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FAST TRACK

Kips Off to Myc: Implications for TGFβ Signaling

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Abstract Loss of sensitivity to the negative growth regulator transforming growth factor β (TGFβ) is a feature of many different tumor types and is likely involved in tumor progression. In some cases this loss of sensitivity to TGFβ has been shown to be manifest in the absence of membrane-associated TGFβ receptor complexes, thus preventing initiation of antiproliferative signals from the cell surface. In others, loss of sensitivity to TGFβ-induced inhibitory signals has been attributed to loss of function of intracellular effectors of TGFβ-induced inhibitory signals due to mutation or allelic loss of effector genes and their products. The intracellular effectors of TGFβ inhibitory signals have been shown to be involved in the normal regulation of progression through the cell cycle, specifically during G_1 phase. In this manner, elucidation of the mechanisms by which TGFβ inhibits cell growth not only helps us identify steps involved in tumor progression, but also allows us to better understand how cells regulate progression through the cell cycle. J. Cell. Biochem. 66:427–432, 1997. © 1997 Wiley-Liss, Inc.

Key words: TGFβ, transforming growth factor β; Cdk, cyclin-dependent kinase; Kip, cdk-inhibitor

INTRODUCTION

A number of cell cycle-regulated factors have been implicated in mediating TGFβ-induced antimitogenic signals and the subsequent G_1 arrest [for review, see Alexandrow and Moses, 1995]. Recently, two of these factors have received much attention concerning their involvement in cell cycle regulation and TGFβ-induced growth arrest, specifically with regard to results showing a possible regulatory function of one factor toward the other. These two factors are the product of the c-*myc* proto-oncogene and a member of the CIP/KIP cyclin-dependent kinase (Cdk) inhibitor family, p27^{Kip1}.

MYC AND CDK INHIBITORS IN THE CELL CYCLE

Although c-*myc* has been the focus of much research for more than a decade, the mechanisms by which c-*myc* contributes to cell proliferation are not completely understood. Expression of the c-*myc* gene is rapidly induced following mitogenic stimulation of resting cells, peaking within a short time, and then decaying to a suprabasal level throughout following cell cycles [Greenberg and Ziff, 1984; Hann et al., 1985; Kelly et al., 1983]. Expression is down-regulated in quiescent and differentiating cells, and many tumor types show deregulated expression of c-*myc* due to activating translocations, amplifications, or overexpression of the gene product [reviewed in Alitalo et al., 1987]. The requirement for c-*myc* in the cell cycle derives largely from experimental data based primarily on antisense approaches in which loss of c-*myc* expression leads to an antiproliferative effect and accumulation of cells in the G_1 phase of the cell cycle [Gai et al., 1990; Pietenpol et al., 1990a; Münger et al., 1992]. In a similar approach, targeted loss of one allele of c-*myc* in a cultured cell system resulted in a slower growth rate that was reversed upon introduction of an exogenous c-*myc* transgene [Hartsough and Mulder, 1995; Hanson et al., 1994]. Cells that express exogenous c-*myc* constitutively or as activated conditional alleles fail to arrest in response to growth factor deprivation in culture [reviewed in Evan and Littlewood, 1993], and mice containing homozygous deletions of c-*myc* do not survive early embryogenesis [Davis et al., 1993]. Together these data

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suggest that c-*myc* normally functions to regulate progression through the G_1 phase of the cell cycle and that loss of regulation of c-*myc* antagonizes at least some of the mechanisms that negatively regulate G_1 progression.

