A balance of signals

Dr G Paolo Dotto discusses his innovative work with notch signalling, an undertaking that will help to lay the groundwork for understanding epithelial cancer development in the skin and beyond

Could you explain what the key objectives of your research are?

The research I am currently involved in is designed to understand the interplay between intracellular and intercellular cell communication pathways that control the normal equilibrium between self-renewing epithelial cell populations and their differentiated daughter cells, using skin as a model. Within this context, I am attempting to understand how alterations in this equilibrium lead to epithelial cancer development, specifically squamous cell carcinoma which is the most frequent type of solid human tumours and a main cause of cancer-related death. I am also investigating how environmental insults, for instance exposure to UV light and ageing, perturb these normal conditions and lead to cancer-predisposing conditions, specifically 'field cancerisation'. The final goal is to devise new ways of intervention for the treatment of squamous cell carcinoma (SCC) and prevention/reversion of their precursor lesions.

Can you describe what is meant by the term 'field cancerisation'? What is the result of this phenomenon?

Tumours are usually thought to result from genetic changes in a discrete group of cells, originating from a single initial progenitor, and of changes in immediately surrounding normal cells. Field cancerisation refers to pro-tumourigenic changes, in both epithelial cells and surrounding stromal cells, that occur in multiple and larger areas of target organs. In fact, very frequently (more than 10 per cent of cases) and in many organs (including the skin, oral cavity, lung, prostate and breast) epithelial tumours are multiple and, when removed, more can develop. This has been linked to the presence of genetic pro-tumourigenic changes in apparently normal 'patches' of epithelial cells that can expand over time. We are testing the possibility that alterations (mostly atrophy and inflammation) of the underlying support stromal tissue play an equally important primary role.

What has led you to explore the novel role of the notch pathway in the mesenchymal compartment of the skin and what promise does this hold for preventing or suppressing SCC formation?

We have spent many years demonstrating that notch signalling plays an important pro-differentiation and tumour suppressing role in epithelial cells of the skin and possibly other organs. As part of our studies, we found signs of Notch activity also in the mesenchymal compartment of the skin, and this prompted us to assess whether this pathway also plays an important role in this compartment. Thus the reason for this current research.

DR G PAOLO DOTTO

Identifying transcription networks for **cancer therapies**

Investigating cancer-predisposing conditions has afforded a research team based at the **University of Lausanne** the opportunity to devise new ways of intervention for the treatment of squamous cell carcinoma

THE MANNER IN which cancer cells typically develop is a result of deregulated control of self-renewing cell populations and alterations in their surrounding environment. This means that slow cycling cancer initiating cells, or putative stem cells, are, by their very definition, difficult to target through the more conventional techniques of chemotherapy which are specifically aimed at more actively proliferating cell populations. In contrast to this, differentiation-inducing agents have the potential to exhaust cancer stem cell populations by recruiting them into differentiation. As a result, transcription factors play a key role in controlling stem cell potential and long-term commitment to differentiation. This means that transcription factors are now among the most sought after targets for potential cancer therapies.

A group of scientists located in the University of Lausanne's Department of Biochemistry in Switzerland have spent the last few years studying the complex and multifaceted field of human epithelial stem cell signalling and carcinogenesis. Dr G Paolo Dotto, who has been investigating genetics for well over 30 years, is leading the research team focusing on the transcription network that promotes keratinocyte commitment to differentiation and suppresses tumorigenesis. When looking at this subject they have specifically been investigating three main topics: crosstalk of Notch with p53/p63 and EGFR signalling pathways in keratinocyte growth/differentiation control and cancer development; a FGFR3/FOXN1 positive loop that underlies benign skin keratosis versus squamous cell carcinoma (SCC) formation; and calcineurin and ATF3 opposite determinants of cancer cell senescence versus stem cell potential.

The notch signalling pathway is at the heart of these investigations. It is an important form of cell-cell communication with a key role in development as well as tissue homeostasis. The pathway's function can vary. Notch activation in mammalian cells was commonly thought to enhance stem cell potential and promote tumourigenesis. Dotto has established that notch signalling promotes commitment of skin epithelial cells towards differentiation, and that compromised notch signalling results in SCC formation.

