Molecular mechanisms in signal transduction and cancer

Meeting on Oncogenes & Growth Control

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Introduction

Good weather and a pint of apple juice (although we thought we had ordered beer) at a farmhouse opposite the European Molecular Biology Laboratory (EMBL) heralded the start of the 15th conference on ‘Oncogenes & Growth Control’. The participants had the opportunity to enjoy the friendly atmosphere at the EMBL, the hospitality of the staff and the tasty food. And of course, a series of excellent talks, which provided an in-depth view of several topics in signal transduction and cancer biology. In this report, we will give you an overview of what was discussed.

Starting at the end

In the last session of the meeting, J. Campisi (Berkeley, CA, USA) looked at cancer from an evolutionary point of view and presented a model in which cancer is not only caused by the accumulation of mutations, but also facilitated by the senescent cells that accumulate in older people. Her model is based on the observation that senescent cells support the growth of preneoplastic cells much better than that of normal fibroblasts. Senescent cells may do so by secreting many products, including growth factors, cytokines such as interleukin-6 (IL-6), and proteases. Older people do indeed accumulate senescent cells as shown by β-galactosidase staining. Furthermore, older people have higher levels of IL-6, which is one of the causes of frailty. Campisi argued that the occurrence of senescent cells is a pleiotropic effect of our fight against cancer cells, and as they occur after reproductive age, there is no selective pressure to eliminate them. This fight against cancer has also resulted in the evolution of tumour suppressor genes, which fall into two groups: the caretakers, which are repair genes that protect the integrity of the genome; and the gatekeepers, which are apoptosis- and senescence-inducing genes that eliminate or arrest potential cancer cells. Caretaker genes are important for sustaining cell renewal. However, gatekeeper genes deplete the organism of cells and essential stem cells, which stalls cell renewal and therefore may cause ageing.

The p53 gene is both a caretaker (by inducing DNA repair) and a gatekeeper (by inducing apoptosis in the case of irreparable damage). Therefore, after a DNA-damaging insult, p53 is activated and the necessary responses are induced. G. Evan (San Francisco, CA, USA) addressed the question of what would happen if p53 is activated only when “it really matters,” that is, when a tumour arises. He generated p53-null mice that express an oestrogen receptor (ER)–p53 fusion protein so that p53 could be switched on and off at will. When these mice were irradiated and p53 was not being expressed, the mice did not show severe damage responses but they developed tumours. However, if p53 expression was restored for just a brief period of time, after the DNA damage had been resolved, no tumours were observed. Evan speculated that if we could repress p53 for most of the time and only activate it when a tumour arises, perhaps we can keep the beneficial effect.
of p53 in fighting tumour formation, but lose its unwanted effects in ageing.

One potential cause of ageing may be the depletion of stem cells, whereas tumour formation may in part be due to the failure of stem cells to differentiate. It is therefore very important to know how stem cells are regulated. The accepted view is that stem cells divide asymmetrically into one cell that remains a stem cell and one that is bound for differentiation. A. Trumpp (Palo Alto, Switzerland) gave an interesting presentation on how stem-cell renewal might occur in mammalian systems and particularly on the role of Myc in this process. First, he indicated that haematopoietic stem cells are restricted to a certain region of the bone marrow—the stem-cell niche—where the stem cells interact with the supporting cells through adhesion anchors, such as N-cadherin and integrins. He showed that a conditional mouse strain that lacks Myc in the bone marrow compartment accumulates stem cells. This accumulation was not due to decreased apoptosis or increased cell proliferation, but rather to a failure to differentiate. Surprisingly, these cells can differentiate normally in vitro. Thus, the failure to differentiate is apparently due to the unique surrounding of the stem-cell niche. Conversely, when Myc is overexpressed in stem cells, the stem-cell niche is depleted of stem cells. This led Trumpp to propose a model in which Myc is involved in the switch of a stem cell to a cell that is on its way to differentiation. It is unclear how Myc is regulated, but the consequence of Myc activation is the repression of the cell-adhesion molecules N-cadherin and the integrins. This repression is likely to be a prerequisite for the cells to leave the niche.

