Dissecting the mechanisms of cancer

Drs Clive Wilson and **Deborah Goberdhan** apply knowledge of the mechanisms of cancer development and progression gained from the manipulation of fruit fly genes to cultured human cells in order to speed up translation into clinical applications

Could you give a brief overview of some of the processes involved when normal cellular regulation goes awry in cancer?

CW: The most widely recognised defect associated with cancer cells is that they lose their normal control of growth and proliferation. But there are other equally important changes: cells that build up lots of gene mutations usually set off a cell suicide programme, which cancer cells evade. In aggressive forms of cancer, cells break contacts that constrain them within a tissue, start migrating and can then embed themselves in other tissues. Intriguingly, these different cellular processes are often partly controlled by shared molecular mechanisms.

Another important feature of cancer cells, which is influencing our work, is their ability to adapt to their environment – when cancer cells proliferate and migrate, they are exposed to unusual adverse conditions, yet they survive and, ultimately, outcompete neighbouring cells.

What is your main focus when studying fruit fly models of cancer?

CW: We study cell biological processes that are implicated in cancer and try to work out which genes control them and what their functions are in normal cells. It's only when we understand what these genes are doing normally that we can determine which are likely to be worth studying in cancer and how to study them. We genetically manipulate cells in living flies, either in large-scale screens or by targeting good candidate genes, and then use several cell-based assays to work out how the cells have changed. A major strength is that we can test many genes in one experiment. This is how we came across the proton-assisted amino acid transporters (PATs). Deborah's most recent work suggests the human versions of these molecules may be biomarkers for aggressive forms of cancer and could be targets for blocking tumour growth.

What is the role of the mammalian target of rapamycin complex 1 (mTORC1)?

DG: When cell growth regulation was studied in yeast – single-celled organisms that don't use endocrine insulin signalling to control their growth – a key role was identified for an intracellular kinase: mechanistic target of rapamycin. It assembles into an active complex called mTORC1 in response to nutrients, particularly amino acids, outside the cell. mTORC1 stimulates protein translation, the basic driver of cell growth. Although humans and flies coordinate growth of all their cells through signalling by insulin-like molecules, it is now clear, again through initial studies in flies, that many of the growth-promoting properties of insulin are transmitted through mTORC1. So mTORC1 is a fundamental regulator of cell growth conserved from yeast to humans and, significantly, it is typically activated at high levels in cancer.

What is the significance of your discovery of the class of proton-assisted amino-acid transporters (PATs)?

DG: In human cells, mTORC1 is sensitive to extracellular amino acids, as it is in yeast, even though it also responds to insulin signalling. We decided to test how mTORC1 senses amino acids. As a first step, we screened many classes of amino acid transporters in flies to try to work out which ones were best at activating mTORC1 and promoting growth. We assumed they would work by transporting amino acids into the cell.

We identified the PATs, and I subsequently showed that human PATs have the same properties and drive cancer cell growth. This was a big surprise because in cancer cells, PATs are often almost exclusively located at the surface of late endosomes and lysosomes, suggesting that they can't drive growth by bringing amino acids into cells. My group's subsequent work has now shown that PATs play a critical role inside the cell by 'sensing' amino acids and activating mTORC1 via a mechanism that is yet to be fully characterised. By collaborating with clinical colleagues at the University of Oxford, we are finding in patients that expression of these transporters is increased in more aggressive tumours.

How have you helped to redefine the role of lysosomes and endosomes, and how did you become interested in the mechanisms underlying exosome regulation?

DG: After we demonstrated that PATs regulate mTORC1, using a completely different

approach, Professor David Sabatini's group at the Massachusetts Institute of Technology in Boston discovered that activated mTORC1 in cancer cells is assembled on late endosomes and lysosomes. Their work and ours pointed to these compartments as hubs for the amino acid sensing that determines cell growth rates.

More recently, many groups have become increasingly interested in the secretion of exosomes from cancer cells. These small vesicles are formed by inward budding of the late endosomal limiting membrane, and released from the cell when the endosomes fuse with the plasma membrane. Since exosomes carry a plethora of secreted, cell surface and intracellular signalling molecules, as well as RNA and DNA, they have the potential to reprogramme target cells. Several reports now indicate that they can stimulate key events in cancer progression, such as the formation of new blood vessels and the priming of new sites for metastasis.