Experimental evidence has led to the general consensus that the c-Myc protein functions most likely as a transcription factor during G_1 phase [Meichle et al., 1992; Dang, 1991; Marcu et al., 1992; Bishop et al., 1991, and references therein]. The observations that c-Myc contains basic/helix-loop-helix/leucine zipper motifs common to the bHLH-LZ family of transcription factors and can bind DNA in a sequence-specific manner as a heterodimer with an unrelated bHLH-LZ protein, Max, strongly support a role for c-Myc in transcriptional regulation [Fisher et al., 1993; Amati et al., 1993]. Experimental evidence has suggested a direct correlation between transformation by c-Myc and the ability of c-Myc to regulate gene expression [Meichle et al., 1992, and references therein]. Mutation of the bHLH-LZ regions as well as at least one highly conserved amino-terminal domain of c-Myc results in both a transcriptionally defective protein as well as a transformation defective protein [Steiner et al., 1995; Meichle et al., 1992]. The expression of key cell cycle proteins, cyclins A, E, and D1, has also been shown, at least indirectly, to be influenced by c-Myc expression [Jansen-Dürr et al., 1993; Shibuya et al., 1992; Philipp et al., 1994; Hanson et al., 1994; Roussel et al., 1995]. Certain studies have shown that expression of cyclin A, in particular, may link c-*myc* to transformation and perhaps to other c-*myc*-induced effects on cell growth [Barrett et al., 1995; Hoang et al., 1994].

Progression through the cell cycle in mammalian cells is governed by several related kinases called cyclin-dependent kinases in complexes with regulatory cyclin subunits [for review, see Sherr, 1994]. Cyclin-dependent kinases have been shown to be subject to negative regulation by proteins that inhibit Cdk activity through stoichiometric mechanisms [for reviews, see Hunter, 1993; Sherr and Roberts, 1995]. These regulatory proteins, known as Cdk inhibitors, apparently act to inhibit Cdk activity either by sterically preventing Cdk-activating kinase (CAK) from phosphorylating the Cdks or by blocking cyclin association with the catalytic subunits. Two families of mammalian inhibitors have been identified. One family includes $p16^{INK4A}$, $p15^{INK4B}$, $p18^{INK4C}$, and $p19^{INK4D}$ [reviewed in Sherr and Roberts, 1995]. The INK4 family appears to prevent Cdk activation, specifically that of Cdk-4 and Cdk-6 associated with D-type cyclins, by blocking binding of the cyclin regulatory subunits. Overexpression of the INK4 inhibitors blocks cell proliferation, and the location of some of the INK4 genes at sites of chromosomal deletions or mutations in certain malignancies suggests that they are candidate tumor suppressor genes and likely involved in regulating cell cycle progression [Sherr and Roberts, 1995; Hunter, 1993, and references therein].

The second family of Cdk inhibitors includes $p21^{WAF1/CIP1}$, $p27^{KIP1}$, and $p57^{KIP2}$. In vitro, these inhibitors have been shown to inhibit the activity of cyclin D/Cdk-4, cyclin D/Cdk-6, cyclin E/Cdk-2, and cyclin A/Cdk-2 [reviewed in Sherr and Roberts, 1995]. These inhibitors appear to prevent CAK from phosphorylating the Cdks but do not appear to alter the activity of CAK itself. As with the INK4 family, CIP/KIP inhibitors have also been shown to cause G_1 arrest when overexpressed in mammalian cells suggesting a likely role in regulation of G_1 progression. As will be discussed below, the participation of one of the CIP/KIP inhibitors, $p27^{KIP1}$ (referred to as Kip), in G_1 progression has itself served as the focus of much research owing in large part to data suggesting a functional role for Kip in the mechanisms underlying TGFβ-induced cell cycle arrest [for review, see Alexandrow and Moses, 1995].

Studies from at least two independent groups have uncovered a potentially important connection between the function of the c-Myc protein in promoting G_1 progression and the function of the Kip inhibitor in restricting G_1 progression. Eilers and colleagues [Steiner et al., 1995] have found that in serum-starved rat fibroblasts activation of a conditional c-Myc protein leads to increased kinase activity of the cyclin E/Cdk-2 complex and that the mechanism underlying this Myc-dependent effect involves loss of total levels of the Kip protein from the cell likely followed by a reduced association of the inhibitor with the cyclin E/Cdk-2 complex. Their data also suggests that the ability of c-Myc to positively regulate the cyclin E/Cdk-2 kinase activity is dependent on the transactivation function of c-Myc as the effect on kinase activity is sensitive to actinomycin and requires the DNA-binding and heterodimerization domains of the c-Myc protein. This makes it interesting to

speculate that loss of Kip from the cells requires Myc-dependent transcriptional stimulation of a factor(s) which, at least indirectly, negatively regulates Kip protein levels in the cells.

