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Distinct Inhibitory Effect of TGFβ on the Growth of Human Myeloid Leukemia Cells

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1. Introduction

Hematopoiesis is the formation and development of blood cells. In a healthy adult person, approximately 10^{11}–10^{12} new blood cells are produced daily in order to maintain steady state levels in the peripheral circulation. Disruption of the steady state may cause the development of hematological diseases, such as acute (AML) or chronic myelogenous leukemia (CML). All mature blood cells ultimately originate from a rare population of primitive pluripotent (multipotential) stem cells. The pluripotent stem cells possess self-renewal and multi-lineage differentiation potential. The latter process leads to two major multipotent progenitors: myeloid progenitors and lymphoid progenitors. The myeloid progenitors have the ability to differentiate into mature white cells, erythrocytes, megakaryocytes/platelets and mast cells; whereas the lymphoid progenitors cells mainly produce mature T, NK, and B lymphocytes. Production of myeloid lineage cells is a highly complex process involving the balance between cell proliferation and differentiation, which is regulated by growth stimulating or inhibiting signals. Transforming Growth Factor-beta (TGFβ) is a pleiotropic growth factor and is one of the major regulators of hematopoiesis (Fortunel NO et al. 2000). A major effect of TGFβ on hematopoietic cells is inhibitory (Hu X et al 1999, Batard P et al. 2000, Ducos K et al. 2000, Fortunel NO 2000) although its stimulatory effect has also been observed in some types of blood cells (Keller JR 1990, 1992). Due to its importance in the understanding human diseases including leukemia, over past 20 years, the mechanisms responsible for TGFβ-induced growth inhibition or stimulation have been a major area of investigation and considerable insights into its action mechanisms have been learned. This review highlights recent progress in understanding regulatory role of TGFβ and its signaling pathways in various types of cells with a focus on human myeloid leukemia cells.

2. Myeloid leukemia and myelogenous leukemia cell lines

Myeloid leukemia is a type of blood and bone marrow cancers, which includes AML and CML. AML is a characterized by the rapid accumulation of abnormal, immature white blood cells in both borrow and blood, which is caused by defects in the progenitors and/or stem cells population. Unlike AML, myeloid cell differentiation is preserved during the chronic phase of CML, a disease which originates from the presence of a genetic abnormality in stem cells/progenitors and the nuclei show an abnormal chromosome called the Philadelphia

chromosome. In a healthy person, bone marrow makes the blood stem cells and progenitors that mature into mature white blood cells, red blood cells and blood platelets.

In order to simplify the study of leukemia and basic research, a number of human AML cell lines were established. These lines provide model systems to study the control of proliferation and differentiation in human myelogenous leukemia and, in a broader framework, the controls of normal myeloid development. More than 10 leukemic cell lines have been used in our lab to study Transforming Growth Factor-β (TGFβ)-mediated cell cycle control and transformed events, among which K562 is the first myelogenous leukemia cell line established. The cell line was isolated from a 53 year old female CML patient in blast crisis (Lozzio CB and Lozzio BB 1975). K652 composed of undifferentiated blast cells that are rich in glycophorin and may be induced to produce fetal and embryonic hemoglobin in the presence of hemin. The rest nine cell lines were isolated from AML patients as described below. MV4-11 cell line was derived from blast cells of a 10-year-old male with biphenotypic myelomonocytic leukemia (Lange B et al. 1987). This cell line expresses mutated FLT3, a member of the type III receptor tyrosine kinase (RTK) family (Quentmeier 2003). KG-1 cell line is composed predominantly of myeloblasts and promyelocytes (Koeffler HP et al. 1978). Recent studies identified a fusion of two genes encoding fibroblast growth factor receptors, making this cell line is a good model for studying gene fusion-linked leukemia (Gu TL et al. 2006). KG-1a (Koeffler HP et al. 1980) is a subline isolated from KG-1. TF-1 is a growth factor-dependent human cell line that was originally isolated from the bone marrow cells of a patient with erythroleukemia (Kitamura T et al. 1989). TF-1 cells express a wide range of cytokine receptors and respond to a variety of cytokines. Thus, this cell line is a useful tool for studying cytokine signaling network (Rosas M et al 2005). TF-1a (Hu X et al. 1998) is growth factor-independent subline isolated from TF-1. A unique characteristic of the KG-1 and TF-1 cells is their dependence on colony-stimulating factor (CSF) or GM-CSF/IL-3 for proliferation, respectively, whereas KG-1a and TF-1a are factor-independent cell lines for their growth, although TF-1a retains its ability to respond to several growth factors. HL-60 is a promyelocytic cell line derived from peripheral blood leukocytes from a female patient with acute promyelocytic leukemia, which can be induced into granulocytes in the presence of DMSO (Gallagher R et al.1979). HL-60 cells differentiate into mononuclear phagocytes when exposed to phorbol esters (Xu YZ et al. 2011). HEL is an erythroleukemia cell line isolated from the bone marrow cells of a patient (Martin P and Papayannopoulou 1982). A mutation in *Janus kinase 2* gene (JAK2) in HEL cells was identified in 2006 (Quentmeier H et al 2006). Mo7e (Avancd GC et al. 1988) is a subline of the M-07 human megakaryoblastic leukemia cell line. The cells express platelet glycoprotein-2b-3a. The growth of Mo7e is dependent on IL-3 or GM-CSF. U937 is a cell line established from a diffuse histiocytic lymphoma of 37 year old male patient (Sundstrom C et al. 1976). A histocyte is a cell that is part of the mononuclear phagocytic system that takes part in the immune responses. Since U937 displays many monocytic characteristics and has thus served as a model for the differentiation of monocytes and macrophage in vitro (Aida J, et al. 2011). This cell line is also a good model for induction of apoptosis (Piedfer M et al. 2011).

