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How to cite

ZUCCA, E., NIGG, Erich A. Cell cycle regulation and the function of cancer genes. In: Annals of Oncology, 1995, vol. 6, n° 10, p. 975–978. doi: 10.1093/oxfordjournals.annonc.a059092

This publication URL: https://archive-ouverte.unige.ch/unige:131164

Publication DOI: 10.1093/oxfordjournals.annonc.a059092

Annals of Oncology 6: 975–978, 1995.

Commentary

Cell cycle regulation and the function of cancer genes

E. Zucca[1] & E. A. Nigg[2]

[1]*Servizio Oncologico Ospedale San Giovanni, Bellinzona;* [2]*ISREC (Swiss Institute for Experimental Cancer Research), Epalinges, and Département de Biologie Moléculaire, Université de Genève, Switzerland*

Key words: cell cycle, cyclin-dependent kinases, cyclins, oncogenes, tumour suppressor genes

The Pezcoller Foundation is a non-profit organisation created in 1979 by Prof. Alessio Pezcoller, then Chief Surgeon of the Santa Chiara Hospital in Trento, Italy. The main purpose of the Foundation is to promote scientific research by awarding prizes to meritorious scientists in the various fields of medicine and organising symposia on issues regarded as pivotal for the advancement of scientific knowledge. Since its inception, the Foundation, as its founder mandated, has dedicated particular attention to oncology, supporting the fight against cancer.

The Seventh Pezcoller Foundation Symposium, focused on functional aspects of cancer genes, took place during the last month of June in Trento, Italy.

The meeting was divided into five sessions for reviewing and discussing the precise relationship between the function of oncogenes and of tumour suppressor genes, the regulation of transcription, cell cycle control and apoptosis, and the possible implications of research programs in these fields for the prevention and therapy of cancer.

Positive and negative regulation of cell division

Normal cell division is activated by signal transduction pathways. These are triggered by extracellular growth factors that bind to cell surface receptors. In response to ligand binding, the receptors recruit and activate a multitude of signalling molecules to the cytoplasmatic surface of the plasma membrane. Prominent among these are the so-called G proteins that function as molecular 'signalling switches' depending on whether they exist in a complex with GTP or GDP. Ultimately, cascades of protein kinases are activated. Some kinases will translocate to the cell nucleus where they phosphorylate transcription factors. The final result is the expression of appropriate genes committing the cell to divide. Many proto-oncogenes code for growth factors, receptors, G proteins, kinases, or transcription factors, and hence exhibit their normal function within signal transduction pathways that activate the cell's entry into the cell division cycle (positive regulation). Of course, cell division is also subject to negative regulation. In normal cells, growth arrest signals play a crucial role in various situations (e.g. response to negative growth-factors, cell differentiation, senescence, repair of damaged DNA, nutrient starvation). Many tumour suppressor genes encode proteins that can inhibit the cell division process at various points. Many, albeit not all, oncogenes and tumour suppressor genes may thus be viewed as, respectively, positive and negative regulators of the cell division cycle.

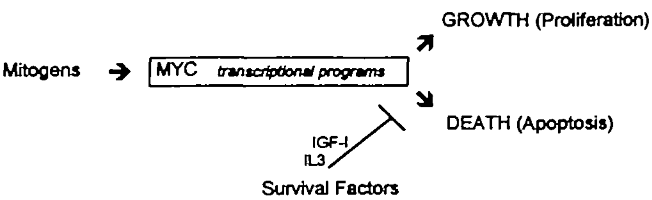
c-*myc* oncogene and tumour suppressor gene?

One important concept which was discussed during the symposium regards the *multifunctional nature of certain key components that regulate cell proliferation.*

One interesting example comes from studies of the c-*myc* function. The c-*myc* proto-oncogene has long been shown to be involved in the control of normal cell growth, and instances of its deregulated expression have been widely found in many different types of tumours. For example, in Burkitt's lymphoma, reciprocal chromosomal translocations deregulate c-*myc* by placing it under the control of immunoglobulin gene enhancer sequences that are transcriptionally active in the B-lymphocytes from which this lymphoma arises. The c-*myc* gene encodes a short-lived nuclear phosphoprotein that exhibits sequence-specific DNA binding and possesses both *trans*-activation and leucine-zipper domains indicating that it acts as a transcription factor. It has well known growth-promoting (and oncogenic) properties that depend on its dimerisation with heterologous partners (named Max and Mad). However the identity of c-*myc* target gene(s) remains unclear. Data were presented at the symposium supporting, the hypothesis that c-*myc*, in certain situations, can induce apoptosis (programmed cell death) instead of proliferation. Apoptosis induced by the c-Myc protein in serum-deprived fibroblasts requires sequence-specific DNA-binding, *trans*-activation and dimerisation with