In support of the model generated from the above data, Amati and colleagues [Vlach et al., 1996] recently showed that overexpression of c-Myc can counteract the negative regulatory effects on cell growth of overexpressed Kip. In contrast to cells that are overexpressing Kip alone, cells overexpressing Kip in the presence of deregulated c-Myc expression progress through the cell cycle and the kinase activities associated with cyclin E and Cdk-2 remain active. Their data also suggest, in partial disagreement with that of the Eilers group, that c-Myc overexpressing cells do not induce Kip degradation nor a modification of the Kip protein that inactivates the function of the inhibitor. As these Myc-expressing cells apparently remain in the cell cycle despite high Kip protein levels, the mechanism underlying the Myc-dependent effects on inhibitor function remains to be elucidated.

Altogether, an attractive model suggesting a novel role for c-Myc in cell cycle progression emerges from the research discussed above (Fig. 1). During the normal progression of cells from a quiescent state into a replicative state, induction of c-Myc early in G_1 may lead to negative regulation of the function of one or more Cdk inhibitors, more specifically Kip. Mechanistically, the c-Myc protein may transactivate a factor(s) that stimulates removal of Kip from the cyclin E/Cdk-2 complex allowing activation of the kinase and progression into S phase. Subsequent to removal from the kinase complex, Kip is either degraded or held in an inactive state until the influence of c-Myc is removed from the cell. In the situation where cells are cycling without entering a G_0 period, c-Myc is continually expressed and thus would prevent association of Kip with the kinase complex as cells progress from mitosis into the following G_1 and S phases. In addition to the studies discussed above, other support for this model comes from recent data showing that elimination of Kip from fibroblasts allows cell cycling in the absence of growth factors [Coats et al., 1996]. As deregulated Myc expression also allows cell cycling in the absence of growth factors [Jansen-Dürr et al., 1993; Bishop et al., 1991], it is intriguing to speculate that one mechanism by which growth factors stimulate progression through the cell cycle involves upregulation of Myc followed by Myc-induced dissociation of Kip from cyclin/Cdk complexes.

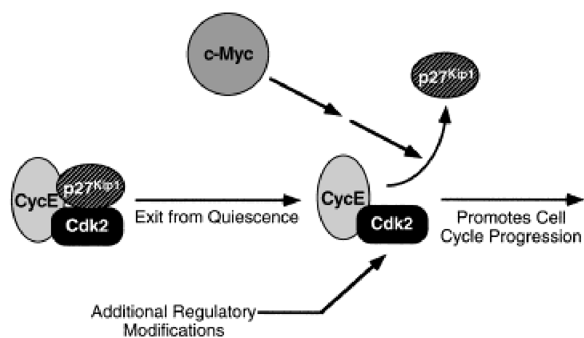

Fig. 1. Proposed model of cyclin-dependent kinase regulation by c-Myc.

