Targeting interferon pathway in TBLR1-RARa-driven promyelocytic leukemia in the context of ATRA and ATO unresponsiveness

Xue Yang
Institute of Hematology & Blood Diseases Hospital

Haiyan Xing
Institute of Hematology & Blood Diseases Hospital

Shaowei Qiu
Institute of Hematology & Blood Diseases Hospital

Kejing Tang
Institute of Hematology & Blood Diseases Hospital

Zheng Tian
Institute of Hematology & Blood Diseases Hospital

Qing Rao
Institute of Hematology & Blood Diseases Hospital

Min Wang
Institute of Hematology & Blood Diseases Hospital

Jianxiang Wang ( wangjx@ihcams.ac.cn )
Institute of Hematology & Blood Diseases Hospital

Research Article

Keywords: Acute promyelocytic leukemia, TBLR1-RARα, interferon pathway

Posted Date: August 3rd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1913410/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

Background

TBLR1-RARα (TR) is a novel oncogene to induce acute promyelocytic leukemia (APL) identified in our previous studies, which elicited differed response and worse prognosis in contrast to the canonical PML-RARα (PR) fusion gene. Despite the textbook rationale of all-trans retinoid acid (ATRA) and arsenic trioxide (ATO) has turned most PR-driven APL from highly fatal to highly curable, TR-driven APL did not yield long-term remission, highlighting the necessity to decipher drug unresponsiveness and explore novel therapies.

Methods

In this study, doxycycline-inducible cell models were established to initiate the expression of TR and PR in U937 cells, respectively. Then RNA-seq was performed to identify differential genes and pathways directly induced by TR and PR oncogene. Based on transcriptomic analyses, different drugs were administered to compare therapy responses and biological phenotypes between TR and PR-induced leukemia through in vitro studies. The efficacy of IFNs and its upstream regulator STING agonist were further validated in TR murine models with survival analysis.

Results

Transcriptome sequencing analyses showed interferon (IFN) pathway were significantly suppressed in TR- rather than PR-induced APL. IFNs as well as the traditional regimen of ATRA and ATO elicit differed responses in biological phenotypes between TR- and PR-induced APL. Specifically, in TR-induced APL, firstly, ATO failed to elicit oncoprotein degradation, apoptosis and loss of self-renewal. Secondly, increasing doses of ATRA further promoted oncoprotein degradation with impaired colony formation capacity, and ultimately conferred survival benefits. Thirdly, type I IFNs is promising, which induced apoptosis, cooperated with ATRA to boost differentiation, and exhibited potential to reduce self-renewal. Finally, type I IFNs combining with ATRA and STING agonist significantly endowed TR mice with extended survival.

Conclusions

For TR-driven APL, the canonical regimen of ATO and lower doses of ATRA are unresponsive and inadequate to elicit oncoprotein degradation, apoptosis and loss of self-renewal, while type I IFNs and STING agonist are promising to endow TR mice with extended survival. Our study aims to gain further understanding of TR-driven APL and integrate insights into leukemogenesis mediated by rare RARα
fusion genes, which may benefit a therapy-resistant population and enable APL to be bona fide curable leukemia.

Introduction

Acute promyelocytic leukemia (APL) is a distinct subtype of acute myeloid leukemia (AML) mostly driven by the t(15;17) translocation that yields the PML-RARα (PR) fusion protein[1]. Apart from PML, other partner genes have been uncovered to fuse with RARα forming rare X-RARα fusion genes[2]. Particularly, the 10th fusion gene TBLR1-RARα (TR) was first identified in our previous studies from rare cases of APL patients carrying t(3;17) chromosomal translocation[3], which is a novel fusion gene acting as an oncogene to induce murine APL with poor prognosis[4].

Despite general similarities between TR and PR fusion protein including dimerization formation and corepressor recruitment, as well as parallel phenotypes of differentiation blockage and increased self-renewal, certain disparity exists. In our previous studies[4], we found although all-trans-retinoic acid (ATRA) could induce terminal differentiation of TR leukemic cells in vitro, TR mice did not benefit from ATRA either as a single agent or combining with arsenic trioxide (ATO), consistent with clinical observations. Why does the paradigmatic regimen of ATRA and ATO that is applicable to the canonical APL with PR fusion gene fail to confer survival advantage in TR-driven APL? What is the mechanism underlying the difference in treatment response? Furthermore, is there any other potential approach to specifically target APL imposed by this rare RARα fusion gene? Given the poor clinical outcome of TR-driven APL, unveiling the unresponsiveness to ATRA and ATO treatment as well as exploring potential therapeutic strategies are of great necessity.

