Anal. Real Acad. Nac. Farm., 2005, 71: 525-534

Revisión

The TGFβ pathway in basic oncology *

JOAN MASSAGUÉ
Cancer Biology and Genetics Program, Howard Hughes Medical Institute, Memorial Sloan-Kettering Cancer Center

ABSTRACT

The transforming growth factor-β (TGFβ) family is particularly prominent among growth factors controlling cell proliferation and differentiation, and fosters tissue growth and morphogenesis during embryogenesis in organisms as diverse as the nematode, fruit fly, and human. To elucidate the basis for the great diversity of TGFβ responses in different cell types, we delineated the pathway linking membrane TGFβ receptors to target genes. TGFβ assembles a receptor complex in which the type II receptor kinase phosphorylates and activates the type I receptor. This type I receptor phosphorylates and activates Smad transcription factors. A receptor-activated Smad complex enters the nucleus to find partners for the recognition and regulation (activation of repression) of specific genes. TGFβ signaling decreases CDK activity and causes the repression of several growth-promoting genes. The TGFβ pathway, when altered, plays an essential role in tumorigenesis and metastasis.

Key words: TGFβ.—Smad.—Cyclins.—CDKs.—Cell cycle.—Metastasis.

RESUMEN

La vía del TGFβ en oncología básica

La familia del factor de crecimiento transformante-β (TGFβ) se destaca particularmente entre los factores de crecimiento como regulador de la proliferación y

* Revisión realizada con motivo de la toma de posesión como Académico de Honor de la Real Academia Nacional de Farmacia, Madrid, 17 de mayo de 2005.

diferenciación celular. El TGFβ promueve el crecimiento de los tejidos y la morfogénesis durante la embriogénesis en organismos tan diversos como nematodos, mosca de la fruta y humanos. Para elucidar las bases de esta gran diversidad de respuestas del TGFβ en diferentes tipos celulares, hemos trazado la vía que comunica los receptores de membrana del TGFβ con los genes diana. El TGFβ forma un complejo receptor en el cual la quinasa del receptor tipo II fosforila y activa el receptor tipo I. Este receptor tipo I fosforila y activa los factores de transcripción Smad. Una vez activados, los factores Smad entran en el núcleo con el fin de formar complejos para el reconocimiento y regulación (activación o represión) de genes específicos. La señalización del TGFβ hace disminuir la actividad CDK y provoca la represión de distintos genes promotores del crecimiento. La vía del TGFβ, cuando se altera, juega un papel esencial en la tumorigénesis y en la metástasis.

Palabras clave: TGFβ.—Smad.—Ciclinas.—CDKs.—Ciclo celular.—Metástasis.

INTRODUCTION

The basic functions of a metazoan cell- its metabolism, proliferation, differentiation, integration into the tissue structure, and eventual death- are controlled by a dense network of polypeptide growth factor signals. The transforming growth factor-β (TGFβ) family of growth factors is particularly prominent among this type of signals. TGFβ fosters tissue growth and morphogenesis during embryogenesis in organisms as diverse as the nematode, fruit fly, and human (1). In the adult, however, TGFβ delivers cytostatic and cell death signals. These responses help maintain tissue homeostasis, and their loss contributes to tumor development (2). Cancer cells that avert TGFβ-mediated cytostasis may then use this factor with impunity to exacerbate their own proliferative, invasive, and metastatic behavior. We are defining the mechanism by which TGFβ and related factors achieve growth control, and how normal control is disrupted in cancer and results in metastasis.

THE TGFβ PATHWAY

To elucidate the basis for the great diversity of TGFβ responses in different cell types, we delineated the pathway linking membrane TGFβ receptors to target genes. At the cell surface, TGFβ activates a

protein complex comprising subunits known as the type I and type II receptors (Figure 1) (3). Both subunits are serine/threonine kinases. Phosphorylation by the type II receptor enables the type I receptor to recognize and phosphorylate Smad2 and Smad3 (4, 5). Phosphorylation by the TGFβ receptor releases the Smad proteins from cytoplasmic retention, allowing their translocation into the nucleus, where they form transcriptional regulatory complexes (Figure 1). These complexes generate hundreds of immediate gene activation and repression responses. Structural analysis of these components has shed light into how they interact and become activated in response to TGFβ (Figure 1) (3). We are now identifying how the TGFβ pathway is integrated into the signaling networks of the cell.

