Adult T-Cell Leukemia and Retinoid

Yasuhiro Maeda*, Atsushi Okamoto, Shin-ichiro Kawaguchi, Akiko Konishi, Kenta Yamamoto, Go Eguchi and Terufumi Yamaguchi

Department of Hematology, National Hospital Organization Osaka Minami Medical Center, 2-1, Kido-Higashi, Kawachinagano, Osaka 586-8521, Japan

Abstract: Adult T-cell leukemia/lymphoma (ATL/ATLL) is an aggressive lymphoid disease caused by human T-cell leukemia virus type 1 (HTLV-1). It is reported that retinoid suppressed the proliferation of malignant cells including ATL cells. In this study, we showed the mechanism of retinoid action for ATL cells. We observed that NF-κB transcriptional activity as well as cell proliferation decreased in HTLV-1-positive T-cell lines by treatment with retinoid. Further, we observed that retinoid reduced HTLV-1 proviral DNA. Interestingly, retinoid significantly inhibited reverse transcriptase (RT) activity similar to azidothimidine (AZT) on HTLV-1-positive T-cell lines. Therefore, AZT was inhibitory of proviral DNA load but not NF-κB transcriptional activity on HTLV-I, however retinoid was inhibitory of both NF-κB and proviral DNA on HTLV-1. Furthermore, we showed cellular senescence in HTLV-I positive T-cell lines and in primary ATL cells obtained from acute ATL patients. The number of senescent cells significantly increased in the HTLV-I positive T-cell lines after treatment with retinoid, but not in the HTLV-I negative ones. These results indicated that retinoid could have three roles, as a NF-κB inhibitor, as a RT inhibitor and as a facilitating cellular senescence.

Keywords: ATL, HTLV-I, senescence, redox potential.

1. INTRODUCTION

We previously reported that retinoid could induce significant growth inhibition of adult T-cell leukemia / lymphoma (ATL/ATLL) [1, 2]. ATL cells finally have been induced apoptosis [3-6]. Retinoid has an ability to influence the growth and differentiation of malignant cells [7-10]. Retinoid, especially, all-*trans* retinoic acid (ATRA) induces differentiation of fresh human acute promyelocytic leukemia cells into normal granulocytes [11-13]. In this article, we showed the mechanism of action of retinoid for ATL cells. In results, retinoid could have two roles, as an NF-κB inhibitor and as a reverse transcriptase (RT) inhibitor [14]. Furthermore, induction of cellular senescence by ATRA in ATL cells has been observed [5]. The grade of cellular senescence was greater for *Tax*-expressing cells than for *Tax*-non-expressing cells. This indicated that the *Tax* gene may act as an oncogene, and that oncogene-induced senescence (OIS) is possible. Taken together, we conclude that ATRA must be a reasonable agent for ATL by facilitating cellular senescence. In this paper, we present the effects of retinoid on 1) cell growth inhibition, 2) NF-κB transcription inhibition, 2) reverse transcriptase activity, 3) redox potential status, 4) cellular senescence induction, and 6) clinical outcomes.

*Address correspondence to this author at the Department of Hematology, National Hospital Organization Osaka Minami Medical Center, 2-1, Kido-Higashi, Kawachinagano, Osaka 586-8521, Japan; Tel: (81)-721-53-5761; Fax: (81)-721-53-8904; E-mail: ymaeda@ommc-hp.jp

2. GROWTH INHIBITION OF ATL T-CELL LINES BY RETINOID

We examined the effects of ATRA on the cell growth of five HTLV-1 (+) T-cell lines (ATL-2, HUT102, MT-2, MT-4, and ED40515) and two HTLV-1 (-) T-cell lines, used as control cells (Jurkat and MOLT-4). Methods are described below. MT-2 and MT-4 cells were gifts kindly provide by Dr. M. Matsuoka of Kyoto University. ED40515 was a gift from Dr. M. Maeda of Kyoto University. In brief, cells (1×10^5/ml) were cultured in flat-bottomed 96-well microplates (Corning, NY) with 10^{-5} M ATRA for 24, 48, and 72 hours. Twenty microliters of CellTiter 96 AQ_{ueous} One Solution (Promega, Southampton, United Kingdom) was added and the cells were incubated for an additional 2 hours. And then, fluorescence intensity was measured at 490 nm using an ELISA plate reader. In results, significant suppression in the cell proliferation of five ATL cell lines with ATRA treatment was observed [15].