3. Effect of TGFβ on the growth of human myeloid leukemia cell lines in culture

Transforming Growth Factor-β (TGFβ) has three isoforms in mammals: TGFβ1, TGFβ2, and TGFβ3. The β1 isoform is the most abundant, universally expressed, which has been intensively studied. This review focuses on TGFβ1 unless indicated otherwise. The TGFβ-

induced biological activities are achieved through the binding of the ligand to its cell surface receptor, a serine-threonine kinase complex, known as type I, II, and III (adaptor) receptors. TGFβ binds to and activates the type II receptor. Then the type II receptor recruits and phosphorylates the type I receptor, which in turn activates Smad family proteins that carry signals from the receptors to the nucleus to control transcription of target genes. These gene products may stimulate or inhibit cell cycle progression. TGFβ plays an essential regulatory role in the control of hematopoiesis (Fortunel NO et al. 2000). First, the development of hematopoietic cells from mesoderm in embryos is regulated by TGFβ signaling. Park et al (Park C et al. 2004) demonstrated that BMP-4, a member of TGFβ superfamily, is able to induce hematopoietic differentiation from mouse embryonic stem cells. The differentiated cells contain all types of myeloid and lymphoid progeny (Olsen AL et al. 2006). Similar roles of TGFβ in human hematopoietic stem cells have been reported (Kaufman DS et al. 2001, Chadwick K et al. 2003, Cerdan C et al. 2004). Second, it has also been found that TGFβ is required for maintaining hematopoietic stem/progenitor cells in quiescent states (Batard P et al. 2000); in contrast, application of anti-endogenous TGFβ antibody induces release of these cells from G1 phase (Ducos K et al. 2000). In TGFβ$^{-/-}$ mice, enhanced numbers of circulating granulocytes, monocytes, platelets, as well as colony forming units, were observed (Kulkarni AB et al 1993, Shull MM et al 1992). In addition, inactivation of the TGFβ signaling pathway leads to malignant transformation of early hematopoietic cells (Le Bousse-Kerdiles MC et al. 1996). These data indicate that TGFβ is inhibitory to hematopoietic cells. However, studies from several groups suggest that the response of myeloid stem/progenitor cells to the inhibitory effect of TGFβ appears to be inversely correlated with their maturation stage (Sing GK 1988, Keller JR 1990, Ottmann OG & Pellus LM 1991, Snoeck H-W 1996). Long-term repopulating stem cells, CD34+ or CD34+/CD38- stem/progenitor cells, early myeloid and erythroid progenitors are markedly inhibited in the presence of TGFβ, whereas more differentiated myeloid progenitors forming granulocyte-macrophage colonies, and late erythroid progenitors forming CFU-e are less inhibited or even stimulated by TGFβ (Keller JR 1990, 1992, 1994, Fortunel NO 2000, Ruscetti FW 2005, Sitnicka E 1996, McNiece IK 1992, Goey H 1989, Morita N 2003, Classen S 2007). The inhibitory or stimulatory role by TGFβ in some cases seems to correlate with the cytokines used to promote cell growth. TGFβ inhibits SCF-, IL-3- and CSF-1-induced colony formation from mouse and human hematopoietic progenitors (McNiece IK 1992, Sitnicka E et al 1996). In contrast, TGFβ enhances GM-CSF-induced colony formation derived from normal bone marrow cells and Lin$^-$ progenitors (Keller JR 1991).