Since both mTORC1 activation and exosome biogenesis are regulated from the same intracellular compartments, we have started to test whether these two processes might be linked. We are now rapidly building up evidence of very complex interactions coordinating nutrient sensing, cancer cell growth and signalling to neighbouring cells.

What progress has been made in understanding prostate cancer as a result of this pioneering work?

CW: A few years ago, spurred on by work from a number of groups showing that stem cells in the *Drosophila* gut shared remarkable parallels with human colorectal stem cells and could be used to study the fundamental mechanisms underlying colorectal cancer, we decided to investigate cells in the fly male accessory gland. Remarkably, we found that some cells in this gland grow disproportionately to other cells as adult flies age, just as the prostate hypertrophies in most ageing men – many of the signals that control this growth also seem to be shared between humans and flies. More recently, our work indicates that the accessory gland, just like the prostate, secretes exosomes, which have an important role in reproduction. We think that cell growth and exosome secretion are linked in the accessory gland and prostate, and the accessory gland provides the first *in vivo* system to work out how these processes are coordinated.

With funding from Cancer Research UK (CRUK), you set up an independent research programme. What challenges did you face when setting out on this initiative?

DG: It certainly was a real challenge! We had found a group of molecules – the PATs – that had a key role in growth control in flies and seemed to regulate cancer pathways. But it wasn't easy to persuade any cancer biologists to devote major resources to testing whether these intracellular transporters actually regulated cancer growth when it was unclear how they might work. So I convinced CRUK to support me and I completely switched fields from the fly to human cell culture. The resulting studies demonstrated that PATs were important in cancer and worked by a conserved mechanism. Since then, we've built up several collaborations with academic clinicians, particularly Adrian Harris (Professor of Clinical Oncology) and Freddie Hamdy (Professor of Urology) in Oxford, and we are beginning to see that PATs are overexpressed in patients in some forms of cancer, and to demonstrate that blocking PATs in experimentally induced tumours in mice can inhibit cancer growth.

Pipeline to success

Harmonising studies of genetics and cellular processes in both the fruit fly and humans, a partnership of two laboratories at the University of Oxford, UK, is fast-tracking identification of new targets for inhibiting the growth and spread of cancer

UNDER NORMAL CONDITIONS, cells require nutrients and oxygen to grow. The ability of cancer cells to avoid their natural fate – apoptosis – in nutrient- and oxygen-depleted environments and to emerge stronger and, apparently, more aggressive, reflects failures in the regulation of the network of signalling cascades that control cell growth. Faulty components in this control mechanism can govern intracellular processes, intercellular communications and traffic molecules such as proteins around the cell, enabling cancer cells to thrive and then navigate to new parts of the body.

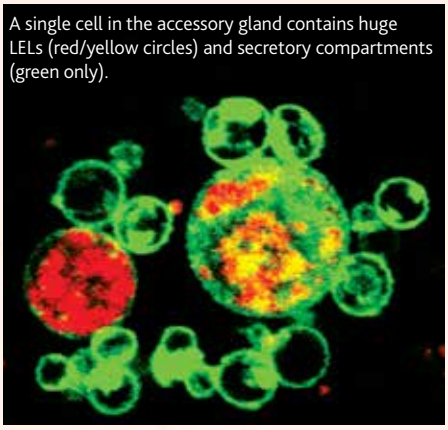
Among the destinations of cargo transported from the cell surface, mature – or late – endosomal and lysosomal compartments (LELs) have traditionally been characterised as merely degradation and recycling centres for unwanted cellular matter. Recently, however, LELs were identified as key elements in cancer, enabling cells to sense the amino acids that surround them and so allowing them to obtain nutrients both internally and externally to fuel their unnaturally high rates of growth. The discovery of this sensing mechanism was the result of efforts at the University of Oxford between Dr Clive Wilson's group, which specialises in the cellular biology and genetics of the fruit fly, *Drosophila melanogaster*, and the group of Dr Deborah Goberdhan, which now specialises in the molecular and cellular metabolic strategies used by tumour cells to survive and in identifying the role of signalling pathway faults in disease.