MYC AND KIP IN TGFβ REGULATION

Regulation of Kip function by c-Myc also has important implications for antimitogenic signaling by TGFβ. Evidence has suggested that both c-Myc and Kip may perform pivotal roles in the mechanisms mediating TGFβ-induced cell cycle arrest. The expression of c-*myc* at the mRNA and protein levels is rapidly downregulated following TGFβ treatment of a variety of cell types [reviewed in Alexandrow and Moses, 1995], and deregulated expression of c-Myc in at least two different cell types overrides the inhibitory effect of TGFβ [Alexandrow et al., 1995; Selvakumaran et al., 1994]. Two groups have shown independently that treatment of mink lung epithelial cells with TGFβ results in the association of Kip with the cyclin E/Cdk-2 complex followed by inactivation of its kinase activity and failure of the cells to progress into S phase [Polyak et al., 1994; Slingerland et al., 1994]. In further support of a role for Kip in the response to TGFβ, it has recently been shown that the adenoviral E1A oncoprotein can bind to and prevent Kip from inhibiting the activity of the cyclin E/Cdk-2 complex [Mal et al., 1996]. As earlier studies have shown that expression of either the E1A or SV40 T antigen oncoproteins can render cells insensitive to the inhibitory effects of TGFβ through inactivation of the pRB tumor suppressor protein [Laiho et al., 1990; Pietenpol et al., 1990b], these recent results suggest that the effects of E1A on cell cycle progression and TGFβ-induced growth arrest may be mediated, at least in part, by direct regulation of the function of the Kip inhibitor.

Perhaps unexpectedly, studies of lymphocytes obtained from mice carrying a homozygous deletion of p27 Kip1 have shown that, although they completely lack Kip, these cells are still sensitive to the growth-inhibitory effects of TGFβ1 [Nakayama et al., 1996], implying that Kip is not essential for TGFβ1-induced growth arrest. In addition, unpublished results from our laboratory have shown that in mouse keratinocytes TGFβ1 can inhibit progression into S phase in the absence of an inhibitory effect on cyclin E/Cdk-2 kinase activity (McDonnell M, Alexandrow MG, Moses HL, unpublished observations, 1997), which is the kinase complex hypothesized to be the TGFβ1-induced target of p27 Kip1 [Polyak et al., 1994; Reynisdottir et al., 1995; Slingerland et al., 1994]. These data also support the idea that function of p27 Kip1 is not necessary for TGFβ1 to induce growth arrest. However, it is likely that TGFβ1 treatment of cells results in multiple negative effects on the cell cycle machinery [Alexandrow and Moses, 1995] and the presence of any one or more negatively regulated pathways may be sufficient to block cell cycle progression, even in the absence of p27 Kip1 function. Additionally, as TGFβ1 elicits multiple effects on cells [Moses et al., 1993], only one of them being growth arrest, it is also possible that Kip functions in some other aspect(s) of TGFβ1 signaling.

PROPOSED MODEL INVOLVING MYC, KIP, AND TGFβ1

As a result of the recent data suggesting that c-Myc may negatively regulate the function of the Kip inhibitor, an attractive model unifying the involvement of c-Myc and Kip in TGFβ-induced inhibitory responses emerges (Fig. 2). In this model, entry of cells into the cell cycle from quiescence in the presence of TGFβ (or treatment with TGFβ during G_1) would result in the lack of induction (or inhibition) of c-Myc expression during G_1 phase, an effect of TGFβ treatment observed in several different cell types, including mink lung epithelial cells [reviewed in Alexandrow and Moses, 1995]. As predicted by the work of the Eilers and Amati groups, one effect of loss of c-Myc expression would be the inability of the cells to negatively regulate the activity of the Kip inhibitor. This effect of c-Myc loss would be manifest in the increased association of Kip with cyclin E/Cdk-2 complexes in cells treated with TGFβ. As discussed above, increased association of Kip with the cyclin E/Cdk-2 complex has been observed in TGFβ-treated cells, and the enzymatic activity of the kinase complex is inhibited in the cells [Polyak et al., 1994; Slingerland et al., 1994].

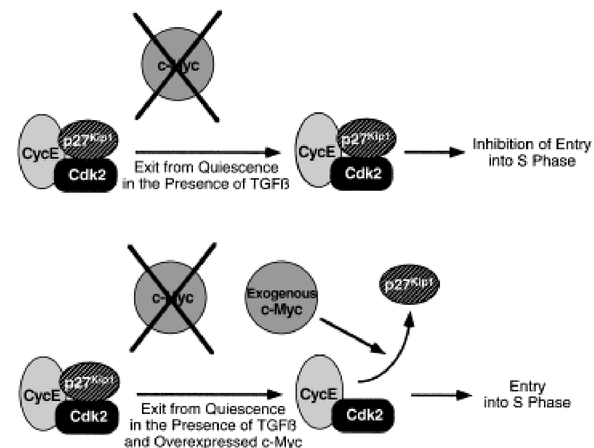

Fig. 2. Proposed mechanisms of TGFβ-induced growth arrest and the effects of deregulated c-Myc expression.