For leukemia cells, both inhibitory and stimulatory effects have been reported (Tessier N & Hoang T 1988, Otten J 2011, Aglietta M 1989, Cashman JD 1992). TGFβ has been shown to inhibit the growth of clonogenic blast cells from patients with acute myeloblastic leukemia (AML) (Tessier N & Hoang T 1988). Mutations in the components of TGFβ signal pathways may relate to the disease development (Imai Y et al. 2001). In contrast, high expression of ALK-1 and ALK-5, two members of TGFβ type I receptors, was reported to be associated with AML (Otten J 2011). An inhibitory effect of TGFβ was suggested by the finding of low levels of TGFβ type II receptors in CML (Rooke HM 1999) and lymphoid neoplasms (Inman GJ et al 2000); whereas, by using TGFβ inhibitors, Naka et al found that TGFβ signal is required for maintaining Leukemia Initiating Cells (LIC) by controlling nuclear localization of transcription factor, FOXO, in a CML mouse model (Naka K et al. 2010).

The data described above suggest that TGFβ has both negative and positive effects on the proliferation of normal hematopoietic and leukemia cells. However, some of the data appear

discordant, which may be due to the complexity of the network in vivo. In the presence of other growth factors, TGFβ-induced stimulation or inhibition could be an indirect result or affected by other factors. Therefore, using a cell line as a research model may provide a direct effect of TGFβ and produce more consistent results. A TGFβ knock out hematopoietic cell line exhibited increased colony formation in response to IL-3, IL-6, and G-CSF (Kim S-J & Letterio J 2003), suggesting that TGFβ is inhibitory to the cells. TGFβ has been reported to inhibit proliferation of mouse myeloid leukemic M1 cells and induce adherence of M1 myeloid leukemia cells to plastic dishes (Okabe-Kado J 1989). In Josk-1 monoblastic leukemia cells, dephosphorylation of pRb and inhibition of c-myc were responsible for TGFβ1-induced G1 arrest (Furukawa Y 1992). In U937 cells, it identified that the inhibition of the cells by TGFβ1 was linked to low levels of transcription factors, C-jun, C-fos and Jun B (Kanatni Y 1996). In order to delineate the effect of TGFβ on more myeloid leukemia cell lines and to reach a general conclusion, we examined more than 10 human myeloid leukemia cell lines, among which MV4-11 and TF-1a are factor-independent but response cell lines; TF-1 and Mo7e are factor-dependent cell lines; and KG-1, KG-1a, K562, U937, HL60, and HEL are factor-independent and insensitive to any growth factors. We demonstrated that TGFβ is inhibitory for all these human myeloid leukemia cell lines in culture, with heterogeneous inhibitory responses being observed (Hu X et al 1999, Hu X & Zuckerman KS 2000). Inhibition of TF-1, KG-1, and K562, Mo7e, and HEL by TGFβ has also been reported by other groups recently (Moller GM et al. 2007, Koutoulaki A et al 2010, Fukuchi Y et al 2001, Kalina U et al. 2001). Our data and some recent discoveries, clearly indicate that the responses of the cell lines to TGFβ are not related to the apparent differentiation stages of the cells (Hu X et al 1999) but could result from differences in the amount of TGFβ receptors expressed or the degrees of the mutations (Quentmeier et al. 2003, 2006, Gu TL et al. 2006) in TGFβ signal components in these cells. The mechanisms by which TGFβ inhibits hematopoietic cells involves in the regulation of pRb pocket proteins-E2F complexes, downregulation of a number of cell cycle regulatory molecules, and upregulation of p21 (Ducos K et al 2000) and p57 (Scandura JM et al 2004) as described in following sections.