Haematopoietic stem cells were also the topic of T. Graf’s presentation (New York, NY, USA), who showed that under normal conditions, blood cells do not contribute to other tissues during mouse development: when these cells were irreversibly labelled with yellow fluorescent protein they could only be detected in the haematopoietic lineage. However, even differentiated haematopoietic cells retain a surprising degree of plasticity. Graf showed that the artificial introduction of the transcription factor C/EBP-α into B cells reprogrammed these cells to become macrophages, and that their immunoglobulin genes were rearranged. The switch involves the induction of the macrophage gene expression programme by C/EBP-α together with the transcription factor PU.1 (already expressed in B cells) and the down-regulation of lymphoid genes through the C/EBP-α-mediated inactivation of the transcription factor Pax5 (Xie et al., 2004).

What tumours do

Angiogenesis is considered to be a key step in tumour growth, but K. Allitalo (Helsinki, Finland) showed that the formation of lymph vessels ( lymphangiogenesis) also has a role in tumour metastasis ( Saharinen et al., 2004 ). Both vascular endothelial growth factor (VEGF)-C and -D are responsible for the induction of lymphangiogenesis by binding to and activating the VEGF receptor VEGFR3, which is a receptor tyrosine kinase. Indeed, the overexpression of VEGF-C induces the formation of lymph vessels and lymph metastasis, and thus provides a route for metastasizing cells to escape from the tumour. By contrast, a soluble VEGFR3 extracellular domain that functions as a decoy to trap VEGF-C and -D inhibits embryonic lymphangiogenesis and lymph metastases. In addition to the role of senescent cells in tumour formation as indicated above, immune cells also have an important role in tumour initiation and progression. Strikingly, chronic inflammation is one of the causes of cancer. Z. Werb (San Francisco, CA, USA) discussed the importance of this interaction and showed that tumours also have a profound effect on the behaviour of lymphocytes and macrophages, as these cells become highly motile in the presence of a tumour.

How to get rid of tumours

At present, the best way to treat metastatic cancer is with chemicals. Although the majority of these drugs are still rather non-specific, more targeted drugs are entering the market. Imatinib (US, Gleevec; non-US, Glivec), an inhibitor of the Ab1 tyrosine kinase, is an example of a drug that is now being used successfully to treat chronic myeloid leukaemia, which develops because of a chromosomal translocation that fuses the B-cell antigen receptor (Bcr) to the Ab1 tyrosine kinase. The resulting kinase is constitutively active and causes a massive proliferation of white blood cells. G. Superti-Furga (Heidelberg, Germany) described the crystal structure of Ab1 in its inactive state. In this conformation, the amino-terminal myristyl group is inserted in the kinase domain causing a conformational change that allows the Src homology SH2 and SH3 domains to interact with and inhibit Ab1. Bcr-Ab1 lacks the N-terminal myristyl group. Imatinib only binds to the catalytic site in its inactive state and thus stabilizes Bcr-Ab1 in the inactive conformation. Imatinib also targets Kit and the platelet-derived growth factor (PDGF) receptor. This led H. Beug (Vienna, Austria) to speculate that imatinib might also prevent the formation of metastatic cells. In his mammary epithelial cell system, the autocrine production of PDGF is required for epithelial–mesenchymal transition (EMT), as well as the induction and maintenance of metastasis. This confirms that EMT is a faithful in vitro correlate of tumour progression and metastasis.

Superti-Furga described further chemical proteomics experiments using imatinib and another kinase inhibitor, PD17. The compounds were immobilized at a permissive group and then used as affinity reagents to ‘pull down’ interacting proteins that were then identified by mass spectrometry and bioinformatics. These experiments nicely displayed the difference in selectivity between the two compounds. Imatinib, the more bulky compound of the two, pulled down all its known targets, as well as a few additional ones that may explain previous reports of imatinib affecting the amyloid precursor protein processing pathway involved in Alzheimer’s disease. PD17 is much more promiscuous and interacts with a whole battery of kinases. This approach can be used to monitor the specificity of kinase inhibitors and other drugs that interfere with signalling pathways.