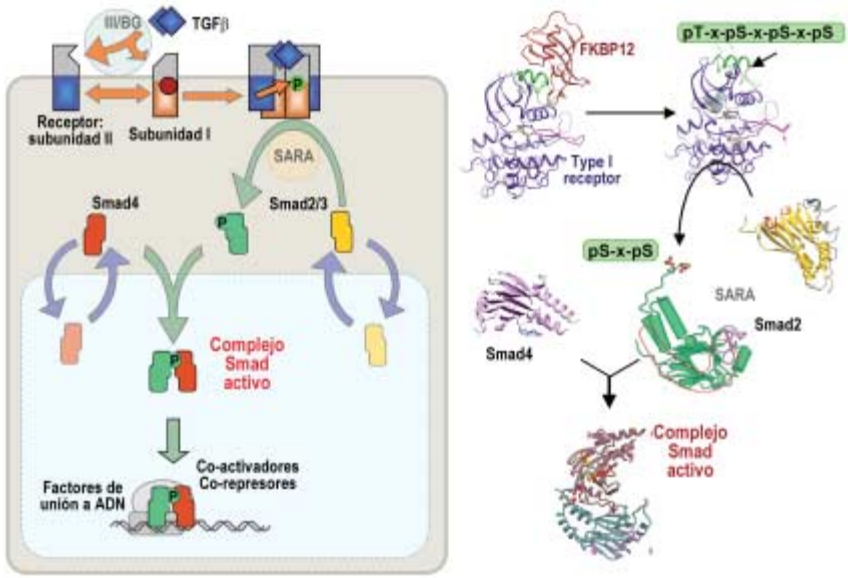

FIGURE 1. *The TGFß/Smad pathway. The ligand TGFß assembles a receptor complex in which the type II receptor kinase phosphorylates and activates the type I receptor. Receptor I phosphorylation by receptor II in the regulatory region (GS region) creates a double pS/T-x-pS motif, turning an inhibitor (FKBP12) binding site into a substrate binding site (18, 19). This allows the type I receptor to phosphorylate and activate Smad transcription factors. Smad phosphorylation by receptor I creates a C-terminal pS-x-pS motif for formation of Smad dimers and trimers that nucleate transcriptional complexes (20-22). A receptor-activated Smad complex enters the nucleus to find partners for the recognition and regulation (activation of repression) of specific genes.*

THE TGFβ CYTOSTATIC PROGRAM

How does an activated Smad complex regulate different genes in different cell types? And how does it activate certain genes while repressing others in the same cell, at the same time? A general framework for the resolution of these important questions is provided by the hypothesis that Smads function in association with different protein partners (6). Each Smad-partner combination would target a particular subset of genes, depending on the DNA-binding specificity of this combination. The availability of such cofactors, which may vary depending on the cell type, partly determines the gene responses to TGFβ in each cell type. Identifying Smad partners therefore is essential for delineating cell-specific programs of TGFβ action and their integration in the signaling networks of the cell.

We have pursued this general problem in the context of delineating the TGFβ cytostatic program. This program involves transcriptional activation of the cyclin-dependent kinase inhibitors $p21^{Cip1}$ and $p15^{Ink4b}$ and repression of the growth-promoting transcription factors c-*myc* and *Id1* (Figure 2) (7). The increased levels of p15 and p21 lead to the mobilization of p27 Kip1, a key CDK inhibitor that blocks the cyclin-Cdk2 complexes (Figure 3) (8). Cooperatively, these responses arrest the cell division cycle. We recently identified DNA-binding cofactors that in association with TGFβ-activated Smad target these genes for activation or repression. A Smad-FoxO complex targets $p21^{Cip1}$ for activation (9), whereas Smad-E2F4/5 and Smad-ATF3 complexes target c-*myc* and *Id1* for repression, respectively (Figure 2) (10, 11). c-Myc plays an integrative role in this process: when present at high levels, as in mitogen-stimulated cells, c-Myc binds to the $p21^{Cip1}$ and $p15^{Ink4b}$ promoters via the zinc finger protein Miz-1, interfering with activation of these genes by TGFβ, the tumor-suppressor p53, and other signals (12, 13). c-Myc down-regulation therefore renders $p21^{Cip1}$ and $p15^{Ink4b}$ competent for activation.