Two different laboratories have shown that deregulated expression of exogenous c-Myc in TGFβ-sensitive cells overrides the negative effect of TGFβ on the cells and allows progression into S phase to occur [Alexandrow et al., 1995; Selvakumaran et al., 1994]. One interpretation of these results is that overexpression of c-Myc in the cells is dissociating or preventing the association of Kip (or other inhibitors) with the cyclin E/Cdk-2 complex allowing normal kinase activity and aiding in cell cycle progression (Fig. 2). However, in light of the recent data suggesting that Kip is not essential for TGFβ1-induced growth arrest, it is likely that overexpression of Myc in TGFβ1-sensitive cells is affecting other cell cycle-regulated events in addition to the Kip pathway and that the cumulative effects of Myc-induced signals are together responsible for abrogation of TGFβ1-induced growth arrest. Although this effect of c-Myc in TGFβ-treated cells, as well as other mechanistic details, will require further experimental verification, the proposed model explaining at least one mechanism by which TGFβ arrests G_1 progression remains a viable possibility for future experimental design. In addition, as recent evidence has shown a possible function of Mad (for Mothers against decapentaplegic) proteins in TGFβ-induced signal transduction pathways [Arora et al., 1995; Savage et al., 1996; Eppert et al., 1996; Zhang et al., 1996], it will also be interesting to determine whether

one or more Mad proteins regulates Myc expression (and subsequently Kip function) in TGFβ-sensitive cells. Such regulation of Myc and Kip by these Mad proteins would demonstrate the first example of a TGFβ receptor-induced signaling cascade leading to downstream negative effects on Cdk function and cell cycle progression.

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REFERENCES

Alexandrow MG, Moses HL (1995): Transforming growth factor β and cell cycle regulation. Cancer Res 55:1452–1457.

Alexandrow MG, Kawabata M, Aakre ME, Moses HL (1995): Overexpression of the c-Myc oncoprotein blocks the growth-inhibitory response but is required for the mitogenic effects of transforming growth factor β1. Proc Natl Acad Sci USA 92:3239–3243.

Alitalo K, Koskinen P, Makela TP, Saksela K, Sistonen L, Winqvist R (1987): Myc oncogenes: Activation and amplification. Biochim Biophys Acta 907:1–32.

Amati B, Littlewood TD, Evan GI, Land H (1993): The c-Myc protein induces cell cycle progression and apoptosis through dimerization with Max. EMBO J 12:5083–5087.

Arora K, Dai H, Kazuko SG, Jamal J, O'Connor MB, Letsou A, Warrior R (1995): The *Drosophila schnurri* gene acts in the Dpp/TGFβ signaling pathway and encodes a transcription factor homologous to the human MBP family. Cell 81:781–790.

Barrett JF, Lewis BC, Hoang AT, Alvarez RJ Jr, Dang CV (1995): Cyclin A links c-Myc to adhesion-independent cell proliferation. J Biol Chem 270:15923–15925.

Bishop JM, Eilers M, Katzen AL, Kornberg T, Ramsay G, Schirm S (1991): MYB and MYC in the cell cycle. Cold Spring Harbor Symp Quant Biol 56:99–107.

Coats S, Flanagan WM, Nourse J, Roberts JM (1996): Requirement of p27Kip1 for restriction point control of the fibroblast cell cycle. Science 272:877–880.

Dang CV (1991): c-Myc oncoprotein function. Biochim Biophys Acta 1072:103–113.

