ADVANCES IN APPLIED BIOTECHNOLOGY SERIES Volume 6

ONCOGENESIS:

ONCOGENES IN
SIGNAL TRANSDUCTION
AND
CELL PROLIFERATION

EDITOR

Takis S. Papas

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Papers delivered at the First International Conference on Jene Regulation, Oncogenesis, and AIDS Loutraki, Greece Sentember 15-21, 1989

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On the Cover	
"Cene Rogulati	on and Oncorposio"

"Gene Regulation and Oncogenesis Computer Generated Graphic

Artist: Dr. Samuel D. Huang, Professor of Biology and noted Riverside, California artist. Commissioned for the First International Conference on Gene Regulation, Oncogenesis, and AIDS held at Louraki, Greece September 15-21, 1989

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In organizing the First International Conference on Gene Regulation, Oncogenesis, and AIDS, we selected the two most currently active areas of disease related research: cancer and AIDS. It goes without saying that in both these areas a vast amount of information has been accumulating over the past few years. Consequently, an extensive amount of information was presented at this Conference. The number of papers, as well as the excellent quality and indepth scope of each paper were such that we felt it would be of greater value and usefulness to the reader to present this information in two separate volumes. The first of the two books comprises the papers presented on the subject of oncogenesis, while the second book comprises topics of gene regulation and AIDS. Together the two books present a unique overview of the interrelationship of research in oncogenesis and AIDS; however, as separate books, each is a comprehensive volume on its respective area of research.

This book, *Oncogenesis*, consists of selected papers presented in the continually expanding field of research on oncogenes and their influence on the occurrence of cancer.

It is generally believed that the products of the normal cellular homologs of the oncogenes perform important functions that are essential to the cell, serving processes that are involved in growth, or its regulation, or in the process of differentiation. Some of these gene products resemble growth factors and their receptors, while others resemble nuclear regulatory factors, but most importantly, these gene products interact with other cellular factors that normally participate in the signal transduction pathway, transmitting signals from the exterior to the interior of the cell and triggering mitogenic or proliferative responses. Thus far we have identified some 55 or so oncogenes. The products of many of these genes have been identified in cells, and great efforts are now being made to understand the biology and role of these oncogene products in cell development and growth.

Just how these gene products interact and affect the initiation of cell growth and proliferation at an abnormal or detrimental level remains to be determined. Therefore, the functions of the protoonco gene products and their cellular targets is of tremendous interest to those of us in the field of oncology. Understanding these functions will enable researchers to pinpoint precisely at which points genetic damage results in the activation of processes that can lead to onco-genesis.

Since the quality of a symposium can be equated with its participants, I believe that the papers presented herein attest to the caliber of research and provide an accurate assessment of the direction and progress being made in this area. The papers are presented in a manner that should orient researchers in fields other than their own. Therefore, this volume, and its companion text, should provide an occasion in which heterogeneous ideas of various experts and their disciplines are brought together and exchanged. Judging the response from the participants, the reader will be able to see why this Conference was considered so successful. Due to space limitations, the active discussions following the sessions have not been included. Nonetheless, it is fair to say that most participants will be looking forward to updating their materials and to presenting new information for the Second International Conference two years hence.

Takis S. Papas Editor

The Editor

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The papers given in this Book were prepared for The First International Conference on Gene Regulation, Oncogenesis, and AIDS, held in Loutraki, Greece, September 15 to 21, 1989.

I would like to acknowledge and thank my co-organizers, Dr. Steve Kottaridis, Dr. Flossie Wong-Staal and Dr. Max Essex for their support and help with the Conference; their advice and assistance enhanced these meetings considerably. I would also like to express my appreciation to my associate, Dr. Richard Ascione, and secretaries, Ms. Karen Cannon and Ms. Cheryl Nolan; they were invaluable to the organization of this conference and made my task considerably easier and more rewarding. My great appreciation is extended to my friend, Dr. Sam Huang of the Riverside City College, for his artistic insight and contribution in illustrating the cover of this volume.

