The TEL/ARG Leukemia Oncogene Promotes Viability and Hyperresponsiveness to Hematopoietic Growth Factors

Keiko Okuda, a Yuko Sato, b Yoshiaki Sonoda, a James D. Griffin c

aDepartment of Health Sciences and Preventive Medicine, Kyoto Prefectural University of Medicine, Kyoto;
Division of Molecular Cytogenetics, Department of Clinical Pathology, Research Institute, International Medical Center of Japan, Tokyo, Japan; 
cDepartment of Adult Oncology, Dana Farber Cancer Institute and Department of Medicine, Brigham and Women’s Hospital and Harvard Medical School, Boston, MA, USA

Received September 9, 2003; received in revised form November 7, 2003; accepted November 14, 2003

Abstract

The TEL/ARG oncogene associated with acute myeloid leukemia is formed by the t(1;12)(q25;p13) reciprocal translocation, which fuses part of the TEL gene to the tyrosine kinase, c-ARG. In an effort to determine the biological effects and investigate signaling of the TEL/ARG fusion protein, multiple sublines of Ba/F3 cells were generated in which a TEL/ARG complementary DNA was expressed under the control of a tetracycline-inducible promoter. Treatment of these cells with doxycycline, a tetracycline analogue, rapidly induced expression of the TEL/ARG protein. TEL/ARG was heavily phosphorylated on tyrosine residues and was also found to rapidly induce tyrosine phosphorylation of multiple cellular proteins, including rasGAP, CBL, STAT5, PI3K, SHP2, Dok, and SHC. The Ba/F3-tet-TEL/ARG cells remained interleukin (IL)-3 dependent without doxycycline but with doxycycline displayed a marked reduction in cell death in the absence of IL-3. TEL/ARG cells also displayed an enhanced proliferative response to IL-3 and to insulin-like growth factor 1. At least in Ba/F3 cells, although the growth rate was much lower compared to that with IL-3, TEL/ARG appeared to induce some cell proliferation as an immediate consequence. Nonetheless, the hyperresponsiveness to growth factors reported here is more likely to contribute to the pathogenesis of leukemia.

©2004 The Japanese Society of Hematology

Key words: Leukemia; Chimeric oncogene; TEL/ARG; Tyrosine kinase; Signal transduction

1. Introduction

The ABL-related gene (ARG), with an overall structure similar to that of ABL, was cloned in 1986 [1,2]. ARG is a widely expressed nonreceptor protein tyrosine kinase, typically detected on Western blot as a series of bands at 135 to 150 kd [3]. There are no signaling pathways currently known to activate ARG, and no ARG substrates have been described in intact cells. The biological function of ARG is also not well understood. ARG (−/−) mice display few defects, but ARG and ABL double−/− mice have severe nervous system defects, suggesting that ABL and ARG perform a cooperative role in the proper development of the nervous system [4]. Recently, the t(1;12) (q25;p13) chromosome translocation observed in some patients with acute myeloid leukemia (AML) was shown to result in a fusion of the TEL and ARG genes, resulting in the production of a fusion protein composed of N-terminal sequences from TEL fused in frame to the ARG gene, including the tyrosine kinase domain [5,6]. TEL has been previously identified as a frequent fusion partner of tyrosine kinase oncogenes in patients with acute leukemia and myeloproliferative syndromes [7-18]. For example, the TEL/PDGFRβ fusion protein has been shown to encode a con-
stitutively activated tyrosine kinase that induces factor-independent proliferation of hematopoietic cell lines and induces a myeloproliferative syndrome in vivo. The pointed domain of TEL has been shown to be particularly important for transformation and may function to induce dimerization and kinase activation. Similar studies with TEL/ABL and TEL/JAK2 support the notion that TEL/tyrosine kinase fusion oncogenes generally function as constitutively activated tyrosine kinases, enhancing proliferation and prolonging viability. However, there are also differences among the various TEL fusions both in murine models and in patients; some are associated with chronic leukemias and others only with acute leukemias. TEL/ARG has so far been associated only with acute leukemia. The mechanisms of transformation associated with TEL/ARG are not well understood. In our previous studies, we have demonstrated that the TEL/ARG protein was heavily phosphorylated on tyrosine, increased tyrosine phosphorylation of multiple cellular proteins, and was associated with factor-independent proliferation when expressed in the factor-dependent murine hematopoietic cell line Ba/F3 [19]. Using the sublines, we also have demonstrated that ARG kinase activity is inhibited by the ABL tyrosine kinase inhibitor, STI571 [20-22].