4. Regulation of pRb-E2F complexes in human myeloid leukemia cells

pRb acts as a signal transducer collecting signals from cell cycle regulatory molecules to transcriptional machinery. G1 protein kinases have been reported to modify pRb by phosphorylation, thereby promoting cell cycle progression toward DNA replication (Suryadinata R et al. 2011, Takaki T et al. 2004). The growth inhibition of human myeloid leukemia cells by TGFβ is linked to suppression of pRb phosphorylation on multiple serine and threonine residues, including Ser249/Thr252, Thr373, Ser780, Ser795, and Ser807/811 (Chen P et al. 1989, Mihara K et al. 1989, Hu X et al. 1999). Dephosphorylated pRb interacts with E2F transcription factors. E2F-binding sites have been found in the promoters of many genes that control cell cycle progression and cell proliferation, thereby E2Fs play a critical role in cell growth control (Iaquinta PJ et al. 2007). Previous studies identified that E2F binds to the pocket domain of pRB. In E2F1, the pRB-binding domain interacts with the C-terminus of the E2F molecule. As a consequence of the pRB-E2F interaction, E2F activity is repressed. pRB, therefore, exerts its growth suppression function mainly through its sequestration of E2F activity. However, one recent report suggests that E2F-1 binding

affinity for pRb is not the only determinant of the E2F1 activity (Sahin F and Sladek TL 2010a). Of the seven E2Fs well documented, E2F1, E2F2, and E2F3 are considered as "activator" E2Fs, whereas, E2F4, E2F5, E2F6, and E2F7 are considered to be "repressor" E2Fs (Attwooll C et al. 2004, Sahin F and Sladek TL 2010b). pRb and two pRb-related proteins, p107 and p130, show a common structure, which are collectively termed the "pocket proteins". E2F1-3 bind to pRb, and E2F5 binds to p130. E2F4 can interact with the all three pocket proteins.

Dephosphorylated pRb preferentially interacts with E2F1-3 (Weinberg RA et al. 1995), and the pRb/E2F complexes are found primarily during the G1 phase of the cell cycle (Shirodkar S et al. 1992, Schwarz JK et al. 1993), during which time E2F-responsive promoters are repressed. The dissociation of the pRb/E2F-repressor complex and liberation of free E2F allows for the activation of these promoters (Hiebert SW et al. 1993, Qin X-Q et al. 1995, Helin K et al. 1993). It was demonstrated that E2F-1 responsive gene activation was inhibited by overexpression of wild-type pRb, but not by a mutant pRb, whereas transactivation mediated by mutants of E2F1, which do not bind to pRb, was not affected by overexpression of wild-type pRb. Loss of E2F1-3 resulted in an acute cell cycle arrest (Wu L et al. 2001). It has been found that Rb gene was often mutated in patients with various cancers, whereas p107 and p130 genes appear to be less frequently mutated in human cancers, suggesting that pRb is a tumor suppressor, whereas p107 and p130 are not. However, recent studies from several labs have shown that p130 and p107 also actively involved in E2F-dependent transcription activities. For example, the major E2F complexes in quiescent fibroblasts were E2F4-p130. As cells enter the cell cycle, E2F4-p130 was replaced by E2F4-p107 and E2F4-pRb (Ikeda M et al. 1996, Moberg K et al. 1996). Subsequent investigators, by performing chromatin immunoprecipitation (ChIP) experiments, were able to locate E2F complexes in a gene promoter. They have demonstrated that in serum-deprived cells, predominantly E2F4, p130, and a histone deacetylase (HDAC) are found to bind to corresponding E2F-responsive promoters (Rayman JB et al. 2002, Ren B et al. 2002, Takahashi Y et al. 2000). Since histones are generally hypoacetylated when a gene is turned off, which is regulated by HDAC, the results described above suggest that p130-E2F4 DNA binding activity is required for the gene inactivity and for arresting cells in quiescent state. As cells progress through mid-G1, E2F4 and p130 are replaced by activators E2F1, 2, and 3. Unexpectedly, loss of pRB had no effect on HDAC recruitment to the promoter or pRb was not detectable in the promoters containing the E2F responsive site in quiescent cells (Rayman JB et al. 2002, Takahashi Y et al. 2000). Thus, these data suggest that pRb may not be a primary negative regulator of E2F activity in control of cell cycle progression in these cells.

