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A new start in Madrid

Symposium on basic and translational cancer research

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This symposium, held on February 6–9, 2002, marked the scientific opening of the Centro Nacional de Investigaciones Oncológicas (Spanish National Cancer Centre). The first national institute in Spain to focus on basic and applied cancer research will organise four conferences each year, covering different aspects of cancer research. These meetings, limited to a total of 20–25 speakers and an equal number of participants, should provide an ideal forum for the exchange of ideas and scientific information surrounding topics of current interest. This first symposium brought together 160 participants including basic researchers and clinicians, who all thoroughly enjoyed the scientific and social opportunities.

Introduction

Without doubt, the sequencing of the human and other important genomes has paved the way for the revolution in biology and medicine that we are experiencing today. Decoding this information in terms of regulation and function (functional genomics) is high on the agendas of governments and funding agencies and is expected to dominate research in the life sciences in the early part of this century. We are moving rapidly from the study of single molecules to the analysis of complex biological processes, and the current explosion of new and powerful technologies promises to accelerate the application of basic discoveries into daily clinical practice.

Cancer affects a significant proportion of the population and has become a prime focus for these new technologies. Indeed, tools for the high-throughput analysis of genes, proteins and their complex networks are being used to identify potential targets for drug discovery and to uncover markers for early detection, recurrence, progression and response to treatment, as well as to develop novel therapies. Molecules being currently investigated include cell-cycle regulators, growth factors, angiogenesis factors and signal transducing proteins, as well as components of apoptosis and DNA repair pathways. The purpose of the first symposium of the Centro Nacional de Investigaciones Oncológicas (CNIO) was to bring together basic researchers and clinicians to discuss the various stages involved in translating basic science into tools that will be useful in the clinic. Therefore, the symposium took the participants on a journey through disciplines as diverse as basic signalling research and clinical disease management. Only a few of the stops along the way are revisited in this report due to space constraints (I apologise to the speakers whose work could not be mentioned). The discussion is broadly organised according to the main emphasis of each talk, whether on basic mechanisms that contribute to the cancer state, genome-wide characterisation of the mutations that lead to cancer, or clinical approaches to treatment.

Setting the stage

Nobel Laureate H. Varmus (New York, NY) delivered the opening lecture of the meeting, providing a model for the sessions that followed with a thoughtful discussion that spanned topics from the need to educate the public about how medical research is conducted to recent findings on the molecular mechanisms behind tumour maintenance. He gave a brief history of various achievements in cancer research, starting with the discoveries of proto-oncogenes and tumour suppressors, and stressed that the recent availability of human and mouse genome information to the scientific community is responsible for the current optimism about developing effective treatments for cancer. His main focus, however, was on the cellular functions that are required to maintain the cancerous state. He used the example of the tyrosine kinase inhibitor STI571 to illustrate that vulnerability in these pathways can lead to the development of better and more effective drugs; STI571 successfully effects

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remission of chronic myelogenous leukemias by knocking out a constitutively active mutant form of the tyrosine kinase Abl and thereby inducing apoptosis. He went on to describe the work of his own group on the role of a *K-ras* oncogene in the initiation and maintenance of lung adenocarcinomas. The group has developed transgenic mice expressing the murine *K-ras4b* (G12D) under the control of doxycycline in type II pneumocytes, and has found the expression of mutant *K-ras* to be necessary for the maintainance of tumour cell viability, even in the absence of tumour suppressor genes (Fisher *et al.*, 2001). Varmus ended by emphasising that, in combination with a better understanding of the basic mechanisms behind cancer, increased knowledge of inherited risk, early detection, diagnosis by microarray analysis versus the microscope, and the individualisation of patient care will all contribute to vastly improved success in cancer treatment in the foreseeable future.

More on mechanisms

On their way to becoming cancerous, cells undergo significant changes mediated by processes ranging from cytoskeletal re-arrangement to cell-cycle deregulation. A number of signalling pathways have been implicated in such processes, and progress in understanding several of these was discussed at the symposium (Figure 1).