The resulting working model of TGFβ action is that each one in a vast repertoire of DNA-binding cofactors endows the Smad complex with the ability to recognize a limited subset of target genes. This model, which is being extended and verified in other gene response

programs, may explain the diversity of responses that each member of the TGFβ family can induce in different cell types (6). It also provides a framework for interventions to alter one TGFβ response without affecting others.

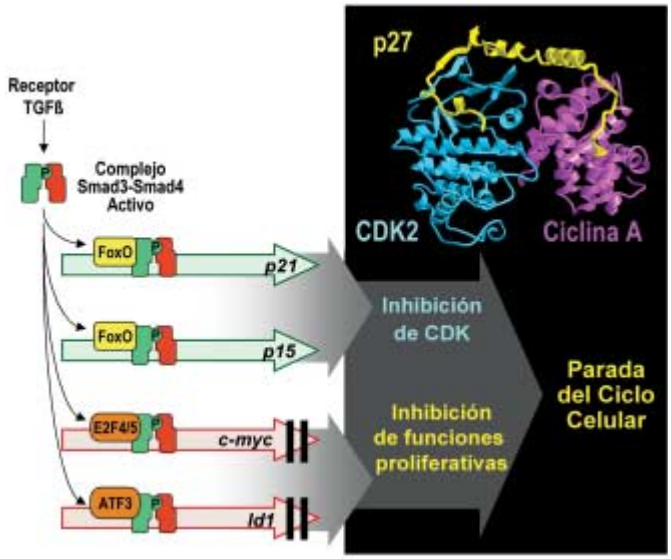

FIGURE 2. *The TGFß/Smad cytostatic program. In the nucleus, receptor-activated Smad3/4 complexes containing FoxO factors as partners, activates genes encoding CDK inhibitors p15Ink4b and p21Cip1. The increased levels of p15 and p21 cooperate with another CDK inhibitor, p27Kip1, to block cyclin-dependent protein kinases. The crystal structure of the p27-cyclinA-cdk2 complex reveals how a CDK inhibitor docks on the cyclin subunit to direct its CDK inhibitory domain to disrupt the catalytic center of the CDK kinase (23). Through this mechanisms, TGFß signaling decreases CDK activity while, through Smad complexes with E2F4/5 and ATF3 as partners, TGFß signaling causes the repression of the growth-promoting genes Myc and ID1.*

MECHANISMS OF METASTASIS

Having shed light on the cytostatic action of TGFβ, its underlying molecular mechanisms, and their disruption in cancer, we are also directing our attention to aberrant gene responses that enable invasion and metastasis in tumor cells. More generally, we are interested in elucidating mechanisms mediating tissue-specific metastasis. Metastasis is the most devastating complication of

cancer, being responsible for a vast majority of deaths from solid tumors. Tumor cells become metastatic by the progressive acquisition of functions enabling invasion, survival and growth under adverse conditions in distant organs. Metastasis, a complex process caused by elaborate interactions between tumor cells and the surrounding normal tissues in different vital organs. The molecular and cellular mechanisms that lead primary tumors to form metastases must be understood in order to better address this major life-threatening problem. Previous work provided a sense of the complexity of the metastasis process (Figure 3), but it did not explain how and why metastasis occurs, what mechanisms make metastasis a tissue-specific process, what events allow dormant metastases to become active and lethal many years after removal of a primary tumor, and what metastasis-mediating genes would eventually constitute worthy therapeutic targets. As a result of such limitations, progress to date has been frustratingly slow.

Our experimental approach is based on the use of moderately metastatic cell lines and a mouse model system for the selection of highly metastatic subpopulations. Live-animal-imaging techniques are used to track the spread, homing, and outgrowth of the metastatic cells in different organs. After harvesting metastatic lesions and verifying that highly metastatic cells have been selected, we use genome-wide transcriptomic profiling to identify metastasis-linked genes. Gene transfer techniques are then used to assess the contribution of individual genes to various steps (invasion, homing, outgrowth, angiogenesis, and stroma adaptation) of the metastasis process. With this approach, we have recently identified different sets of genes that cooperatively mediate breast cancer metastasis to the bone and to the lung (Figure 3) (14, 15). Ongoing studies are directed at establishing the clinical relevance of these findings and identifying additional tissue-specific metastasis genes in other tumor types and for other metastasis sites.