Max, events that are also required for the oncogenic and growth-promoting activities of c-Myc. Therefore, c-Myc seems to be able to promote functionally different genetic programs (cell growth and programmed cell death), possibly through the transcriptional modulation of different types of target genes. Several lines of evidence support the concept of a physiological regulation of apoptosis by c-*myc* in certain circumstances. The c-*myc* apoptotic program seems to be present but not active in fibroblasts when these grow in highly concentrated serum. Serum contains different cytokines, among which the insulin-like growth factor-I, IGF-I, was found to be the most potent inhibitor of c-Myc-mediated apoptosis. In certain cell lines treated with etoposide (a topoisomerase II inhibitor) or thymidine (a DNA synthesis inhibitor) activation of c-Myc induces apoptosis that can be reduced and delayed by IGF-I. IGF-I thus appears to protect cells from apoptosis.

A 'Dual Signal model' has been proposed to explain the multifunctional activity of c-Myc:



This model needs further validation. However, if proven correct, it might have important implications for cancer therapy. In fact, inhibition of apoptosis may result in a kind of multi-drug resistance, since it allows the survival of cells despite acquired DNA damage, and promotes genetic instability (thus contributing to tumour progression). If the c-Myc protein in normal cells acts not only as a growth promoter, but also as an inducer of apoptosis (thereby preventing the continued proliferation of cells with damaged DNA), then the suppression of c-*myc* function might make cells more resistant to drugs. Hence, therapeutic approaches to the regulation of this gene, should perhaps not be aimed at impairing *myc* function *per se*, but should selectively target the growth-promoting pathway downstream of *myc*.

Dysregulation of cell cycle and cancer development

Another important issue on which many presentations at the symposium were focused, was the *interaction between the machinery that regulates the cell division cycle and the growth-regulatory pathways that are subverted as a consequence of oncogenic events.*

At a molecular level, the development of human tumours is a complex multi-step process involving multiple genetic alterations that abrogate the normal mechanisms of control of cell proliferation. A characteristic of most tumour cells is their ability to enter and progress through the cell cycle under conditions in which normal cells would be quiescent.

The timing of the cell cycle is based to a large extent on the synthesis and degradation of proteins called cyclins. The name of these proteins comes from the observation that their abundance periodically increases during one phase of the cell cycle and then decreases. Many cyclins have been described and grouped into families denoted by letters (A, B, C, D, E, etc.). It is now well established that the positive regulation of the cell division cycle is primarily controlled by a family of serine/threonine protein kinases, known as CDKs (cyclin dependent kinases), which form complexes with their regulatory cyclin partners. Thus far, eight CDKs have been identified in humans, designated CDK1 (Cdc2), CDK2, CDK3, and so on. Each of these CDKs can form a binary complex with one or more different cyclins. CDKs by themselves are inactive, and although binding to a cyclin protein is required for their activity, it is not sufficient. In fact, the function of CDKs is also regulated in a complex manner by other kinases and phosphatases. In their catalytically active state, the cyclin/CDK complexes allow the cell to enter the cell cycle and progress through the specific phases: DNA synthesis (S) and mitosis (M), separated by the corresponding preparatory phases (G1 and G2).

The first cyclins to be discovered are called A and B cyclins. The Cdc2/cyclin B complex (also known as maturation promoting factor, MPF) regulates the transition from G2 to mitosis, whereas. cyclin A is required for the progression through S phase. The G1 cyclins (the cyclin D family and cyclin E) promote movement through the earliest phases of the cycle and are essential for the G1-S transition. Of particular interest, CDK complexes with members of the cyclin D family are modulated by exogenous growth factors (e.g. CSF-1 and TGF-β) and play a key role in abrogating the growth-inhibiting and tumour-suppressor function of the retinoblastoma gene product (pRb).