With attempts to decipher specific molecular pathways involved in the pathogenesis of TR-driven APL, we established two Tet-on inducible lentiviral model to initiate the expression of TR and PR fusion protein in U937 leukemia cells. Analyses based on RNA-seq showed interferon (IFN) pathway was significantly suppressed in TR rather than PR-induced leukemia. Then we utilized a series of in vitro and in vivo assays to compare the efficacy of IFNs as well as the classic regimen of ATRA and ATO in leukemia cells induced by TR and PR, providing comprehensive mechanistic interpretations on differed drug response and therapeutic implications for APL harboring TR fusion gene. Our study aims to gain further understanding of TR-driven APL and integrate insights into principles underlying leukemogenesis mediated by rare RARα fusion genes, which may benefit such a therapy-resistant population and enable APL to be a bona fide curable leukemia.

Materials And Methods

Animals and cell culture

HEK293T cells were cultured in DMEM and used to generate TR and PR producing lentiviral particles. TR and PR-transfected U937 cells were maintained in RPMI 1640 medium. Cell lines were tested for
mycoplasma contamination and proved to be mycoplasma free. Mouse models of TR were constructed as previously described[4]. Procedures involving animal experiments were approved by the Institutional Animal Care and Use Committee of Peking Union Medical College.

**Establishment of doxycycline-inducible leukemia cell lines**

Lentiviruses were produced according to the protocol described in Lenti-X™ Tet-On Advanced Inducible Expression System User Manual (Clontech). U937 cells are co-transduced with two lentiviruses derived from the pLVX-Tet-On Advanced regulator vector (rtTA) and the pLVX-Tight-Puro-GOI response vector, respectively. Full-length cDNA of TR (GeneBank KF589333) and PR was cloned into pLVX-Tight-Puro-GOI with a Flag tag to yield pLVX-Tight-Puro-TR-flag and pLVX-Tight-Puro-PR-flag. After infection for 72 hours, GFP-positive U937 cells transduced with rtTA lentivirus were sorted by FACS. Then GFP positive cells were cultured with puromycin at a concentration of 2.5 μg/mL to select the pLVX-Tight-puro-TR or pLVX-Tight-puro-PR stably transduced U937 cells, namely U937-TR and U937-PR. Upon induction with 500ng/mL doxycycline (Dox) for 48h, cells were harvested for subsequent analysis.

**RNA-seq and data analysis**

Total RNA was extracted from U937-TR and U937-PR cells. Cells were cultured with Dox (+Dox) as experimental group, or without Dox (-Dox) as control group. RNA samples were sent to Novogene Co., Ltd. (Tianjin, China) for library preparation and sequencing. All RNA-seq experiments have two biological replicates. Differential expression genes (DEGs) were determined using DESeq2. Padj<0.05 was used as the threshold to judge the significance of gene expression difference. Volcano plots were generated using R package ggplot2. Gene Ontology (GO) enrichment was performed and visualized using R package clusterprofiler. Pathway analyses were performed using Ingenuity Pathway Analysis (IPA) (Qiagen, http://www.ingenuity.com/products/ipa).

**Cell treatment and protein analyses**

Cells were induced with Dox for 48h and treated with different agents for additional 48h. Drugs used for Western blottting include ATRA (sigma), ATO (HARBIN YIDA PHARMACEUTICAL CO., LTD.), Universal Type I Interferon (PBL) and Recombinant Human IFN-γ (R&D). Total protein was extracted with RIPA lysis buffer (Beyotime). Western blot experiments were performed for protein analyses using antibody including anti-β-actin (Sigma) and anti-flag (Sigma). Western blot densitometry was performed using Image J.

**Differentiation, apoptosis and colony formation assays**

Myeloid differentiation of U937 cells was assessed by CD11b expression and apoptosis was detected by Annexin V/PI staining. Differentiation and apoptosis assays were performed by flow cytometry (Novocyte). For colony formation assay (CFA), TR murine cells were plated in 24-well plates (3000 cells/well) and cultured in M3434 methylcellulose (StemCell Technologies) in the presence or absence of ATRA, ATO, Universal Type I Interferon as described and Recombinant Mouse IFN-γ (R&D). After 7 days,
colonies were counted and equal number of cells were reseeded for serial plating. Cell colonies were photographed under Operetta CLS High Content Analysis System (Perkin Elmer).

In vivo drug treatment

Mice used in drug-treatment experiments were non-irradiated recipient C57BL/6 mice generated by intravenous inoculation of 1×10^6 GFP^+ spleen cells of TR leukemia mice. For each experiment, six mice were randomly assigned to each treatment group with ATRA, ATO, Universal Type I Interferon and Recombinant Mouse IFN-γ. The percentage of GFP^+ cells of peripheral blood and body weight were measured dynamically. Survival time of each group was monitored and compared.