Davis AC, Wims M, Spotts GD, Hann SR, Bradley A (1993): A null c-*myc* mutation causes lethality before 10.5 days of gestation in homozygotes and reduced fertility in heterozygous female mice. Genes Dev 7:671–682.

Eppert K, Scherer SW, Ozcelik H, Pirone R, Hoodless P, Kim H, Tsui L-C, Bapat B, Gallinger S, Andrulis IL, Thomsen GH, Wrana JL, Attisano L (1996): MADR2 maps to 18q21 and encodes a TGFβ-regulated MAD-related protein that is functionally mutated in colorectal carcinoma. Cell 86:543–552.

Evan GI, Littlewood TD (1993): The role of c-*myc* in cell growth. Curr Opin Genet Genet Dev 3:44–49.

Fisher F, Crouch DH, Jayaraman P-S, Clark W, Gillespie DAF, Goding CR (1993): Transcription activation by Myc and Max: Flanking sequences target activation to a subset of CACGTG motifs in vivo. EMBO J 12:5075–5082.

Gai X, Rizzo M-G, Valpreda S, Baserga R (1990): Regulation of c-myc mRNA levels by insulin or platelet-poor plasma. Oncogene Res 5:111–120.

Greenberg ME, Ziff EB (1984): Stimulation of 3T3 cells induces transcription of the c-*fos* proto-oncogene. Nature 311:433–438.

Hann SR, Thompson CB, Eisenman RN (1985): c-*myc* oncogene protein synthesis is independent of the cell cycle in human and avian cells. Nature 314:366–369.

Hanson KD, Shichiri M, Follansbee MR, Sedivy JM (1994): Effects of c-*myc* expression on cell cycle progression. Mol Cell Biol 14:5748–5755.

Hartsough MT, Mulder KM (1995): Transforming growth factor β activation of p44*mapk* in proliferating cultures of epithelial cells. J Biol Chem 270:7117–7124.

Hoang AT, Cohen KJ, Barrett JF, Bergstrom DA, Dang CV (1994): Participation of cyclin A in Myc-induced apoptosis. Proc Natl Acad Sci USA 91:6875–6879.

Hunter T (1993): Braking the cycle. Cell 75:839–841.

Jansen-Dürr P, Meichle A, Steiner P, Pagano M, Finke K, Botz J, Wessbecher J, Draetta G, Eilers M (1993): Differential modulation of cyclin gene expression by *MYC.* Proc Natl Acad Sci USA 90:3685–3689.

Kelly K, Cochran BH, Stiles CD, Leder P (1983): Cell-specific regulation of the c-*myc* gene by lymphocyte mitogens and platelet-derived growth factor. Cell 35:603–610.

Laiho M, DeCaprio JA, Ludlow JW, Livingston DM, Massagué J (1990): Growth inhibition by TGF-beta linked to suppression of retinoblastoma protein phosphorylation. Cell 62:175–185.

Mal A, Poon RYC, Howe PH, Toyoshima H, Hunter T, Harter ML (1996): Inactivation of p27Kip by the viral E1A oncoprotein in TGFβ-treated cells. Nature 380:262–265.

Marcu KB, Bossone SA, Patel AJ (1992): Myc function and regulation. Annu Rev Biochem 61:809–860.

Meichle A, Philipp A, Eilers M (1992): The functions of Myc proteins. Biochim Biophys Acta 1114:129–146.

Moses HL, Arteaga CL, Alexandrow MG, Dagnino L, Kawabata M, Pierce DF Jr, Serra R (1993): TGFβ regulation of cell proliferation. Princess Takamatsu Symp 24:250–263.

Münger K, Pietenpol JA, Pittelkow MR, Holt JT, Moses HL (1992): Transforming growth factor β1 regulation of c-myc expression, pRB phosphorylation, and cell cycle progression in keratinocytes. Cell Growth Differ 3:291–298.