The regulation of the cell cycle is clearly important for maintaining a homeostatic balance between cell growth, differentiation, survival and death. Therefore, it does not come as a surprise that multiple lines of evidence now indicate a link between dysregulation of the cell cycle and development of cancers. In particular, the excessive, or temporarily inappropriate expression of cyclins during the cell cycle appears to play an important role in the development of different malignancies. The cyclin D1 gene maps to 11q13, closely linked to a chromosomal region where loci have been identified (PRAD1, *bcl*-1) that are implicated in the aetiology of different tumours. Translocation and rearrangement with consequent overexpression of the cyclin D1 gene is characteristic, for instance, of mantle cell lymphomas in which the t(11;14)(q13;q32) chromosomal translocation results in deregulation of the cyclin D1 gene. This may perturb the G1-S transition of the cell cycle, thereby contributing to lymphoma development. Overexpression of cyclin D1 is consistently found in mantle cell lymphoma, even when *bcl*-1 rearrangement cannot be demonstrated. The cyclin D1 gene was also found to be amplified in approximately 20% of breast cancers, and amplification or overexpression of the gene has been detected in oesophageal , bladder, lung, and head and neck cancers. Abnormalities of the cyclin D3 gene have been described in retinoblastomas, lymphocytic leukaemias and lymphomas. Overexpression of other cyclins has also been reported in breast cancer and in several other tumours. Therefore, detection of the aberrant expression of one or more cyclins or the loss of CDK inhibition (see below) might in the future become useful as diagnostic and prognostic molecular markers for many cancers. Abnormal cyclin mRNAs and proteins might also provide molecular targets for novel therapeutic interventions.

Cells also possess negative regulatory mechanisms to control the cell cycle, balancing the growth-promoting activities of the cyclin/CDK complexes. Several cell cycle inhibitory proteins capable of physically interacting with cyclins or CDKs have recently been discovered, and a striking connection between some of these inhibitors and tumour-growth suppression has been demonstrated. Currently at least two distinct families of CDK inhibitors, represented by the two prototypes, *p21* and *p16*, can be recognised.

The inhibition of CDKs appears to be very important for the proper functioning of checkpoints that can arrest the cell cycle when cells are exposed to DNA-damaging agents. The term checkpoint refers to pathways that are activated in response to damage or failure during the cell cycle (e.g. damage to DNA or failure to assemble the spindle). Defects in checkpoint control genes have been demonstrated to be associated with increased rates of mutations and chromosomal instability in many tumours. Normal proliferating cells can respond to DNA damages induced by exposure to ionizing radiation, UV, alkylating agents or metabolic poisons by delaying the transitions from G1 to S or from G2 to mitosis.

By activating checkpoint mechanisms, normal cells can delay progression through the cell cycle and thus gain time for repairing DNA or for correctly aligning their chromosomes on the mitotic spindle. Alternatively, checkpoint mechanisms may eliminate damaged cells by causing apoptosis. In cancer cells, checkpoint mechanisms are frequently defective, and cells continue cell cycle progression in spite of damage to their genome.

The p21 CDK inhibitor (WAF1) works downstream of the tumour suppressor gene p53 and was first identified in normal human fibroblasts as a component of a cyclin D1-CDK quaternary complex that also contains the proliferating cell nuclear antigen (PCNA). In virally transformed cell lines as well as in p53-deficient cells from patients with Li-Fraumeni syndrome, p21 and PCNA are not detected in the cyclin-CDK complexes. It has been demonstrated that p21 expression is transcriptionally activated by wild-type, but not mutant p53 genes. The p53 tumour suppressor gene is the most frequently mutated gene in human cancers. In cancer cells with p53 loss or mutations, the failure to induce p21 expression (and hence failure to inhibit the cyclin/CDK complex and/or the PCNA dependent DNA replication) results in the suppression of the G1-S checkpoint that normally halts cell cycle progression when cells are exposed to DNA damaging agents. As a consequence, the lack of time to repair DNA damage may increase the frequency of mutations being perpetuated during DNA replication. The mechanism of p21 activation by the p53 gene, therefore, demon-

Table 1. List of speakers, and discussed issues at the 7th Pezcoller Foundation Symposium devoted to the functional aspects of cancer genes, held in Trento, Italy on June 14–16, 1995.

The 7th Pezcoller Foundation Symposium, Trento, Italy, June 14–16, 1995

Cancer genes: functional aspects

Session I, Oncogenes
- Integrated control of cell growth and cell death by oncogenes (G. Evan, *Imperial Cancer Research Fund, – London, U.K.*)
- Control of invasive cell growth by the MET family oncogenes (P. Comoglio, *Università di Torino, Italy*)
- Src family kinases and the cell cycle (S.A. Courtneidge, *Sugen Inc., – Redwood City, U.S.A.*)

Session II, Suppressor Genes
- CDK Inhibitors (Y. Xiong, *University of North Carolina – Chapel Hill, U.S.A.*)
- p53, transcriptional activation, and apoptosis (M. Oren, *Weizmann Institute of Science – Rehovot, Israel*)
- The Wilm's tumour suppressor in tumorigenesis and development (D. Hausman, *MIT – Cambridge, U.S.A.*)