Although commonly used to investigate the biochemical activities of leukemia oncogenes, these factor-independent cells may not accurately reflect the biological activities of tyrosine kinases, especially if the cell lines were selected for certain properties, such as factor-independent proliferation. An alternative approach is to express oncogenes under the control of inducible promoters so that expression can be regulated. A particular advantage is the ability to generate cell lines with reduced likelihood of new mutations in unknown genes. Here, we report studies on a set of sublines of Ba/F3 cells in which a TEL/ARG complementary DNA (cDNA) was expressed under the control of a doxycycline.

2. Materials and Methods

2.1. Plasmid Constructs

The cDNA encoding the TEL/ARG (AL) was generated by reverse transcriptase polymerase chain reactions from HT93A cells with FLAG epitope tags at their 3’ ends, as described previously [6,19]. The complete TEL/ARG cDNA was ligated into pTRE (Clontech, Palo Alto, CA, USA) containing a tet-responsive promoter, forming pTRE TEL/ARG.

2.2. Cell Lines and Cell Culture

Ba/F3 is an interleukin (IL)-3-dependent murine hematopoietic pro-B cell line [23]. TonB1 is a Ba/F3 subline, which has been stably transfected with a plasmid containing the reverse tetracycline transactivator pUHD172-1 and is also IL-3 dependent [24]. Both Ba/F3 and TonB1 cells were maintained in RPMI 1640 medium (GIBCO BRL, Gaithersburg, MD, USA) supplemented with 10% (vol/vol) fetal calf serum (FCS) and 1.0 ng/mL recombinant murine IL-3 (Kirin, Tokyo, Japan). TEL/ABL- and TEL/ARG-transformed Ba/F3 cells, which have been previously described [19,25], are IL-3 independent and were maintained in culture without IL-3.

To generate TonB1 cells inducibly expressing the TEL/ARG, 20 μg pTRE TEL/ARG and 2 μg pTK-Hygro (Clontech) were electroporated into these cells (0.27 kV, 960 μF) using a gene pulser (Biorad, Hercules, CA, USA). Cells were grown for 48 hours in 10% FCS-RPMI 1640, supplemented with 1 ng/mL IL-3 before addition of hygromycin-B (Boehringer Mannheim, Indianapolis, IN, USA) 400 μg/mL. After 2 to 3 weeks of initial selection, individual subclones were obtained by limiting dilution.

2.3. Antibodies

Antiphosphotyrosine monoclonal antibody (4G10) was provided by Dr. Brian Druker (University of Oregon Health Sciences Center, Portland, OR, USA). Rabbit polyclonal anti-ARG antibody, which was provided by Dr. Gary D. Kruh (Fox Chase Cancer Center, Philadelphia, PA, USA), was raised against the c-terminus (amino acids 432-446) of ARG and used for immunoblotting [3]. Anti-ARG goat polyclonal antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and used for immunoprecipitation. Anti-Dok, SHP2, CBL, ras-GAP, and CRK-L antibodies were purchased from Santa Cruz Biotechnology. Anti-PI3 kinase and Paxillin antibodies were purchased from Upstate Biotechnology (Lake Placid, NY, USA). Anti-Shc antibody was purchased from Transduction Laboratories (Lexington, KY, USA). An anti-phospho-STAT polyclonal antibody recognizing only tyrosine phosphorylated STAT 1 and 5 was provided by Dr. David Frank (Dana-Farber Cancer Institute, Boston, MA, USA).

2.4. Immunoblotting and Immunoprecipitation

Cells were deprived of growth factors by culturing in medium containing 10% FCS in RPMI1640 and were then stimulated with either murine recombinant IL-3 or doxycycline (Sigma, St. Louis, MO, USA) as indicated in each experiment. Immunoprecipitation and immunoblotting were performed as previously described [25].

2.5. Proliferation and Viability Assays

The number of viable cells was determined by trypan blue (Sigma), and viable cells were counted using a hemacytometer. Cell concentrations were calculated as number of cells × 10,000/mL. Cell viability was reported as a percentage of total cells.

2.6. Migration Assay

Migration assays were performed using Transwell plates (8 μm–pore-size polycarbonate membrane; Corning Coster, Cambridge, MA, USA). Both sides of the membrane were coated with human fibronectin (5 μg/mL). The lower chamber contained 600 μL of RPMI 1640 medium containing 0.5% bovine serum albumin (BSA), 0.2 × 106 cells in 100 μL