Nakayama K, Ishida N, Shirane M, Inomata A, Inoue T, Shishido N, Horii I, Loh DY, Nakayama K-I (1996): Mice lacking p27Kip1 display increased body size, multiple organ hyperplasia, retinal dysplasia, and pituitary tumors. Cell 85:707–720.

Philipp A, Schneider A, Väsrik I, Finke K, Xiong Y, Beach D, Alitalo K, Eilers M (1994): Repression of cyclin D1: A novel function of MYC. Mol Cell Biol 14:4032–4043.

Pietenpol JA, Holt JT, Stein RW, Moses HL (1990a): Transforming growth factor β-1 suppression of c-*myc* gene transcription: Role in inhibition of keratinocyte proliferation. Proc Natl Acad Sci USA 87:3758–3762.

Pietenpol JA, Stein RW, Moran E, Yaciuk P, Schlegel R, Lyons RM, Pittelkow MR, Münger K, Howley PM, Moses HL (1990b): TGF-β1 inhibition of c-*myc* transcription and growth in keratinocytes is abrogated by viral transforming proteins with pRB binding domains. Cell 61:777–785.

Polyak K, Kato J, Solomon MJ, Sherr CJ, Massagué J, Roberts JM, Koff A (1994): p27^{Kip1}, A cyclin-Cdk inhibitor, links transforming growth factor-β and contact inhibition to cell cycle arrest. Genes Dev 8:9–22.

Reynisdottir I, Polyak K, Iavarone A, Massagué J (1995): Kip/Cip and Ink4 Cdk inhibitors cooperate to induce cell cycle arrest in response to TGFβ. Genes Dev 9:1831–1845.

Roussel MF, Theodoras AM, Pagano M, Sherr CJ (1995): Rescue of defective mitogenic signaling by D-type cyclins. Proc Natl Acad Sci USA 92:6837–6841.

Savage C, Das P, Finelli AL, Townsend SR, Sun C-Y, Baird SE, Padgett RW (1996): Caenorhabditis elegans genes sma-2, sma-3, and sma-4 define a conserved family of transforming growth factor β pathway components. Proc Natl Acad Sci USA 93:790–794.

Selvakumaran M, Lin H-K, Tjin Tham Sjin R, Reed JC, Liebermann DA, Hoffman B (1994): The novel primary response gene *MyD118* and the proto-oncogenes *myb, myc,* and *bcl-2* modulate transforming growth factor β1-induced apoptosis of myeloid leukemia cells. Mol Cell Biol 14:2352–2360.

Sherr CJ (1994): G1 phase progression: Cycling on cue. Cell 79:551–555.

Sherr CJ, Roberts JM (1995): Inhibitors of mammalian G_1 cyclin-dependent kinases. Genes Dev 9:1149–1163.

Shibuya H, Yoneyama M, Ninomiya-Tsuji J, Matsumoto K, Taniguchi T (1992): IL-2 and EGF receptors stimulate the hematopoietic cell cycle via different signaling pathways: Demonstration of a novel role for c-*myc.* Cell 70:57–67.

Slingerland JM, Hengst L, Pan C-H, Alexander D, Stampfer MR, Reed SI (1994): A novel inhibitor of cyclin-Cdk activity detected in transforming growth factor β-arrested epithelial cells. Mol Cell Biol 14:3683–3694.

Steiner P, Philipp A, Lukas J, Godden-Kent D, Pagano M, Mittnacht S, Bartek J, Eilers M (1995): Identification of a Myc-dependent step during the formation of active G1 cyclin-cdk complexes. EMBO J 14:4814–4826.

Vlach J, Hennecke S, Alevizopoulos K, Conti D, Amati B (1996): Growth-arrest by the cyclin-dependent kinase inhibitor p27 Kip1 is abrogated by c-Myc. EMBO J 15: 6595–6604.

Zhang Y, Feng X-H, Wu R-Y, Derynck R (1996): Receptor-associated Mad homologues synergize as effectors of the TGFβ response. Nature 383:168–172.