Signal transduction. T. Hunter (La Jolla, CA) discussed recent progress in understanding the regulation of the actin cytoskeleton by receptor and non-receptor tyrosine kinases. He reported that activating the EphA2 receptor tyrosine kinase in fibroblasts by plating cells on surfaces coated with its ligand, Ephrin A1, leads to cell spreading involving the formation of a circumferential ring of F-actin filaments. This occurs concomitantly with the appearance of peripheral structures containing the focal adhesion components paxillin and p130Cas and increased tyrosine phosphorylation of p130Cas and FAK. Fibroblasts from FAK tyrosine kinase, Src/Fyn/Yes tyrosine kinase and p130Cas knock-out mice all fail to spread in this assay, implicating these proteins in a signalling pathway downstream of EphA2. Thus, whereas neuronal Ephrin/Eph signalling generally results in F-actin disassembly, growth cone collapse and repulsion, the work with fibroblasts suggests that the cellular response to Ephrins is more complicated. Hunter went on to describe a dual role for the non-receptor tyrosine kinase Abl with regard to its interaction with the actin cytoskeleton. Wild-type Abl is inhibited by F-actin both *in vitro* and *in vivo*, and a mutant form lacking the F-actin binding domain promotes the formation of F-actin-containing microspikes or filopodia on the surfaces of fibroblasts in suspension, independent of the activity of the Rho family of small G proteins. Abl activity also stimulates the formation of F-actin branches/filopodia on axons of rat embryonic cortical neurons in culture, and the Abl kinase inhibitor STI571 reduces the number of branches. Thus, Abl and F-actin exhibit reciprocal regulation, in which activation of Abl promotes F-actin assembly, but F-actin then self-limits this process by inhibiting Abl activity (Woodring *et al.*, 2002). Continued progress in understanding the mechanisms touched on here may eventually lead to better control of cell migration events that contribute to metastasis.

K. Alitalo (Helsinki, Finland) spoke in a similar vein, describing growth factor regulation of the lymphatic vascular system, defects in which can lead to efficient transport of tumour cells throughout the body. Angiogenesis and the permeability of blood vessels are regulated by vascular endothelium growth factor (VEGF) via its two known receptors, VEGFR-1 and VEGFR-2. Alitalo showed that two other members of the family, VEGF-C and VEGF-D, in association with their receptor VEGFR-3, are necessary and sufficient for the embryonic development of the lymphatic vessels. Interestingly, human lymphoedema, a condition characterised by lymphatic insufficiency, is associated with inactivating missense mutations in the VEGFR-3 tyrosine kinase domain, and VEGF-C overproduction in various tumours increases lymphatic metastasis. Alitalo also provided evidence that blocking the VEGFR-3 signal transduction pathway inhibits this process.

P. Chambon (Strasbourg, France) described the role of retinoids and their potential for the treatment of various skin disorders and cancer. The actions of retinoids are mediated by the retinoic acid receptors (RARα, β and γ) and the retinoid X receptors (RXRα, β and γ), all of which belong to the nuclear receptor (NR) superfamily. The complexity of NR-mediated 'hormonal' signalling and the functional redundancies among receptor isotypes, as well as *in utero* or post-natal lethality of germline mutation of certain NRs, preclude a simple genetic analysis of the *in vivo* function of NRs using conventional gene knock-out approaches. Thus, Chambon's group has generated conditional somatic mutations, using the bacteriophage P1-Cre/lox system and the epidermally expressed *Keratin 14* (*K14*) promoter, eliminating retinoid receptor function specifically during skin development (Li *et al.*, 2000). Selective disruption of RXRα in epidermal keratinocytes of the mouse resulted in alopecia (hair loss), hyperproliferation of interfollicular epidermis, abnormal differentiation of keratinocytes and inflammation. Alopecia is also seen when the vitamin D3 receptor (VDR) is disrupted, suggesting a crucial function for RXRα/VDR heterodimers in the hair cycle. In contrast, none of these skin abnormalities are exhibited by mice in which RARα and RARβ are disrupted in epidermal keratinocytes, demonstrating that retinoic acid is not required for their homeostatic renewal. Finally, topical application of carcinogen to the skin of mice lacking RXRα in epidermal keratinocytes promotes an increased formation of epidermal tumours (papillomas) and an increased formation of melanocytic growths (nevi), lending support to the idea that retinoids may be useful in the prevention of cancer.