3. INHIBITION OF NF-κB TRANSCRIPTIONAL ACTIVITY BY ATRA ON HTLV-I (+) T-CELL LINES

The effects of ATRA on NF-κB transcriptional activity were observed. The transcriptional activity of NF-κB in five ATL cell lines described above was significantly decreased in the presence of ATRA [14-20]. However, in the presence of azidothimidine (AZT), the transcriptional activity was not decreased in ATL cell lines. In results, the transcriptional activity of NF-κB in all ATL cell lines (including ED40515 of a HTLV-1-positive T-cell line that does not express *Tax* mRNA) decreased by treatment with ATRA.

E-ISSN: 2312-5411/16

4. EFFECTS OF ATRA ON HTLV-1 PROVIRAL DNA LOAD IN ATL CELL LINES

To examine the anti-retroviral effect of ATRA, the HTLV-1 proviral DNA load was determined using real time PCR in five ATL cell lines before and after treatment with ATRA or reverse transcriptase (RT) for 48 and 72 hours [14]. In results, the HTLV-1 proviral DNA load was significantly decreased by AZT in ATL cell lines. Furthermore, the HTLV-1 proviral DNA load was also significantly decreased by ATRA in ATL cell lines. These results indicated that ATRA may act as an RT inhibitor resulting in cell growth arrest.

5. CELLULAR SENESCENCE INDUCTION BY RETINOID IN ATL CELLS

We also focused on the role of ATRA in inducing cellular senescence ATL cells. Cellular senescence was detected by staining for senescence-associated β-galactosidase (SA β-Gal) [22]. SA β-Gal-positive cells were observed during spontaneous culture without ATRA in five HTLV-I (+) T-cell lines described above, but not in HTLV-I (-) T-cell lines (Jurkat and MOLT-4). On treatment with ATRA, the number of SA β-Gal-positive cells significantly increased in the HTLV-I (+) T-cell lines, but not in the HTLV-I (-) ones [5]. The same trend was observed in fresh ATL cells obtained from 3 acute ATL patients [5]. Furthermore, the mRNA expression levels of CDK inhibitors (p21cip1 and p16ink4a) were observed. The $p16^{INK4a}$ mRNA expression level was enhanced in all ATL cell lines, but not in the non-ATL cell lines. The cellular senescence grade was greater for HUT102, MT-2, MT-4, and ATL-2 cells than for ED40515 cells, which do not express *Tax* mRNA because of a nonsense mutation. This is an additional report pointing to *Tax* as an oncogene, and OIS may have occurred. OIS does occur in malignant tumor cells *in vivo* [23, 24]. These cells cannot re-enter the cell cycle or undergo tumorigenesis once senescence is triggered [24, 25]. This DNA damage is caused by oncogene-derived accumulation of reactive oxygen species (ROS) [26]. Chemotherapy using antineoplastic agents that decrease OIS and reduce cellular senescence may rejuvenate these cells and ultimately induce chemotherapy resistance. In conclusion, retinoid, ATRA may be a reasonable agent for the treatment of ATL by facilitating cellular senescence [5].