FROM TUMOR SUPPRESSOR TO METASTASIS MEDIATOR

Metastatic functions may be acquired by abnormal utilization of cellular pathways that otherwise play important roles in normal

tissue development and maintenance. One of these is the TGFβ pathway, which induces cytostasis and apoptosis under normal conditions and is disabled by inactivating mutations in the TGFβ receptors or the signal transducer Smad transcription factor in various types of tumors.

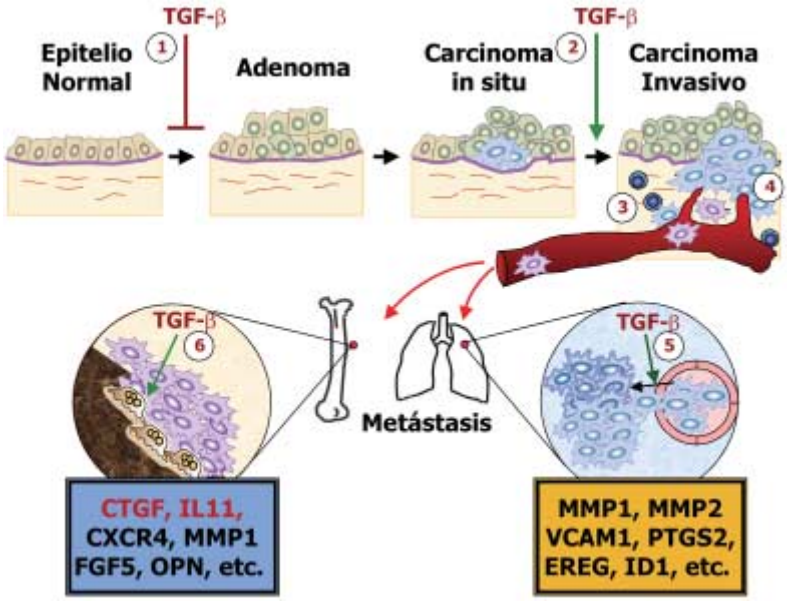

FIGURE 3. *Mechanisms of metastasis. 1) TGF-β limits the growth of normal epithelium and early-stage tumors. 2) Loss of growth inhibitory responsiveness by loss of TGF-β receptors or Smad proteins, or by specific loss of cytostatic gene responses, selects for more aggressively growing tumors, facilitating the acquisition of additional oncogenic mutations. 3) Tumor cells that have lost the cytostatic response but retain TGF-β signaling components can undergo epithelial-mesenchymal transdifferentiation in response to TGF-β, becoming more invasive. 4) Tumor-derived TGF-β creates an immunosuppressive environment by suppressing T cell function, allowing tumor cells to escape CTL-mediated clearance. 5) TGF-β can induce an angiogenic response, facilitating the recruitment of new blood vessels that sustain tumor growth and systemic spread. 6) Adherence of tumor cells to the endothelium and/or extravasation of tumor cells at sites of metastasis, such as the lung, can be augmented by TGF-b signaling. 7) TGF-β stimulates the expression of genes such as the osteoclast differentiation factor IL11 and the angiogenic factor CTGF, which promote osteolytic bone metastasis by breast cancer cells. Genes that mediate organ-specific metastasis by breast cancer cells have been identified by a combination of in vivo selection of metastatic cells, bioinformatics identification of metastasis-linked genes, functional verification of the metastatic activity of these genes, and clinical validation of their relevance to human disease (15).*

Often, however, the strong selective pressure for loss of TGFβ growth inhibitory responsiveness in cancer leads to the accumulation of defects in cytostatic mechanisms downstream of the Smads. Tumor cells that become resistant to antimitogenic control in this manner may display a corrupt sensitivity to TGFβ undergoing tumorigenic progression in response to this cytokine (2). Patients whose pancreatic or colon tumors express TGFβ receptors fare less well than those with low or absent TGFβ receptor expression in the tumor. In mouse models of breast cancer, TGFβ signaling promotes extravasation of circulating tumor cells in the lung (16) and formation of bone metastasis by human breast cancer cells (17).