Cell cycle and genomic instability. Cell proliferation is an obvious requirement of all cancers. Whereas a great deal is known about the players that are directly involved in carrying out the cell cycle, the complexity of the stimuli that impact on it and therefore may play a role in the establishment of cancer has left many questions unanswered. It was only fitting that a number of talks at the symposium focused on the mechanisms involved in proliferation-related responses to various sorts of cellular stress or DNA damage. For example, M. Serrano (Madrid, Spain) described his group's efforts to identify candidate tumour suppressor genes that are specifically activated during Ras-induced senescence (a cellular protective response against the oncogene) using microarrayed filters

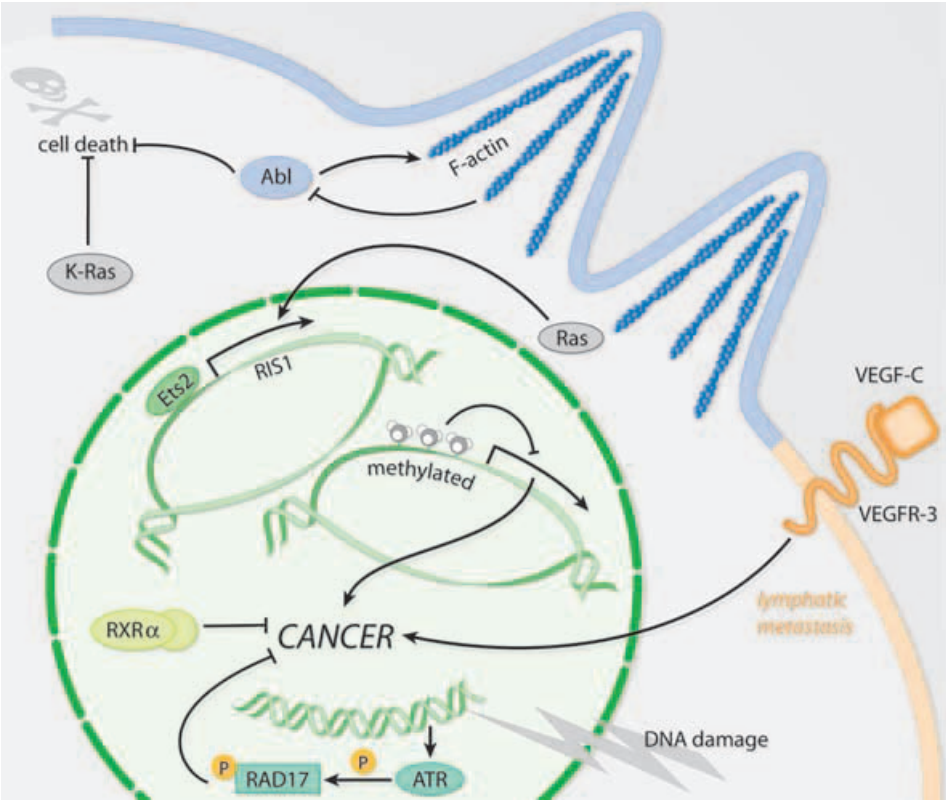

Fig. 1. Illustration summarising the effects of some of the signalling pathways that have been implicated in cancer and were covered at this meeting (but not necessarily acting in the same cell): K-Ras and Abl contribute to cancer maintenance; Abl and F-actin exhibit reciprocal regulation in cell shape remodelling; VEGF-C stimulates VEGFR-3 signalling to promote lymphatic vessel development, and its overproduction stimulates lymphatic metastasis; the ATR kinase responds to DNA damage by phosphorylating Rad17, thereby leading to a delay in the cell cycle; RXRα loss leads to increased cancer rates; Ras-induced senescence is mediated in part by the Ets-2 regulated *RIS1* gene; and methylation defects can contribute to cancer by silencing tumour suppressor and DNA repair genes.