Another approach to treat tumours in the future may be to interfere with the hedgehog pathway. P. Beachy (Baltimore, MD, USA) elegantly described the important role of this signalling pathway in metastatic prostate cancer ( Lum & Beachy, 2004 ). Hedgehog binds to the cell surface receptor patched (Ptc) thereby releasing smoothened (Smo) from inhibition by Ptc (Fig 1). Overexpression of two hedgehog ligands, Sonic hedgehog and Indian hedgehog, and constitutive activation of Smo, either by mutation of Smo or loss of Ptc, is observed in many tumours. Indeed, Beachy calculated that the hedgehog pathway is involved in as many as 25% of all cancer deaths. In prostate cancer, Smo expression seems to correlate with the metastatic behaviour of cells, leading to the proposal that the hedgehog
pathway is promoting the metastatic programme of prostate cancer cells. Importantly, the natural compound cyclopaamine, which was isolated from a poisonous plant that causes a developmental defect resulting in a cyclopic eye, binds to Smo and inhibits the pathway. This drug is now in clinical trials, as are other drugs that inhibit Smo.

The hedgehog signalling pathway also has a role in skin tumour formation. F. Watt (London, UK) showed that stem cells in the skin differentiate in different lineages depending on whether the β-catenin–T-cell-factor (Tcf) signalling pathway is switched on or off: high levels of stabilized β-catenin promote hair follicle formation, intermediate levels promote sebocyte differentiation and low levels promote interfollicular epidermal differentiation. Hair follicle tumours arise in mice that have a high epidermal expression of β-catenin and sebaceous tumours develop when β-catenin signalling is blocked by N-terminally truncated lymphoid-enhancer binding factor 1 (Lef1). This correlates with the expression of either Sonic hedgehog (in hair follicle tumours) or Indian hedgehog (in sebaceous tumours).

The spectacular recent finding that the B-Raf kinase is mutated in many tumours, such as melanomas, adenocarcinoma of the colon and papillary thyroid tumours, is another indication of the importance of the Ras–B-Raf–MEK–ERK pathway in human tumour formation. D. Barford (London, UK) presented the recently described crystal structure of B-Raf and explained the nature of most of the observed mutations (Wan et al, 2004; Fig 2). Most mutations can be explained by the destabilization of the inactive conformation or the stabilization of the active conformation. Interestingly, one group of mutations results in a B-Raf with reduced activity in vitro, but this protein still activates ERK in vivo, perhaps through the formation of heterodimers with Raf1. A candidate drug exists that can inhibit B-Raf and this Bayer–Onyx compound is now in clinical trials. Interestingly, the elongation factor eEF–4E, also induces lymphomas. As eEF–4E functions downstream of Tor, these tumours are insensitive to rapamycin/adriamycin treatment (Wendel et al, 2004).

**p53 still has its surprises**

The p53 protein is probably the most extensively studied molecule in the cancer field. One of the proteins that is involved in the regulation of p53 is ADP-ribosylation factor (Arf). In a series of elegant experiments, J. Lees (Cambridge, MA, USA) presented evidence that Arf is regulated both positively and negatively by members of the E2F family. It was shown previously that E2F1 is a transcriptional activator of Arf, but by using knockout cell lines, Lees showed that E2F1a is a transcriptional repressor of Arf, which directly binds to the Arf promoter. Thus, in normal cells, E2F1 represses Arf, whereas in tumour cells, in which E2F1 is frequently activated due to the absence of retinoblastoma protein (Rb), E2F1 takes over and induces Arf expression (Aslanian et al, 2004).