As indicated in the previous section, TGFβ dramatically inhibits the growth of several human myeloid leukemia cell lines in culture with a maximal growth inhibition being observed in MV4-11 cells. We asked how pRb-E2F complexes were regulated in MV4-11 cells. We found that in actively proliferating MV4-11 cells, p130 and pRb were expressed as peptides that migrated in multiple bands in SDS-PAGE, whereas in the cells treated with TGFβ, p130 and pRb were dephosphorylated. However, TGFβ did not dephosphrylate p107 but inhibited expression of the molecule (Hu X et al. 2000). Subsequently, we found that TGFβ-treated MV4-11 cells had very low levels of pRb-associated E2F1 and E2F3. The low levels of pRb-E2Fs are resulted from a low expression of E2F1 and E2F3 in response to TGFβ that arrested cells in G1 phase. Unexpectedly, proliferating cells without TGFβ contained

substantial quantities of pRb-E2F1 and pRb-E2F3 complexes. In contrast, the cells treated with TGFβ (i.e. G1 cells) showed increased levels of pRb-E2F4 and p130-E2F4 and low level of p107-E2F4, as detected by immunoprecipitation and Western blotting. Since TGFβ treatment inhibited the expression p107 but not E2F4, thus, the low level of p107-E2F4 complexes is a result of the inhibition of p107. Our data indicate that E2F4 was switched from p107 to pRb and p130 when p107 was inhibited in response to TGFβ treatment. By performing electrophoretic gel mobility shift assay (EMSA), we were not able to detect pRb-E2F4 DNA binding activities either in proliferating cells or in G1 cells but exhibited the existence of p107-E2F4 and p130-E2F4 DNA binding complexes in G1 cells. which is consistent with the reports from the ChIP experiments described above. Taking together, our data suggest that p107 and p130, but not pRb, and the repressor E2F, but not activator E2Fs, play a critical role in regulating E2F-responsive gene expression in TGFβ-mediated cell cycle control in human myeloid leukemia cells. This conclusion is consistent with several studies that have failed to detect pRb at E2F-responsive genes, whereas p107 and p130 are readily detectable (Aslanian A 2004, Rayman JB 2002, Takahashi Y 2000, Wells J 2000). In addition, many cell cycle-regulated E2F targets are depressed in p107-/- and p130-/- cells but are not depressed in Rb-/- cells (Herrera RE 1996, Hurford Jr RK 1997, Mulligan GJ 1998). It is not clear if traditional activator E2Fs have any significant impact on cell cycle progression of MV4-11 cells, because pRb-E2F1 and pRb-E2F3 complexes have been found in the proliferating but not in G1 phase cells. Previous studies suggest that pRb may bind other regulatory regions of DNA or uses other mechanisms (Sun H et al. 2011), directly or indirectly, for its inhibitory function as a tumor repressor. pRb may also have both positive or negative role on cell growth, depending on the transcription factors it interacted (Calo E et al. 2010).

5. TGFβ inhibits both G1 and G2 protein kinases in human myeloid leukemia cells

In mammalian cells, the cell cycle progression is regulated by a group of cyclin dependent kinases (cdks) and their regulatory subunits in sequential order: cyclin D-cdk4/cdk6 and cyclin E-cdk2 complexes act on G1 and G1-S transition, respectively, followed by cyclin A-cdk2 on S and cyclin B-cdc2 at G2-M transition. TGFβ is well known to be a negative regulator of G_1 cyclin/cdk activity in many types of cells. This has been reported to be a major mechanism that is responsible for TGFβ-induced growth inhibition of cells. In epithelial and fibroblasts, the negative role of TGFβ includes inhibition of cdk4 and disruption of cyclin E-cdk2 assembly (Ewen ME et al. 1993, Koff A et al 1993). Although TGFβ is known to be a G1 cdk inhibitor, studies from several groups suggest potential effect of TGFβ on G2 checkpoint kinase. For instance, the mouse epithelial cells treated with TGFβ expressed a lower level of cdc2 as compared with the cells without TGFβ (Fautsch MP et al 1995); in a hepatoma cell line, TGFβ activates cdc2 and cdk2 followed by phosphorylation of pRb which in turn triggers apoptosis of the cells (Choi KS et al 1999). A new report published in 2008 presented evidence that disruption of Smad 3, a key component of TGFβ-induced signal pathway, elevated cdk 1(cdc2) activity via upregulating the expression of cyclin B. As a result, the uncontrolled cdk1 delayed mitotic progression (Fujita T 2008). In human myeloid leukemia cells, we found that inhibition of multiple cdks including cdc2 and cdc25C is a dominant event in TGFβ-induced growth inhibition of the cells (Hu X et al.