(Barradas *et al.*, 2002). Microarrays were generated from cells transfected with oncogenic Ras, which triggers an irreversible proliferation arrest that is reminiscent of replicative senescence and is considered a relevant tumour suppressor mechanism. Among the initial set of genes selected from the microarrays was that encoding the known cell-cycle inhibitor p21 (Cip1/Waf1), validating the potential of the screen. Six other genes that were also highly upregulated were further evaluated for specificity, in cells overexpressing oncogenic Ras but rendered resistant to Ras-induced senescence by the concurrent expression of the viral oncoprotein E1a. These studies led to the identification of a novel gene, *RIS1* (for Ras-induced senescence 1), which is upregulated exclusively during Ras senescence by the transcription factor Ets2, a known mediator of Ras-induced senescence. Interestingly, *RIS1* is located at position 3p21.3, within a short segment of just 1 Mb that had previously been defined by others for its tumour suppressor activity.

E.Y.-H.P. Lee (San Antonio, TX) described the protein network involved in the DNA damage response, which also results in cell-cycle arrest. ATM (Ataxia telangiectasia mutated) and ATR (ATM- and Rad3-related) are protein kinases involved in DNA damage-induced cell-cycle checkpoints and the DNA replication checkpoint. Lee showed that human Rad17, which shares significant homology with the replication factor C, is preferentially phosphorylated by ATR. Phosphorylation of Ser635 and Ser645 of Rad17 is critical for ionising radiation-induced G_1/S and G_2/M checkpoints and is likely to be involved in the DNA replication checkpoint as well (Post *et al.*, 2001). Lee also reported on mouse models of mammary tumorigenesis that her group has generated by conditional inactivation of the *p53* tumour suppressor gene using the Cre/loxP system. Quantitative models of mammary tumorigenesis with nearly complete penetrance and a high frequency of metastasis have been established, and these display many characteristics of human breast cancer, including oncogene amplification and variation in the expression of estrogen receptor (ER)-α. Thus, these models are proving useful for evaluating genetic events during mammary tumour progression and for preclinical therapeutic studies.

M. Blasco (Madrid, Spain) discussed telomeres, specialised protein–DNA structures at the chromosome ends, and their role in cancer and ageing. Telomeres are essential for chromosomal stability; their dysfunction results in end-to-end chromosome fusions and loss of cell viability. These structures naturally become dysfunctional with ageing, due to progressive loss of telomeric sequences during cell division, or from mutation of telomere-binding proteins. Blasco's group has demonstrated that mice lacking the mouse telomerase RNA component Terc (González-Suárez *et al.*, 2001) are viable for only four to six generations depending on the genetic

background, each generation exhibiting progressively more severe defects in the germline, the skin, the gut and the haematopoietic system. Future studies will evaluate the impact of short telomeres on tumorigenesis and on chromosomal stability, the effects of telomerase upregulation (which occurs in >90% of all human tumours) and the possible negative effects of a putative gene therapy for age-related diseases based on telomerase reconstitution.

M. Esteller's (Madrid, Spain) presentation focused on cancer epigenetics, the inheritance of information based on gene expression levels rather than on the basis of gene sequence (Esteller and Herman, 2002). The main epigenetic modification in humans is cytosine methylation, which occurs in 'CpG islands', the guanine and cytosine-rich sequence tracts that are enriched in the 5′ regions of many genes. Methylation generally serves to silence gene transcription, whereas hypomethylation leaves the genes 'open' for transcription. Esteller's group has been characterising changes in methylation patterns in transformed cells, hypothesising that in some cases reversing these defects might be easier, and ultimately more effective, than correcting the genetic defects. Esteller's studies have illustrated that the epigenetic equilibrium of the cell suffers a dramatic transformation in cancer. Tumour suppressor genes such as *p16*^{INK4a}, *p14*^{ARF}, *APC* and *BRCA1* have all been found to be hypermethylated, and thereby silenced, in certain tumour types. The same is true for a number of DNA repair genes such as the mismatch repair gene *hMLH1* and *O6-methylguanine DNA methyltransferase* (*MGMT*), resulting in microsatellite instability in the former case, and in mutations in genes like *K-ras* and *p53* in the latter. Hypomethylation is generally a more global phenomenon and contributes to carcinogenesis by causing chromosomal instability, reactivation of transposable elements and loss of imprinting. Genetic defects in methyl-chromatin related genes have been shown to derepress DNA methyltransferases, methyl-binding proteins responsible for recruiting histone deacetylases, and chromatin factors that methylate CpGs. Many are known to be involved in diseases like Rett syndrome, pediatric cancers and Rubinstein–Taybi syndrome. The epigenetic profiles on which Esteller's group is working should be useful in refining and developing new cancer treatments that are based on demethylation strategies, establishing biomarkers that can be used in the detection of cancer cells, and developing prognostic indicators for subclasses of cancers (e.g. the methylation-associated silencing of the DNA repair gene *MGMT* in gliomas and lymphomas is a good indicator of which patients are sensitive to certain chemotherapies).