The TGFβ signaling mechanisms that foster metastasis and their relevance in human cancer, are important open questions. In the case of osteolytic bone metastasis by breast cancer cells, it has been proposed that TGFβ released from the decaying bone matrix stimulates neighboring tumor cells, establishing a vicious cycle that exacerbates the growth of the metastatic lesion. The role of TGFβ as a pro-metastatic factor raises questions about the signal transduction pathways involved. Probably owing to selective pressure against this cytostatic action in cancer, *SMAD4* (also known as *Deleted in Pancreatic Carcinoma locus 4, DPC4*) and, to a lesser extent *SMAD2* are mutationally inactivated in cancers of the colon, pancreas and other sites (7). Because Smads function as quintessential tumor suppressors, one might think that the pro-invasive and metastatic effects of TGFβ are mediated by Smad-independent pathways. However, the possibility that the Smad pathway may mediate metastatic effects has not been ruled out, nor has it been tested in vivo.

Among the bone metastasis genes that we recently identified, two genes, interleukin 11 (IL11) and connective tissue growth factor (CTGF) are of special interest (14). IL11 is an enhancer of osteoclast differentiation and osteolysis, and CTGF is an angiogenic and growth-promoting factor. *IL11* and *CTGF* are inducible by TGFβ, providing a possible mechanism for the pro-metastatic activity of TGFβ in breast cancer. Our work has shown that the Smad pathway mediates *IL11* and *CTGF* activation and the development of bone metastasis in breast cancer (14). Thus, the tumor suppressor Smad pathway becomes corrupted in breast cancer, acting as a mediator of metastasis.

Through this combined approach, and encouraged by its recent validation in clinical samples, we hope to provide a better understanding of the mechanisms mediating metastasis, a better definition of the role of corrupt TGFβ signaling in this process, and a better rationalization for the therapies that could be applied.

REFERENCES

(1) MASSAGUÉ, J. (1990) The transforming growth factor-beta family. *Annu. Rev. Cell Biol.* 6: 597-641.
(2) SIEGEL, P. M.; MASSAGUÉ J. (2003) Cytostatic and apoptotic actions of TGF-beta in homeostasis and cancer. *Nat. Rev. Cancer* 3: 807-821.
(3) SHI, Y.; MASSAGUÉ, J. (2003). Mechanism of TGF-beta signaling from cell membrane to the nucleus. *Cell* 113: 685-700.
(4) WRANA, J. L.; ATTISANO, L.; WIESER, R.; VENTURA, F.; MASSAGUÉ, J. (1994) Mechanism of activation of the TGF-beta receptor. *Nature* 370: 341-347.
(5) LIU, F.; HATA, A.; BAKER, J. C.; DOODY, J.; CARCAMO, J.; HARLAND, R. M.; MASSAGUÉ, J. (1996) A human Mad protein acting as a BMP-regulated transcriptional activator. *Nature* 381: 620-623.
(6) MASSAGUÉ, J. (2000) How cells read TGF-beta signals. *Nat. Rev. Mol. Cell Biol.* 1: 169-178.
(7) MASSAGUÉ, J.; BLAIN, S. W.; LO, R. S. (2000) TGFbeta signaling in growth control, cancer, and heritable disorders. *Cell* 103: 295-309.
(8) POLYAK, K.; LEE, M. H.; ERDJUMENT-BROMAGE, H.; KOFF, A.; ROBERTS, J. M.; TEMPST, P.; MASSAGUÉ, J. (1994) Cloning of p27Kip1, a cyclin-dependent kinase inhibitor and a potential mediator of extracellular antimitogenic signals. *Cell* 78: 59-66.
(9) SEOANE, J.; LE, H. V.; SHEN, L.; ANDERSON, S. A.; MASSAGUÉ, J. (2004) Integration of Smad and forkhead pathways in the control of neuroepithelial and glioblastoma cell proliferation. *Cell* 117: 211-223.
(10) CHEN, C. R.; KANG, Y.; SIEGEL, P. M.; MASSAGUÉ, J. (2002) E2F4/5 and p107 as Smad cofactors linking the TGFbeta receptor to c-myc repression. *Cell* 110: 19-32.
(11) KANG, Y.; CHEN, C. R.; MASSAGUÉ, J. (2003) A self-enabling TGFbeta response coupled to stress signaling: Smad engages stress response factor ATF3 for Id1 repression in epithelial cells. *Mol Cell* 11: 915-926.
(12) SEOANE, J.; POUPONNOT, C.; STALLER, P.; SCHADER, M.; EILERS, M.; MASSAGUÉ, J. (2001) TGFbeta influences Myc, Miz-1 and Smad to control the CDK inhibitor p15INK4b. *Nat. Cell Biol.* 3: 400-408.
(13) SEOANE, J.; LE, H. V.; MASSAGUÉ, J. (2002) Myc suppression of the p21(Cip1) Cdk inhibitor influences the outcome of the p53 response to DNA damage. *Nature* 419: 729-734.