6. EFFECTS OF REDOX POTENTIAL ON RETINOID SENSITIVITY

In ATL, ATL derived factor (ADF) that is homologous to thioredoxin (TRX) [27] has been reported to be an active reducing molecule for active oxygen species [27]. It has been reported that the activity of thioredoxin reductase (TRX-R) from melanoma tissue is inhibited remarkably by 13-*cis* RA [28]. Cellular redox potential changes various aspects of cellular function when oxidative stress occurs. The balance of oxidative/anti-oxidative status may play an important role in cellular function. Previous reports suggest that L-cysteine and L-cystine act as buffers for the redox potential in cell or serum environments [29, 30]. Intracellular L-cystine is converted to L-cysteine, which is an active thiol compound that is utilized for GSH synthesis [29] and depletion of L-cystine results in a reduction of intracellular GSH content [30]. However, no restoration of growth was obtained in thiol-untreated ATL-2 cells. These reports suggested that the L-cystine/GSH and ADF/TRX systems cooperate to adjust intracellular redox states following exposure to several oxidants, thereby promoting the growth and viability of lymphocytes. We preincubated ATL-2 cells with each thiol compound (1μg/ml recombinant ADF, 1μg/ml TRX, 10^{-5} M L-cystine, and 10^{-4} M GSH) for 24 hr, and 10^{-5} M ATRA or 13-*cis* was added to ATL-2 cells in a thiol-depleted medium. The reduction rate was decreased significantly by preincubation with the thiol compounds. In particular, preincubation of ATL-2 with L-cystine or GSH resulted in complete recovery of growth despite the inhibitory effects of RA, suggesting that they acted to increase the redox potential of the intracellular environment. Our results suggest that the imbalance of intracellular redox potential in ATL cell lines may be associated strongly with sensitivity to RA and that the cystine/GSH and ADF/TRX redox systems may act against RA action [31, 32].

7. CLINICAL TRIAL OF RETINOID TREATMENT FOR ATL PATIENTS

Twenty patients diagnosed with ATL (7 with acute ATL, 4 with chronic ATL, 3 with lymphoma-type ATL, and 6 with smoldering-type ATL) were selected for this study. All patients gave written informed consent according to the Declaration of Helsinki before participating in this study. Patients were scheduled to receive a daily oral administration of ATRA at a dose of $45mg/m^2$ for 4 weeks. During ATRA treatment, no chemotherapy, glucocorticoid therapy, or radiation therapy was administered. Patients were monitored for safety and anti-tumor effects by regular laboratory testing including standard chemistry performed at the baseline and repeated every week [33]. The median age of patients was 56 years (range, 35–73). Briefly, the treatment efficacy was as follows: CR, 0% of the

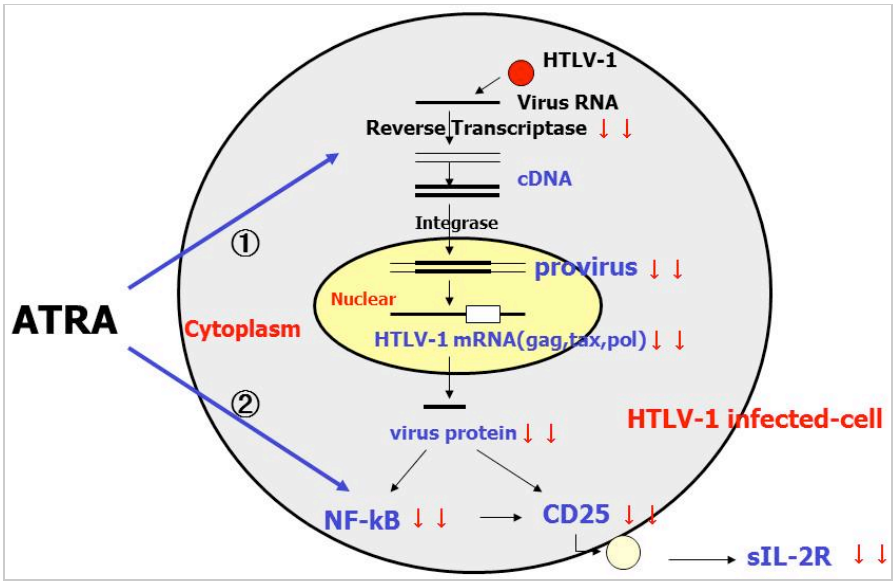

Figure 1: The mechanism of ATRA for ATL cells.