2001). Our recent studies, using TF-1 and MV4-11 human myeloid leukemia cells, demonstrated that TGFβ1-induced inhibition of leukemia cell growth is not due solely to downregulation of G_1 cyclins and cdks but also G2 check point kinases (Hu X 2007). In MV4-11 cells, TGFβ caused a rapid and transient dephosphorylation of cdc2 (Tyr15) and cdc25c (Ser216), with 60% and 70% inhibition of phosphorylation of the both proteins by 2-3 hours, respectively, followed by returning to near normal by 12 hours, and 70-100% inhibitions again by 24-72 hours, repectively. The dephosphorylation of cdc2 and cdc25c was followed by a dramatic decrease of total cdc25c (100% decrease at 48 and 72 hours) and cylcin B1 and cdc2 (70-90% reduction at 24 to 72 hours). Consistent with these data, cdc2 kinase activity, as determined by ability to phosphorylate histone H1 and GST-Rb in vitro, was markedly reduced in the leukemia cells treated with TGFβ for 24-48 h. Cyclin B1 and cdc25c were degraded in a ubiquitin/26S proteosome assay system in vitro, which suggests strongly that ubiquitination and proteasomal degradation may be the key mechanism of degradation that leads to block of G_2-M transition. The downregulation of cdc2, cdc25c, and cyclin B1 was not a result of G1 arrest but a direct effect of TGFβ, because the time required for the inhibition of the cells released from G2 is much shorter than the cells released from G1. Using immunoprecipitation and Western blotting we detected that the levels of cyclin B1-cdc2 complexes were much lower in extracts of TGFβ-treated cells than in control cells. Based on our data, we suggest that TGFβ suppresses growth of human myeloid leukemia cells through multiple pathways, by inhibiting both G1 and G2 checkpoint kinases. Since the G1 and G2 checkpoint kinases are able to phosphorylate pRb in proliferating cells, the down regulation of G1 and G2 checkpoint kinases would prevent pRb phospharylation, leading to G1 arrest. The data also suggest that cdc2, a traditional G2 regulator in mammalian cells, may participate in G1 regulation. Fig. 1 illustrates diagrammatically the negative roles of TGFβ in the cell cycle control in myeloid leukemic MV4-11 cells.