Session III, Transcription Regulation
- Cellular and viral transcriptional activators (M. Green, *Univ. of Massachusetts Medical center, Worcester, U.S.A.*)
- CREM and the transcriptional response to cAMP (P. Sassone-Corsi, *Laboratoire Genetique Moleculaire – Strasbourg, France*)
- Signal-mediated activation of transcription by tenary complex factors (A. Nordheim, *Hannover Medical School, Germany*)
- Involvement of the TEL gene in hematologic malignancy (G. Gilliland, *Brigham and Women's Hospital – Boston, U.S.A.*)

Session IV, Cell Cycle Regulation
- Cyclin-dependent Kinases regulation in normal and cancerous cells (G. Draetta, *Mitotix Inc. – Cambridge, U.S.A.*)
- Retinoblastoma protein, gene expression and cell cycle control (J. Azizkhan *Roswell Park Cancer Institute-Buffalo, U.S.A.*)
- Gene products which regulate Go/G1 exit (D. Livingston, *Dana-Farber Cancer Institute – Boston, U.S.A.*)
- Homeostatic mechanisms governing the Go phase (S. Schneider, *LNCIB Area Science Park – Trieste, Italy*)

Session V, Clinical Implications
- Selective killing of BCR-ABL positive cells with a specific inhibitor of the ABL tyrosine kinase (B. Druker, *Oregon Health Sciences University – Portland, U.S.A.*)
- Genetic manipulation of anti-tumour immunity (G. Dranoff, *Dana-Farber Cancer Institute – Boston, U.S.A.*)
- BCL2 and response to therapy, (C. Croce, *Jefferson Cancer Institute – Philadelphia, U.S.A.*)

strates a critical link between the tumour suppressor function of p53 and the cell cycle control. Further evidence that CDK inhibitors may play a pivotal role in tumour development and growth is provided by studies of p16. This CDK-inhibitor was first identified as a small protein which forms a binary complex with CDK4 (and CDK6) in virally transformed cells that lack functional p53 and retinoblastoma protein (pRb). The gene encoding p16, the so called multiple tumour suppressor-1 (MTS1, also named INK4, CDK4I, and CDKN2) is located on chromosome 9p21, where the locus for familial melanoma has also been mapped. Alterations of the p16 gene have been described in several specific types of primary tumours, including melanoma, lymphocytic leukaemias, and cancers of the lung, breast, bladder, ovary, skin, and brain, and also in many human tumour-derived cell lines.

The targets of the p16 inhibitory activity are CDK4 and CDK6. Both have been identified as the physiological kinases regulating the function of another tumour suppressor gene product, the retinoblastoma protein, pRb. When pRb is not phosphorylated extensively, it sequesters transcription factors (E2F and DP1) that are required for the activation of S-phase genes. Phosphorylation of pRb by cyclin D-CDK4/CDK6 complexes causes the release and functional activation of the E2F/DP1 transcription factors. By preventing the transcription of S phase genes, pRb is an important negative regulator of entry into S phase. In turn, the tumour-suppressor activity of p16 results from its ability to inhibit the kinase activities associated with cyclin D-CDK4/CDK6 complexes: when pRb cannot be phosphorylated by these CDKs, cell cycle progression from G1 to S cannot occur.

In addition to p21 and p16, other small proteins associated with CDK inhibition have been identified and their number is increasing. The expression of different CDK inhibitors seems to vary in different human tissues, indicating a tissue-specific regulation of their expression, and a possible role in causing cell cycle exit during terminal differentiation.

In this commentary we have tried to summarise just a few of the scientific topics discussed at the meeting, focusing on new developments related to the comprehension of the molecular regulation of cell proliferation. Table 1 lists the contributions presented at the meeting. The most meritorious aspect of the symposium was the opportunity for a detailed discussion of the many conceptual and practical advances in molecular medicine that are changing our perception of cancer pathogenesis. This in time might provide the opportunity to develop novel, more efficacious therapeutic strategies to alter the biological behaviour of many tumours. However, at present, we still wait for these discoveries to produce their complete clinical benefit, and the fulfillment of the gap between our steadily increasing molecular knowledge and the prevention and treatment of cancer remains a fundamental challenge.

Detailed proceedings of the meeting, edited as usual, by E. Mihich, will be soon published.

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Received 14 November 1995; accepted 21 November 1995.

Correspondence to:
Emanuele Zucca, M.D.
Servizio Oncologico Cantonale
Ospedale San Giovanni
6500 Bellinzona
Switzerland