A DYNAMIC APPROACH

The research group's main hypothesis is that modulation of squamous differentiation can be a double-edged sword for SCC, and that more precise insights into this issue will have major preventive and therapeutic value. They are addressing this issue by a combination of both genetic and pharmacological studies as well as *in vivo* assays which are closely approaching the clinical situation. On one hand, they have developed a number of mouse genetic models of skin carcinogenesis, the latest of which recapitulates to a striking extent the field cancerisation process found in the clinic. On

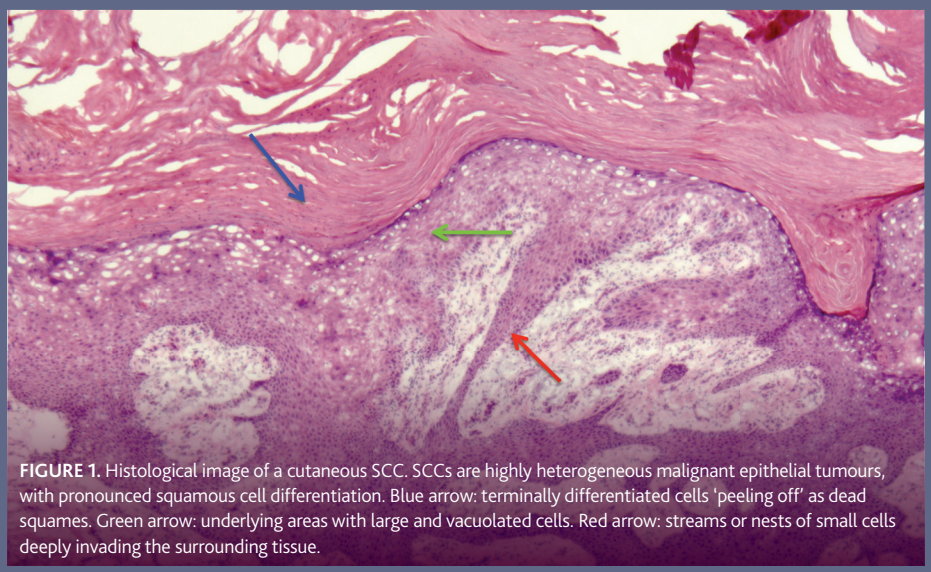

FIGURE 1. Histological image of a cutaneous SCC. SCCs are highly heterogeneous malignant epithelial tumours, with pronounced squamous cell differentiation. Blue arrow: terminally differentiated cells 'peeling off' as dead squames. Green arrow: underlying areas with large and vacuolated cells. Red arrow: streams or nests of small cells deeply invading the surrounding tissue.

How will the project help to build cooperation between researchers, clinicians and the pharmaceutical industry?

We have established and already greatly benefited from extensive interactions and collaborations with colleagues in clinical departments with a keen interest in squamous cell tumours, their prevention and treatment. Besides the skin model that we have been extensively using, we have now started an intensive effort aimed at validating and expanding our findings in lung SCCs, which account for between 30-40 per cent of all forms of lung cancer. We have started this effort in close connection and collaboration with pathologists, oncologists and thoracic surgeons involved in the diagnosis and treatment of the disease. We are also employing a chemical genetic approach to identify small molecular weight compounds that may be beneficially used in the treatment and/or prevention of the disease. We have already identified, and published, a few such molecules that are already considered or used in the clinic, such as inhibitors of the epidermal growth factor receptor or EGFR pathway. As a consequence, we have been in contact with a couple of biotechnology companies for possible further joint developments. In addition to our interests and insights into ways to intervene against SCC development, our recent work is also pointing in the direction of ageing and ways to interfere with this process, which may be of substantial interest to the pharmaceutical industry.

What are the next steps in your research and when do you believe the transfer of your work into a treatment for patients will be possible?

We plan on further developing our research along two main directions. Firstly, as previously mentioned, we have started an intensive effort aimed at validating and expanding the findings that we have obtained so far for skin SCCs in the lung, developing a whole new line of research for this organ. Secondly, we will be probing further into the cellular and molecular aspects of field cancerisation and its connection with stromal changes linked with inflammatory/degenerative processes and/or ageing.