The secretory epithelium of the accessory gland contains two cell types: main cells (green) and round secondary cells (unstained).

TRANSLATING THE BIOLOGY OF FLIES

Many key questions about human cell biology have been first answered through experimentation with fruit flies, since about 70 per cent of human genes are shared with *Drosophila melanogaster*. From the standpoint that mutually exchanging findings and knowledge in both systems will expedite discovery, and that emerging ideas about the causes of disease gleaned from animal studies need to be validated and verified in the human context, Wilson and Goberdhan have established a translational pipeline that both ensures efficient experimental design and leads to rapid testing of concepts.

Wilson's group establishes fly models with the genetic makeup required for testing a certain hypothesis and can rapidly examine the function of a particular gene within a selected living cell. These results can then be used to help inform studies in Goberdhan's group: "Our fly work highlights really good candidates to test in human cells and suggests how they function," reveals Wilson. "With this collaborative approach, fly researchers can see how their progress is being translated, and those working in human cell culture can devise new hypotheses that can then be tested in just a few weeks." This arrangement thus enables them to identify new mechanisms of cellular growth regulation, characterise them in fruit flies and then confirm that they are conserved and operate similarly in human cells.



A single cell in the accessory gland contains huge LELs (red/yellow circles) and secretory compartments (green only).

DISCOVERIES ABOUT CANCER SIGNALLING

During her doctorate studies, Goberdhan used the fruit fly in an exploration of the then recently cloned human tumour suppressor gene – *PTEN* – and its effects on PI3-kinase (PI3K), a major target of insulin signalling. The use of the fruit fly demonstrated *in vivo* that *PTEN* is a direct antagonist of PI3K and highlighted the importance of its role in restricting cell growth. Goberdhan also found that when *PTEN* was altered, its ability to control growth disappeared: "All tissues grew more and the cells were in some cases more than three times their normal size," she recalls. *PTEN* malfunction is now implicated in inducing hyperactivity of the intracellular P13K/protein kinase B/mechanistic target of rapamycin (PI3K/AKT/mTOR) signalling and transduction pathway; when a fault in this pathway occurs, particularly at the point where the complex that detects ambient nutrients,

Proton-assisted amino acid transporters may be viable biomarkers of the presence of disease and an effective target for disrupting amino acid sensing in cancer cells

called mTOR complex 1 (mTORC1), is involved, dysfunctional cells, such as tumour cells, survive even under extremely adverse conditions.

Goberdhan discovered in 2010, also following *in vivo* genetic screens of *Drosophila melanogaster*, that members of the proton-assisted amino acid transporter (PAT) family are fundamental to orchestration of the intracellular response to local amino acids, increasing insulin signalling via control of mTORC1. This finding paved the way to the notion that PATs, which are largely concentrated in LELs, may be viable biomarkers of the presence of disease and an effective target for disrupting the amino acid sensing mechanism. Though there are still questions about how the mechanism facilitates the progression of cancer, Goberdhan is now urgently pursuing this notion with clinical colleagues and support from Cancer Research UK.