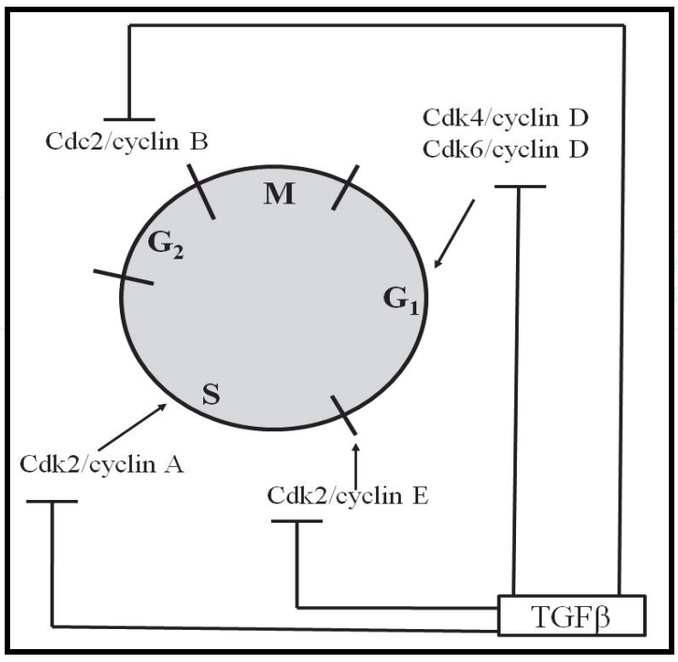

Fig. 1. TGFβ inhibits both G! and G@ cdks in MV4-11 cells

6. Dual roles of cdk inhibitors in human myeloid leukemia cells

It is well known that activity of cdks is counterbalanced by cdk inhibitors (ckis). The cdk inhibitors can be classified into two different families: Ink4 (p16, p15, p18 and p19) and cip/kip (p21, p27, and p57), based on their structure similarity. Among these cdk inhibitors, p27 and p21 have been intensively studied in various types of cells. In quiescent cells or cells treated with TGFβ show high level of p27, whereas proliferating cells or cells treated with mitogens express low level of p27 (Moller MB 2000). Overexpression of p27 in cultured cells arrests the cells in G1 phase and antisense inhibition of p27 reverses cell cycle arrest induced by p27 (Coats S et al 1996; Rivard N et al. 1996). In vivo, p27 is a critical regulator of cellular proliferation. Mice lacking p27 gene are 15–30% larger than their wild-type or hemizygous ($p27^{+/-}$) littermates (Fero et al. 1996; Kiyokawa et al. 1996). p27 was initially thought to interfere with the activities of cyclin D-, E-, and A-dependent kinases. The crystal structure of p27 bound to cyclin-cdk2 reveals that a p27 fragment inserts itself within the cdk2 catalytic site, blocking ATP access (Russo et al., 1996). These data lead to a popular model in which anti-proliferative stimuli up-regulate p27, followed by inhibition of cyclinD-cdk4, cyclinE-cdk2, cyclin A-cdk2 activities, and by G1 arrest/growth inhibition. Similar to p27, as a negative regulator of the cell cycle progression, p21 is thought to bind and inhibit cdk2/cyclin E and/or cdk4/cyclin D complexes, thereby arresting cells at the G1 phase. The induction of p21 is regulated by the tumor suppressor gene, p53, in response to DNA damage.

The traditional model of p27 or p21 as a cdk inhibitor has been challenged by a number of studies from several labs in which p21 or p27 were demonstrated to be required for a cdk/cyclin kinase activity or to be a dual player in cell cycle control (Zhang H et al 1994, LaBaer et al 1997). It was reported that p21, at low concentration, promotes the assembly of active kinase complexes, whereas a high concentration of p21 causes the opposite effect in SAOS-2 cells derived from osteosarcoma (LaBaer et al., 1997). In an animal model, it was found that low concentration of p21 could promote tumor progression in the presence of oncogenic factors (Jones JM 1999). Primary mouse fibroblasts that are p21-/- and p27-/- were failed to assemble cylcin D-CDK complexes and were unable to direct cyclin D nuclear translocation (Cheng M 1999). Consistent with this report, p27 was demonstrated to be essential for mouse mammary gland morphogenesis and function (Muraoka RS et al. 2001); an increased level of p21 was found to be parallel with S-phase entry of oligodendrocytes (Bansal R et al. 2005). In another report published in the same year, p27 in mouse T cells was found to acts as a promoter during the early stage of the cell cycle (Rowell EA et al 2005). In addition, it has been reported that in MCF-7 cells, cyclin D1 promotes cellular migration through inducing the expression of p27 and by physically associating with p27 to promote cellular migration (Li Z et al. 2006). These dual roles of p21 and p27 in non-hematopoietic cells are consistent with what we found in human hematopoietic cells a few years ago. By using several human myeloid leukemia cell lines as research models, we demonstrated that p27 has differential effect on cdk2 and cyclin E activities, which provides supplementary information to the evidence of p27 and p21 having dual roles in cell cycle control described above (Hu X et al. 2001). Although TGFβ greatly upregulated p27 in both cytosol and nucleus in MV4-11 cells, the association of p27 with cylcin E and cdk2 showed different patterns with increased amount of cyclin E-p27 and decreased levels of cdk2-p27 being detected by Western blot and immunoprecipitation. Surprised, a significant amount of p27 was found to associate with cdk2 in proliferating cells, which was downregulated in