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Takis S. Papas Editor

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Platelet-Derived Growth Factor in Human Proliferative

Diseases

Harry N. Antoniades

The Center for Blood Research Division of Biological Sciences and Department of Nutrition Harvard School of Public Health Boston, MA 02115 Human PDGF normally circulates in blood stored in the α granules of platelets. These cells have an affinity for injured sites, aggregating there and releasing PDGF, where it contributes to wound repair. The *inappropriate* expression of PDGF has been linked to proliferative diseases and appears to be part of the autocrine mechanism leading certain responsive cells to unregulated growth.

In investigating the expression of PDGF family genes in tissues derived from patients with primary human astrocytomas and meningiomas and patients with idiopathic pulmonary fibrosis (IPF), we found the co-expression of the c-sis/ PDGF-2 protooncogene and PDGF-receptor (PDGF-R) gene in the human astrocytoma and meningiomas tumor cells. Nonmalignant brain tissue and normal dura expressed the PDGF-R gene but not the c-sis protooncogene. The coexpression of a potent mitogen and its receptor in the tumor cells appears to contribute to the development and unregulated growth of these tumors. Studies also showed a strong expression of the c-sis/PDGF-2 protooncogene by the lung epithelial cells. Epithelial cells in lung tissues derived from individuals without IPF did not express c-sis mRNA. Thus, the expression of PDGF by epithelial cells and macrophage of lung tissue appears to contribute to the excessive growth of responsive lung fibroblasts and to the accumulation of interstitial collagen.

Introduction

Human platelet-derived growth factor (hPDGF) represents the major growth factor activity in human clotted blood serum. It normally circulates in blood and is stored in the α-granules of platelets. Human PDGF is a potent mitogen for mesenchymal-derived cells such as diploid fibroblasts, osteoblasts, arterial smooth muscle cells, and glial cells.¹⁻³ It consists of two homologous polypeptide chains (PDGF-1 and PDGF-2) linked together by disulfide bonds. The PDGF-2 chain is encoded by the sis oncogene, 5.6 which is localized in chromosome 22, and the PDGF-1 chain is encoded by a gene localized in chromosome 7.7 In cells infected with the simian sarcoma virus (SSV), the transforming protein product (p28st) undergoes discrete processing steps to yield a disulfide-linked sis/PDGF-2 homodimer that is structurally, immunologically, and functionally similar to hPDGF.89 The connection of PDGF-2 to the sis oncogene has had far-reaching consequences with respect to understanding the functional role of the transforming gene (v-sis) itself. The mechanism by which this oncogene transforms cells appears to involve the constitutive expression of a potent mitogen, the sis/PDGF-2 homodimer, with the results of unregulated, sustained cell proliferation. 5,8,10 Thus, such studies brought two important areas of research, oncogenes and growth factors, into a common area of investigation.

Isoforms of PDGF

PDGF-1 is also known as PDGF-A, and PDGF-2 is known as PDGF-B. Human PDGF represents a heterodimer of these two chains, as suggested originally from our sequence studies. The sis/PDGF-2 and the PDGF-1 homodimers represent two additional forms of biologically active PDGF. Thus, as shown in Figure 1, there are three isoforms of PDGF. The mitogenic action of these isoforms is mediated through binding to specific cell surface PDGF-receptors. The receptor that binds the human PDGF heterodimer and the sis/PDGF-2 homodimer has been cloned from mouse and human fibroblasts^{11,12} and is currently referred to as the "a/b" or b receptor. This receptor

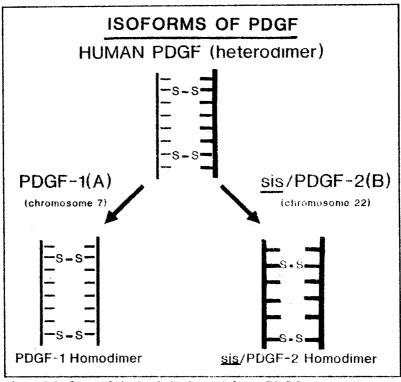


Figure 1. Isoforms of platelet-derived growth factor (PDGF).

does not recognize the PDGF-1 homodimer which binds to a separate, "a" receptor. This "a" receptor has been cloned recently from human cells and was shown to bind to all three PDGF isoforms (Table 1). In view of the information just summarized, investigations on the functional role of PDGF must take into account the differences in the specificity of receptor binding among its various isoforms.