The effects of p53 on cell-cycle inhibition and apoptosis are produced by several downstream transcriptional targets. K. Voulsen
(Glasgow, UK) showed further evidence for the role of Puma in p53-induced apoptosis. This protein has a Bcl-homology domain and interacts with Bcl2 to induce apoptosis. However, a deletion mutant that fails to induce apoptosis still retains some ability to bind to Bcl2, indicating that this interaction is not the sole function of Puma in inducing apoptosis. The key role of Puma in p53-mediated apoptosis was also investigated by Lowe in his Epi-Myc-induced mouse lymphoma model system. The use of RNA interference (RNAi) through the introduction of small hairpin RNA (shRNA) molecules that are homologous to either p53 or Puma strongly accelerated the formation of these tumours, indicating that in this model system, Puma phenocopies the effect of p53.

More kinases
J. Downward (London, UK) described the use of an shRNAi library containing three different constructs for all protein kinases. This library was used to identify novel intermediates in Ras-induced senescence including p110ct-Pi3 kinase, p70S6 kinase and the kinase target Mink. Mink is an STE20/GCK-like kinase that activates the p38 and Jun N-terminal kinase (Jnk) pathways. Mink shRNA inhibits the Ras-transformation of certain cell lines, indicating that this kinase also has a role in Ras-induced cell transformation.

LKB1 is a kinase that is mutated in patients with Peutz Jeghers syndrome, which is characterized by multiple benign and malignant tumours. The protein functions in a complex with the Ste20-related adaptor protein (Strad) and the scaffolding protein Mo25. D. Alessi (Dundee, UK) discussed their recent result that LKB1 is the upstream kinase for the AMP-activated protein kinase (AMPK): LKB1 phosphorylates AMPK in the T-loop and in LKB1-null cells, AMPK can no longer become activated (Hawley et al, 2004; Fig 3).

In the transcription factor field, R. Treisman (London, UK) reported further on his recently published work to decipher how serum response factor (SRF) is regulated by actin. One of the proteins involved in this process is Mal, a transcriptional coactivator (Miralles et al, 2003).

Interestingly, P. Rørth (Heidelberg, Germany) reported that Drosophila Mal has a crucial role in guiding border cells to their proper location in the oocyte. This process is induced by epidermal growth factor (EGF) or by PDGF/VEGF, depending on the destination. Border cells migrate as a group of six cells, and tension between the cells activates Mal to coordinate migration. This tension is induced by long extensions of the cells towards their destination. Indeed, when Mal is mutated, the cell body does not migrate, but the extensions break loose and continue to do so.

Lessons from the beginning
Our understanding of cancer-related processes often originates from studies in Drosophila. M. Bienz (Cambridge, UK) discussed the role of Pygopus (Pygo) in the regulation of the Wnt signalling pathway in Drosophila. Pygo is a protein that forms a complex
with Legless (Lgs), and together they interact with Armadillo (Arm; the *Drosophila* homologue of β-catenin). This interaction enhances the localization of Arm to the nucleus, where Arm interacts with Tcf to induce transcription. In addition, Pygo itself can interact with transcriptional coactivators and enhance transcription. Indeed, a fusion of the carboxyl-terminus of Pygo to a mutant of Tcf rescued the dominant-negative phenotype of this mutant.

B. Conradt (Hanover, NH, USA) reported on the mechanisms by which *Caenorhabditis elegans* regulates the apoptosis of cells that are destined to die. During the development of serotonergic neurons, the BH3-domain-containing protein egg laying abnormal 1 (EGL-1) is induced in the daughter cell that undergoes apoptosis. This induction is transcriptionally regulated through two helix–loop–helix proteins, HLH-2 and HLH-3, which compete with the snail-like transcription repressor CES-1 for binding to the EGL-1 promoter. How HLH-2 and -3 are induced is still unclear.

**Concluding remarks**

The field of signal transduction and cancer is still progressing at a rapid pace. It is encouraging that the translation of this knowledge into the development of potential therapeutics is also continuing to take place. This meeting was certainly a stimulating experience in the continuing efforts to combat cancer. We are already looking forward to the next one.

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**REFERENCES**


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