Genome-wide characterisation of cancers

A critical issue that was highlighted at this symposium is the lack of a unifying principle behind cancer. As more basic research is performed, the fact that numerous strategies will continue to be required for effective treatment, and that these will have to reflect not only the source of the cancer, but also its stage of progression, is becoming more obvious. Accordingly, many of the talks reflected the growing need for detailed characterisation and cataloguing of the differences between various cancerous states.

C. Lopez-Otin (Oviedo, Spain) made a strong case for this requirement in his discussion of matrix metalloproteinases (MMPs). He summarised major advances in our understanding of the structural and functional diversity of these enzymes, which comprise >20 distinct family members with potential relevance to disease. MMPs are not only involved in the extracellular matrix (ECM) destruction required for cell migration during metastasis, but also play essential roles in early stages of angiogenesis and growth factor activation. The pharmaceutical industry has made an impressive effort to develop MMP inhibitors; however, most studies with the first series of drugs have failed to demonstrate positive results in patients with advanced cancers. Lopez-Otin discussed possible causes for these failures, including underestimation of the importance of MMPs in early stages of the disease, as well as problems derived from the selection of both inhibitors and the proteases to be targeted by them. He also gave examples of MMP functions that are required by the host, and inhibitors with the ability to block the activities not only of MMPs, but also of other beneficial metalloproteinases. He concluded by introducing the concept of the tumour degradome, which he has developed in collaboration with C. Overall (Vancouver, Canada). The degradome is defined as the complete set of proteases produced by a particular tumour and is of great relevance for its progression and potential treatment; clinicians may in future be able to use a database containing this kind of information to more effectively design individualised therapeutic regimes.

Although much of the information that is now available for different tumour types is being generated by high-throughput technologies, F. Mitelman (Lund, Sweden) stressed the continuing value of the cytogenetic data that have been painstakingly compiled over the last 20 years. He explained that studies on human neoplasias (chromosome aberrations have now been reported in >40 000 human tumours; see the US Cancer Genome Anatomy Project at http://cgap.nci.nih.gov/Chromosomes/Mitelman) originally led to the realisation that many tumour types can be subdivided on the basis of characteristic rearrangements. An increasing number of the recurrent aberrations are specifically associated with distinctive morphological or clinical disease characteristics, making cytogenetics an increasingly important clinical tool for effective diagnosis, as well as prognosis prediction. Furthermore, the cytogenetic information has proved invaluable for identifying genes (presently at least 100) involved in the carcinogenic process, as well as in determining mechanisms by which they exert their actions through, for example, the deregulation of a seemingly normal gene at one of the breakpoints.

M.-A. Piris (Madrid, Spain) presented his group's work on cell-cycle control in non-Hodgkin's lymphoma. Cell-cycle deregulation in lymphomas is the result of multiple alterations in the genes encoding Bcl-6, c-Myc and cyclin kinase inhibitors (CKIs), concurrent alterations giving additional advantages to the tumour cells. Piris' use of expression profiling has revealed sets of correlations in B-cell lymphomas. Some of these have mechanistic implications. For example, the 'Major Mutational Cluster' intronic mutations contribute to Bcl-6 deregulation, and the expression of c-Myc and Bcl-6 are closely related, which is consistent with a proposed role for Bcl-6 induction of c-Myc. Other correlations, such as the fact that inactivation of CKIs is a feature of aggressive lymphomas, have predictive value.