The Expression of the Mitogenic Action of PDGF is Enhanced by the Synergistic Action of Progression Factors

An important consideration in studies with PDGF is the understanding of its mode of action. Although PDGF is considered to be a

Table 1
Platelet-Derived Growth Factor (PDGF)—Receptor Competition Among the Isoforms of PDGF

Receptor	Competes Only With
PDGF-1	PDGF-1
PDGF-2	PDGF-1, PDGF-2, hPDGF
hPDGF	hPDGF and PDGF-1

potent mitogen, expression of its mitogenic activity requires the synergistic action of additional growth factors. In vitro studies using cultured 3T3 cells provided a new understanding of cell growth. The significant finding was that the transition from G₀/G₁ phase to the S phase of the cell cycle could be subdivided into two stages. One, called competency, is controlled by PDGF and allows cells to enter the G/ G, phase of the cell cycle. The other, called progression, is controlled by other growth factors present in plasma that enable progression of the PDGF-induced competent cells into the S phase¹⁷⁻²⁰ (Figure 2). Insulin-like growth factors (IGFs) appear to be part of the progression factors, although in vitro, in cell culture, IGFs alone did not induce the progression of competent cells into the S phase, indicating the need of additional factors present in plasma. 18 In contrast, in vivo studies have shown that the synergistic action of pure PDGF with pure IGF-1 can induce in animals a dramatic increase in connective tissue cell proliferation and collagen synthesis and an increased amount of epithelium²¹ (Figure 3). Thus, in vivo, the synergistic action of IGF-1 was necessary for the expression of the biologic action of PDGF. Transforming growth factor alpha (TGF-α) could replace IGF-1, and the combination of PDGF/TGF-\alpha produced in vivo effects similar to those seen with the PDGF/IGF-1 combination.²¹

The studies just described imply that PDGF is the limiting factor for the *in vivo* growth of normal target cells. Progression factors are available *in vivo* from circulating blood. In contrast, PDGF is transported by platelets and is available only selectively, in small amounts, at the site of injury during platelet degranulation. For this reason, cells that are targets for PDGF action and acquire the ability to produce

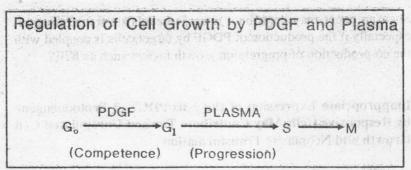


Figure 2. Growth of 3T3 fibroblasts in culture is regulated by synergistic action of PDGF and other factors present in platelet-poor plasma (plasma). PDGF makes cells competent to enter the G_n/G₁ phase of cell cycle (competence factor); transition to S phase is controlled by progression factors in plasma. (References 17 and 18).

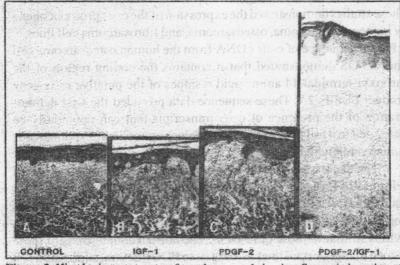


Figure 3. Histologic appearance of ten-day wounds in pigs. Synergistic action of pure recombinant PDGF-2 homodimer and insulin-like growth factor (IGF)-1is necessary for in vivo regeneration of connective tissue and epithelium in this wound healing model. All wounds received a single 500ng application of each growth factor after wounding. PDGF-2 or IGF-1 alone caused significantly smaller effects than their combination. (Reproduced from Reference 21 with permission of the American Society for Clinical Investigation.)

their own supply of PDGF escape this regulatory restriction. They are then capable of entering into a continuous and unregulated growth.