NOTCH SIGNALING AS A KEY DETERMINANT OF EPITHELIAL-MESENCHYMAL INTERACTIONS IN THE SKIN

OBJECTIVES

- To understand control of epithelial tissue homeostasis and development of squamous cell carcinomas, the most frequent solid human tumours and main cause of death
- To understand how environmental insults and ageing lead to cancer-predisposing conditions and devise new ways for treatment of squamous cell carcinomas and prevention/reversion of precursor lesions

KEY COLLABORATORS

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G PAOLO DOTTO received his MD from Turin University and PhD from Rockefeller University. After postdoctoral training at Whitehead Institute/MIT, he joined Yale University and then Harvard Medical School, where he became full Professor in 2000. While maintaining an active laboratory at Harvard/MGH, since 2002 he has been Professor of Biochemistry at the University of Lausanne. He is an elected EMBO member.

the other hand, they have developed an *in vivo* 'intradermal tumourigenicity assay' that enables the researchers to reproduce and analyse early steps of human cancer formation by testing human cells freshly derived from normal tissues (primary cells) with specific genetic alterations and in combination with other cells back in the *in vivo* situation, in a dermal-epidermal junction site that closely approximates that of clinically developing tumours.

OVERCOMING SCCs RESILIENCE TO THERAPY

SCCs are malignant solid tumours that originate from various epithelial organs (skin, oral cavity, oesophagus, lung, bladder and cervix) and, as Dotto explains: "These are very resistant to conventional chemotherapy as well as more recent drug-targeted approaches". These tumours are characterised by a high heterogeneity of epithelial tumour cells with some key features: firstly, there are a small number of self-renewing populations that divide infrequently, but are resistant to chemotherapy and can reform a tumour even when most or all other tumour cells are killed; secondly, there are actively dividing cells that can comprise the majority of the tumour and are targeted and killed by treatment; and finally, there are differentiating cells that, like in a normal tissue, have stopped dividing. "In tumours, unlike in normal tissues, differentiation has the potential to be a reversible process, so that tumour differentiated populations may also give rise to more proliferating tumour cells," he affirms. In addition to the tumour epithelial cells, an important component of these tumours are normal cells of other origins, like providing the necessary support (such as fibroblasts), nutrients (endothelial cells forming blood vessels) and reactive inflammatory cells, which in some cases can restrict tumour formation but in others can actually enhance it.

A key distinguishing feature of SCC tumours is their elevated degree of heterogeneity and differentiation, which Dotto notes may explain their resilience to conventional as well as novel targeted therapy. These tumours contain self-renewing cell populations mixed with cells at various stages of commitment to differentiation. Cancer initiating cells in these tumours are typically slow cycling and difficult to target by conventional chemotherapy that is aimed at actively proliferating cells. In this context, 'differentiation therapy' with differentiation-inducing agents has the potential to exhaust tumour stem cell populations by recruiting them into the process. However, countering these beneficial effects, Dotto's research, as well as other scientists' work, has found that the induction of squamous differentiation can also increase cell survival and render cells more resistant to chemotherapeutic agents. In addition, his research group has been able to show that in tumour cells, in contrast to normal primary cells: "Squamous differentiation is a reversible process, so that differentiated cells could be a 'quiescent reservoir' of novel cancer initiating populations," Dotto notes.

FULL STEAM AHEAD FOR SCC RESEARCH

Encountering setbacks during the course of research is a common concern for most research investigations. To date, Dotto's team has been lucky enough to have steered clear of major obstacles in their work, but this doesn't mean their research is not without its challenges: "We obviously work in a very competitive field and progress is often slower than I would like," he admits. On the positive side, they have been able to uncover some interesting results since the project's commencement, including obtaining some patents of potential significance, one on the role of Notch signalling in skin ageing and the other on a 'mouse gene chip technology' for the *in vivo* screening of drugs. Signalling by the calcium-dependent phosphatase calcineurin is another important pathway that they have implicated in control of keratinocytes in concert with Notch and p53. Dotto's recent demonstration that calcineurin signalling plays an intrinsic tumour suppressing function in these cells, upstream of p53, is of substantial clinical significance.

In fact, the efforts that Dotto's team are putting into understanding more about the tumour suppressing function of calcineurin is very valuable. This work has major clinical relevance for the large number of patients who undergo treatment with immune suppressive drugs which can develop skin SCC as a major complication and potentially a cause of death. The future is looking very positive for the team's research and their ongoing work is aimed at further exploring their current topics as well as assessing possible translational potential by high throughput genetic and chemical functional screens aimed at prevention and/or suppression of SCC formation through induction of differentiation.