(14) KANG, Y.; SIEGEL, P. M.; SHU, W.; DROBNJAK, M.; KAKONEN, S. M.; CORDON-CARDO, C.; GUISE, T. A.; MASSAGUÉ, J. (2003) A multigenic program mediating breast cancer metastasis to bone. *Cancer Cell* 3: 537-549.
(15) MINN, A. J.; GUPTA, G. P.; SIEGEL, P. M.; BOS, P. D.; SHU, W.; GIRI, D. D.; VIALE, A.; OLSHEN, A. B.; GERALD, W. L.; MASSAGUÉ, J. (2005) Genes that mediate breast cancer metastasis to lung. *Nature* in press.
(16) SIEGEL, P. M.; SHU, W.; CARDIFF, R. D.; MULLER, W. J.; MASSAGUÉ, J. (2003) Transforming growth factor beta signaling impairs Neu-induced mammary tumorigenesis while promoting pulmonary metastasis. *Proc. Natl. Acad. Sci. U. S. A.* 100: 8430-8435.
(17) YIN, J. J.; SELANDER, K.; CHIRGWIN, J. M.; DALLAS, M.; GRUBBS, B. G.; WIESER, R.; MASSAGUÉ, J.; MUNDY, G. R.; GUISE, T. A. (1999) TGF-beta signaling blockade inhibits PTHrP secretion by breast cancer cells and bone metastases development. *J. Clin. Invest.* 103: 197-206.
(18) HUSE, M.; CHEN, Y. G.; MASSAGUÉ, J.; KURIYAN, J. (1999) Crystal structure of the cytoplasmic domain of the type I TGF beta receptor in complex with FKBP12. *Cell* 96: 425-436.
(19) HUSE, M.; MUIR, T. W.; XU, L.; CHEN, Y. G.; KURIYAN, J.; MASSAGUÉ, J. (2001) The TGF beta receptor activation process: an inhibitor- to substrate-binding switch. *Mol. Cell.* 8: 671-682.
(20) WU, G.; CHEN, Y. G.; OZDAMAR, B.; GYURICZA, C. A.; CHONG, P. A.; WRANA, J. L.; MASSAGUÉ, J.; SHI, Y. (2000) Structural basis of Smad2 recognition by the Smad anchor for receptor activation. *Science* 287: 92-97.
(21) WU, J. W.; HU, M.; CHAI, J.; SEOANE, J.; HUSE, M.; LI, C.; RIGOTTI, D. J.; KYIN, S.; MUIR, T. W.; FAIRMAN, R., et al. (2001) Crystal structure of a phosphorylated Smad2. Recognition of phosphoserine by the MH2 domain and insights on Smad function in TGF-beta signaling. *Mol. Cell.* 8: 1277-1289.
(22) SHI, Y.; WANG, Y. F.; JAYARAMAN, L.; YANG, H.; MASSAGUÉ, J.; PAVLETICH, N. P. (1998) Crystal structure of a Smad MH1 domain bound to DNA: insights on DNA binding in TGF-beta signaling. *Cell* 94: 585-594.
(23) RUSSO, A. A.; JEFFREY, P. D.; PATTEN, A. K.; MASSAGUÉ, J.; PAVLETICH, N. P. (1996) Crystal structure of the p27Kip1 cyclin- dependent-kinase inhibitor bound to the cyclin A-Cdk2 complex. *Nature* 382: 325-331.