response to TGFβ. The downregulation is a result of inhibition of total cdk2 by TGFβ. However, TGFβ had no effect on the expression of cyclin E. By carrying out p27 immunodepletion and transfection experiments, we further demonstrated that that at least some cdk2 kinase activity is associated with p27 in MV4-11cells. Additional support for this possibility was the observation that cells transfected with anti-sense p27 cDNA but not empty plasmid, downregulated cdk2 kinase activity by more than 60%. Although p27 also may act as an adapter of cyclin E activity, as suggested by expression of cyclin E- p27 complexes in proliferating MV4-11 cells, p27 mainly acts as an inhibitor of cyclin E activity in response to TGFβ. This is indicated by the fact that TGFβ1 upregulated cyclin E-associated p27 and was accompanied by the inhibition of cyclin E-dependent kinase activity. Moreover, p27 immunodepletion dramatically upregulated cyclin E-dependent kinase activity in TGFβ1 treated cells. The effect of p27 on cyclin E-dependent kinase activity is dose-dependent, with only high concentrations of p27 being inhibitory to cyclin E activity. This may explain the observation that immunodepletion of p27 or anti-sense p27 cDNA did not significantly increase cyclin E-dependent kinase activity in proliferating MV4-11 cells. These results are against the notion that p27 is a negative regulator of cdk2 kinase *in vivo,* at least in these human myeloid leukemia cells. Our data are consistent with previous findings, in which cell cycle arrest mediated by TGFβ remained intact in p27-/- cells (Nakayama et al., 1996) and p21 and p27 has dual roles in cell cycle control (Rowell EA et al. 2005, LaBaer et al. 1997, Zhang H et al. 1994). The difference is that the negative or positive role of p27 in our model is related to the molecules p27 interacted but not the concentrations of p27 as reported in the case of p21 (Matsushime et al., 1992). Taken together, we interpret our results to suggest that in proliferating cells p27 is associated with cdk2 and cdk4 as an adapter. TGFβ1 downregulates cdk2, cdk4, and D-type cyclins, which leaves $p27^{kip1}$ free to bind and saturate cyclin E, inhibiting cyclin E-cdk2 assembly, rendering cyclin E-dependent kinase inactive. By using these two different regulatory mechanisms, TGFβ1 magnifies its cell cycle inhibitory efficiency in human myeloid leukemia cells (Hu X et al 2001).

7. Conclusion

New findings in recent years have provided insight into the complex roles and action mechanisms of TGFβ in various types of cells. TGFβ exhibits its distinct effect on the growth of human myeloid leukemia cells. Although both positive and negative roles of TGFβ in hematopoiesis have been reported, the major role of TGFβ appears to be inhibitory with heterogeneous responses being observed in human myeloid leukemia cells. The heterogeneous responses of the cells to TGFβ are not related to the apparent differentiation stages of the cells but could result from differences in the expression of TGFβ receptors or degrees of the mutations in the components of TGFβ signal pathways. TGFβ, a traditional G1 cdk inhibitor, represses not only G1 but also G2 protein kinases in human myeloid leukemia cells. pRb has been reported to be a tumor suppressor in various types of cells. The new studies suggest that p130, whose gene is less mutated in cancer patients, plays a critical role in maintaining cells in quiescent status and in TGFβ-mediated growth inhibition. Traditionally, p27 and p21 are G1 cdk inhibitors. However, new evidence suggests that p27 and p21 have both positive and negative effect on G1 cdk activities.

Based on the data described in this review, it is clear that TGFβ signals have a defined role in regulating normal hematopoiesis, and disruption of the signaling components could lead

to hematologic malignancies including myeloid leukemia. These are suggested by the observations described above: (1) The development of hematopoietic cells from mesoderm in embryos is regulated by TGFβ signaling; (2) inactivation of the TGFβ signaling pathway leads to malignant transformation of early hematopoietic cells; (3) abnormal expressions of the components of TGFβ signaling pathway have been found in AML and CML; and (4) a number of mutations of genes that encode TGFβ signaling components have been detected in several myeloid leukemia cell lines. Thus, the mutated components may be the targets for therapeutic strategy of myeloid leukemia in future.

Although new information on TGFβ and myeloid leukemia have been emerged at an incredibly rapid pace, it is unclear whether some information obtained from studies performed *in vitro* mimic a physiological status *in vivo*. In addition, the detail about dual role of cdk inhibitiors and the role of pRb in leukemia transformation are also need to be clarified in future.

8. References

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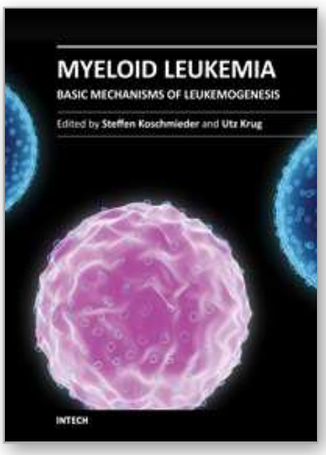

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The current book comprises a series of chapters from experts in the field of myeloid cell biology and myeloid leukemia pathogenesis. It is meant to provide reviews about current knowledge in the area of basic science of acute (AML) and chronic myeloid leukemia (CML) as well as original publications covering specific aspects of these important diseases. Covering the specifics of leukemia biology and pathogenesis by authors from different parts of the World, including America, Europe, Africa, and Asia, this book provides a colorful view on research activities in this field around the globe.

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