Piris also reported that sequestration of the CKI p27 by complexes containing Cyclin D and CDK4/CDK6 is secondary to the loss of the CKIs p21 and p16, and p14ARF nuclear over-expression in aggressive B-cell lymphomas is a sensor of malfunction of the common tumour suppressor pathways (Sánchez-Aguilera *et al.*, 2002). Further characterisation along these lines will surely continue to enhance the effectiveness of patient treatment.

P. Meltzer (Bethesda, MD) gave a comprehensive talk about the benefits of microarray technology, starting with a history of its development. He emphasised that for clinical correlative studies using this technique it is essential to define the question, choose the right patient samples and use appropriate and rigorous statistical analysis, as well as to validate the results using additional technologies. He presented studies in which the phenotypes associated with ERα expression in 58 node-negative breast carcinomas discordant for ER status were investigated using microarray technology, in combination with artificial neural networks and standard hierarchical clustering techniques for analysis of the data. The results showed that $ER\alpha^+$ and $ER\alpha^-$ tumours display profound differences in target gene expression, again illustrating the potential impact that tumour databases will have with regard to cancer treatment. Finally, Meltzer stressed that all the information that is currently being collected can only be effective if new computational tools are developed to efficiently analyse gene expression data, to link genomic with expression data to define regulatory elements and to assure that these data reach the clinical practice.

Clinical issues

In addition to the talks about investigations into the basic mechanisms whereby cancers arise and about defining the features of particular cancer types, there was also a great deal of discussion about patient needs and clinical policy. B. Ponder (Cambridge, UK) gave a particularly interesting presentation on the need to address the genetics of cancer predisposition, not only for high-risk cases that have been the focus to date because the genes involved have been relatively straightforward to identify, but also of predispositions resulting from a combination of genetic variants with only weak individual effects. The latter are likely to be quite widespread through the population (in the case of breast cancer, for example, the origins of ~80% of cases are unknown). Although the value of identifying such variants has been controversial because their individual impact is unclear, Ponder argued that this kind of predisposition could lead to a population risk distribution of considerable significance when multiple variants are present simultaneously. As the identification of the genes that make up the total risk profile becomes possible with emerging technologies, such approaches should be undertaken because they are likely to bring tangible benefits to society.

D.W. Golde, Physician-in-Chief of the Memorial Sloan-Kettering Cancer Center (New York, NY), focused on the need to more efficiently translate basic discoveries to the benefit of the patient, discussing the problems involved in providing first-class cancer care delivery and sharing his vision of the future. He described a model of super sub-specialisation which, in his experience, is the key to outstanding cancer care. The approach relies on integrated, multi-disciplinary disease management teams whose members, including not only the physicians and nurses but all of the support staff, essentially deal with only one kind of cancer and are thus extremely efficient at treating patients with a particular type of cancer. This methodology relies on highly organised treatment pathways, an advanced electronic information system and, of course, state-of-the-art equipment and infrastructure.

Perspectives

The inaugural symposium of the CNIO was a unique meeting that encouraged its participants to think more about the mutual benefits of, and the need for, cross-disciplinary discussion when it comes to cancer research. For basic researchers, it highlighted the clinical endpoints of some of our efforts, as well as reminding us of the enormous complexity of disease, which often makes the cure much more complicated than the laboratory experiment. For clinicians—and there were many in the audience who normally have little contact with basic research laboratories—it was a chance to catch up on the latest information on the mechanisms that lead to the tumours that they see in the pathology laboratory, or for the therapies that they are implementing in the wards. For all, it was also an opportunity to think about the factors and policies that are currently shaping medical care.

Finally, as was expressed by a number of the speakers, it was a privilege to speak at this opening symposium of the CNIO. The presentations of the young Spanish speakers left no doubt that cancer research in Spain is on its way up, and the healthy representation of both PhDs and MDs on the staff of the CNIO is sure to lead to it becoming a strong presence in the fight against cancer.

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Mariano Barbacid (Symposium organiser and Director of the CNIO) & Julio E. Celis in front of the newly established Cancer Centre.

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