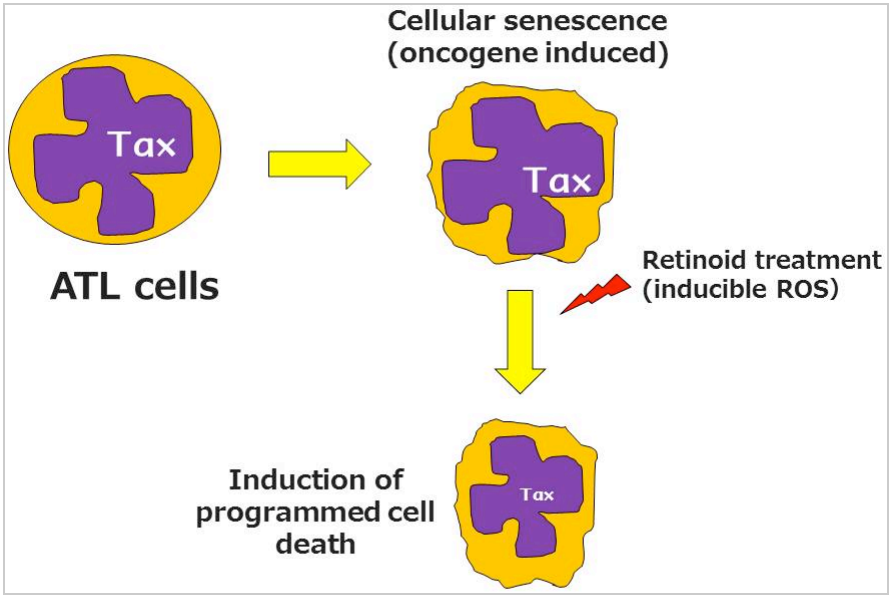

Figure 2: Possible role of Tax for cellular senescence *and* ATRA-induced programmed cell death.

patients; PR, 40%; NC, 45%; and PD, 15%. In the 7 acute ATL patients, PR was achieved in 2 (28.5%), NC in 2 (28.5%), and PD in 3 (42.8%) patients. In all 3 (100%) lymphoma-type patients, PR was achieved. In the 4 chronic-type patients, PR was achieved in 1 (25%) patient, while NC was observed in the remaining 3 (75%) patients. Among the 6 smoldering-type patients, PR was achieved in 2 (33.3%) and NC was observed in 4 (66.6%) patients. Adverse effects were noted in 10 of the 20 patients (50%). These effects were generally mild (headache in 5 patients; liver dysfunction, 2; hyperlipidemia, 2; and anorexia, 1). No hematological toxicity was observed. Moreover, our clinical trial of ATRA for skin involvement demonstrated that ATRA was effective in the treatment of skin involvement in 6 of 8 patients (74%) [34]. Haji *et al,* reported that synthetic retinoid ST1926 reduced tumor burden in murine ATL model [35]. Further, they also showed that ST1926 were potent inducer of cell death in ATRA resistant HTLV-I positive T-cell lines. In conclusion, we firmly believe that treatment with ATRA can provide some important benefits to ATL patients [35].

CONCLUSION

Figure **1** showed the mechanism of ATRA for ATL cells. ATRA must be dichotomized into inhibition of

NF-κB transcriptional activity related to HTLV-1 and inhibition of RT. Finally, we found evidence of cellular senescence in HTLV-I (+) T-cell lines and in fresh primary cells obtained from patients with acute ATL. As shown in Figure **2**, it is possible that *Tax* has an OIS function associated with induction of ROS and ATRA-induced programmed cell death. In summary, ATRA suppressed the growth of HTLV-1-positive T-cell lines, reduced HTLV-1 proviral DNA loads and inhibited NF-κB transcriptional activity in HTLV-1-positive T-cell lines, and enhanced cellular senescence [5].

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Received on 08-02-16 Accepted on 21-03-16 Published on 31-12-2016

DOI: http://dx.doi.org/10.12974/2312-5411.2016.03.01.1