PROSTATE CANCER AND *DROSOPHILA MELANOGASTER*'S ACCESSORY GLAND

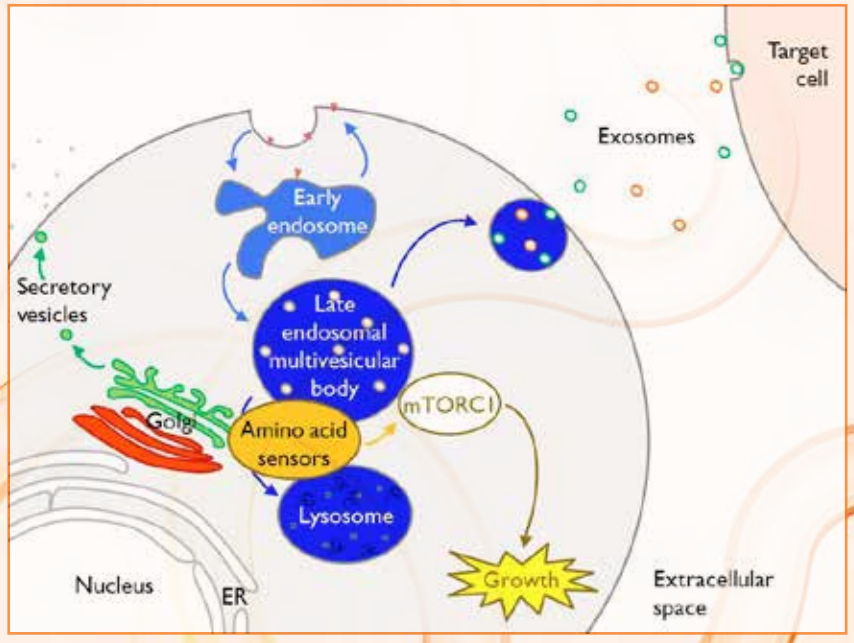
The male fruit fly's accessory gland is a structure that appears to equate in function to the prostate in human males. On the premises that biological similarities likely exist between accessory gland and human prostate cells, and that late endosomes in the prostate and other tissues release exosomes – vesicles that mediate signals between cancer cells and their neighbouring normal cells – Goberdhan and Wilson have been delving into what happens when the control system for exosome production goes awry: "If exosome secretion changes when we drug key growth pathways, we need to understand its impact on the tumour and surrounding cells," explains Goberdhan.

By studying other aspects of the biology of the fly accessory gland, Wilson and Goberdhan hope to uncover new mechanisms that may be relevant to prostate biology. One recent finding is particularly curious: cells in the accessory gland have developed a mechanism that leads female flies to reject other suitors for a period after mating: "There is evidence that the cellular processes causing this effect also exist in the prostate: "Who knows what the implications are for human reproductive biology!" exclaims Goberdhan.

CELL GROWTH MEDIATORS

Wilson and Goberdhan are now further testing their discovery that amino acid transporters in LELs are major sensors required for cell growth and exploring the role that LELs have as a hub that manages intercellular signalling in disease states. Wilson's group has developed a new fly cell system that allows the study of LELs in living tissue at ultra-high resolution: "We can look at how these compartments work and determine how they can go wrong in a way that has not previously been possible," he enthuses.

Goberdhan and Wilson are also analysing the means whereby trafficking to LELs is regulated, since these may present new targets for blocking cancer growth and metastasis; considering the possibility that the PATs may actually act as receptors rather than as transporters; and exploring the amino acid sensing process further, as they have recently identified intracellular transporters that affect cell growth situated other than in LELs.



INTELLIGENCE

GROWTH CONTROL IN HEALTH AND DISEASE

OBJECTIVES

- To identify and characterise key novel growth regulatory mechanisms in flies
- To confirm that these processes are conserved in human cells and potentially highlight new aspects to tumour growth regulation
- To further characterise the roles of late endosomes and lysosomes in growth and cell-cell signalling
- To assess how trafficking to late endosomes and lysosomes is controlled, since these trafficking events may be key new targets for blocking cancer growth and metastasis

KEY TEAM MEMBERS

Shih-Jung Fan • Mihindukulasuriya Perera • Mark Wainwright • Carina Gandy • Laura Corrigan • Aaron Leiblich • Siamak Redhai

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CLIVE WILSON received his PhD from the University of Warwick, UK, in 1986. Postgraduate studies followed in Professor Walter Gehring's lab at the University of Basel, Switzerland, before moving to the Massachusetts Institute of Technology (MIT), USA, in the lab of Professor Hermann Steller, where he began his collaboration with Goberdhan. Wilson returned to the UK as Lecturer at the University of Kent, UK, and in 2001 moved to the University of Oxford as Lecturer, where he remains today.

DEBORAH GOBERDHAN is Departmental and University Research Lecturer in the Department of Physiology, Anatomy and Genetics at the University of Oxford. She started to use genetic approaches to address biological questions in flies while working in the US in labs at Harvard and MIT, before undertaking a PhD at the University of Kent.