their complete animal model, Rudland and Barraclough know that they have discovered another MIP – as well as a potential target for fighting cancer. In fact, through this streamlined pathway to discovering more MIPs, the scientists have confirmed two further candidates with several possibilities still open.

Although a number of potential MIPs had already been linked with metastasis before this study, it was only by using their unique stem cell line in intact rats that the researchers were able to prove that certain proteins could directly induce metastasis. Moreover, the MIPs confirmed using this assay were of a different class to those responsible for inducing the primary tumours. Alone, they could not induce the development of a primary tumour; they could only work in concert with existing oncogenes to induce metastasis.

CREATING CANCER CURES

The current research programme of the CPRF Group at the University of Liverpool is focusing on two main points. First, the team is developing predictive tools to identify individuals likely to suffer from metastatic disease. Second, the researchers are helping to create therapies for eradicating metastatic cells.

Accordingly, Rudland and Barraclough are currently utilising a wide array of investigative approaches to advance the present understanding of MIPs and their workings. The Biosciences Building where the team is based allows for access to Nuclear Magnetic Resonance equipment, which has facilitated the characterisation of the atomic structure of AGR2, and the structure of S100A4's interaction with a cytoskeletal protein. Simultaneously, it places the researchers in a perfect position

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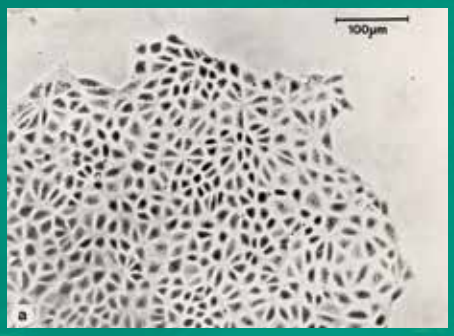
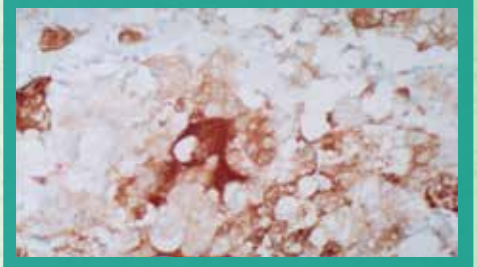
to collaborate with scientists in other fields, for example, to map the effects of MIPs in transgenic flies with Dr D Bennett, allowing them to gauge the downstream pathways induced by these proteins and to test inhibitors directly by introducing them into developing larvae.

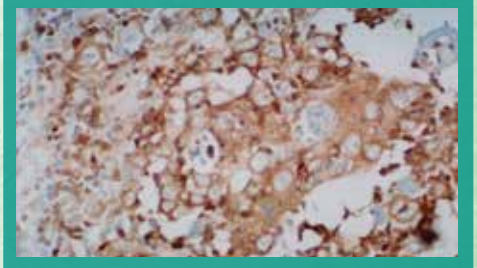
FURTHER DISCOVERIES

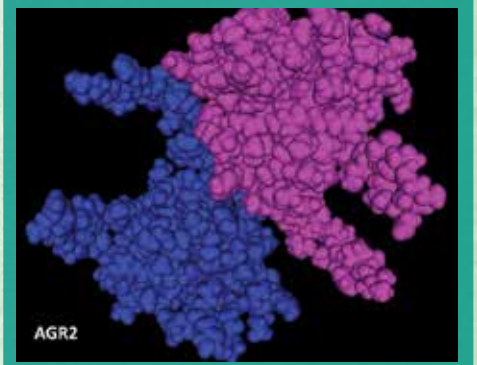
The more recent discoveries of the Liverpool group have fallen into two categories: clinical and molecular. On the molecular level with Dr N Jones, the researchers have been able to determine that the probable cause of MIP overexpression is failure of the homologous double-strand DNA repair mechanism, which destabilises the cancer genome and promotes the selection of metastatic cells. Two of the intracellular MIPs under study have been found to stimulate cell migration and invasion by binding to parts of the cytoskeleton, while two of their extracellular cousins, it has emerged, stimulate cell adhesion to extracellular material, enhancing the cell's ability to survive without the tumour's protection.

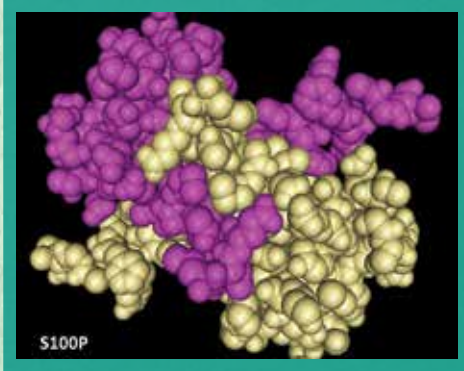
Perhaps the most important clinical finding has been the simple demonstration that when MIPs are overproduced, patients die more quickly. Using tissue specimens collected by cancer tissue banks such as the Liverpool Tissue Bank, which was originally set up in part by Rudland, the cancer researchers have been using immunohistochemical staining to identify MIPs and correlate their presence with patient outcome. Rudland and Barraclough's goal for this process is to reveal more about the diagnostic potential of MIPs, and although the MIPs may take many years to study comprehensively, it seems likely that such studies will be well worth the wait.











Benign tumour cells growing in culture; A metastasis in the rat stained for MIP AGR2; Antibody staining for MIP S100P in human breast cancer; Antibody staining for MIP S100A4 in human breast cancer; 3D structure of AGR2 dimer; 3D structure of S100P dimer.

INTELLIGENCE

DISCOVERY AND USE OF METASTASIS-INDUCING PROTEINS IN DIAGNOSIS AND TREATMENT OF BREAST AND OTHER CANCERS

OBJECTIVES

To help doctors identify patients most at risk of dying from cancer and to develop more effective drugs to combat metastatic disease.

KEY COLLABORATORS

Professor David Fernig; **Professor Rob Beynon**; **Professor Neil Hall**; **Professor Lu-Yun Lian**; **Dr Igor Barsukov**, Institute of Integrative Biology, University of Liverpool, UK • **Dr Andrew Carnell**, Department of Chemistry, University of Liverpool, UK • **Professor Terry Jones**; **Dr Daniel Crooks**; **Mr Michael Jenkinson**, Fazakerley Hospital, UK • **Professor Chris Foster**; **Dr Nitin Khirwadkar**; **Professor Christopher Holcombe**; **Mr John Winstanley**, Royal Liverpool University Hospital, UK • **Professor Ross Sibson**, CRUK Centre for Cancer Research/Beijing Genomics Institute, Hong Kong • **Professor Sarah Coupland**, Liverpool Tissue Bank, UK • **Dr Carol Walker**, Walton Brain Tissue Bank, UK • **Dr Stephane Gross**, Aston University, UK• **Dr Mohamed El-Tanani**, Queens University, Belfast, UK • **Dr Barry Davies**, AstraZeneca, UK

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PHILIP RUDLAND is currently Professor of Biochemistry at the University of Liverpool where he co-discovered the metastasis inducing proteins (MIPs), S100A4, osteopontin, S100P and AGR2.

ROGER BARRACLOUGH joined the University of Liverpool where he co-discovered MIPs and worked with Rudland to discover the mechanisms of their regulation and activity in relation to their metastasis-inducing properties.

Helping to end the threat of secondary cancers

UNIVERSITY OF LIVERPOOL