Statistics

Statistical analyses were performed using GraphPad Prism 8. Statistical comparisons were made using Student’s t-test. Survival was plotted by Kaplan–Maier curves. Significant differences were indicated with asterisks (*P<0.05; **P< 0.01; ***P< 0.001). Relative intensities (RI) were quantified by Image J.

Results

Interferon pathways are significantly suppressed in TR rather than PR-induced leukemia

To explore the molecular pathway directly regulated by TR in comparison with the canonical PR fusion gene, we used a tetracycline-controlled Tet-On Advanced system in U937 leukemia cell line to induce the expression of TR and PR oncoprotein. Two doxycycline-inducible leukemia cell lines were successfully established, namely U937-TR and U937-PR (Figure 1A). As is shown in Figure 1B, TR and PR were induced stably in the presence of 500ng/mL Dox for 48 hours, the dose of which was applied in the subsequent experiments. Then RNA-seq was carried out on U937-TR and U937-PR with or without the administration of Dox for 48 hours, as the experimental and control groups, respectively. Volcano plots exhibited differential expression genes (DEGs) in U937-TR (Figure 1C) and U937-PR (Figure 1D) upon the expression of fusion proteins, with the former emerging more downregulated genes, implying a more severe transcriptional repression exerted by TR fusion gene. Strikingly, GO analysis revealed that downregulated genes in U937-TR were significantly enriched in categories related to IFN pathway (Figure 1E), which was not predominant in U937-PR (Figure 1F). Then pathway analysis was performed on downregulated genes of U937-TR by IPA, displaying enrichment in both Type-I (IFN-α/β) and Type-II (IFNγ) IFN pathways (Figure 1G). Top ten IFN-stimulated genes (ISGs) downregulated by TR were listed in Table 1, with the expression level displayed in contrast to PR.

ATRA, ATO and IFN elicit different responses in terms of TR and PR oncoprotein degradation

To further determine the distinct role of IFN pathway in TR-induced APL, we administered type I IFNs and IFNγ to U937-TR and U937-PR leukemic cells. To determine differed drug responses with the investigation of combinational efficacy, we also added ATRA and ATO as contrast agents. Since in PR-driven APL,
oncoprotein degradation is critically involved in disease cure[5], we first examine the expression of fusion proteins with the administration of different drugs. In U937-TR, apparent oncoprotein degradation could be elicited by ATRA rather than ATO (Figure 2A). While in U937-PR, both ATRA and ATO could degrade PR fusion protein (Figure 2B). With increasing doses of ATRA, TR fusion protein could be further degraded, while ATO exerted no effect even with higher doses (Figure 2C). Notably, IFNs demonstrated no obvious protein degradation effect on both TR and PR. The degradation effect on fusion protein elicited by ATRA, ATO and IFNs were summarized in Table 2.

IFNs rather than ATO promote apoptosis robustly in TR-induced leukemia

Next, we examined the effect of IFN on cellular phenotypes of TR and PR-induced leukemia. U937-TR and U937-PR cells were treated with 1000U/mL type I IFNs and IFNγ in vitro. Then the level of apoptosis and differentiation were assessed. In the apoptosis assay, 1μM ATO was added as a control with known efficacy to induce apoptosis in PR[6]. The baseline apoptosis and differentiation level of U937 cells with and without Dox (±dox) were also tested. Figure 3A and Figure 3B presented the apoptosis level of U937-TR and U937-PR treated with different drugs, respectively. As evidenced, ATO significantly triggered apoptosis in U937-PR, which was consistent with previously reported studies, while U937-TR did not respond to ATO. Single-agent type I IFNs and IFNγ could both induce apoptosis in U937-TR and U937-PR cells, while the pro-apoptotic effect of type I IFNs and IFNγ were more significant in U937-TR compared with that of U937-PR. It is worth mentioning that an increase in the baseline apoptosis level was observed in U937-PR after dox induction, which was not seen in TR, implying the tendency of TR expression towards apoptosis suppression. Representative flow cytometric graphs are shown in Supplementary Figure 1A.

IFNs synergize with ATRA to induce differentiation in TR-induced leukemia

In the differentiation assay, we added ATRA (100nM and 1μM) as positive controls for differentiation induction, with or without the combination of IFNs. CD11b expression of U937-TR and U937-PR administered with different drug are shown in Figure 3C and Figure 3D, respectively. Single drug of either type I IFNs or IFNγ had no obvious pro-differentiation effect on U937-TR and U937-PR. However, in U937-TR with dox induction, IFNs synergized with 100nM ATRA to induce differentiation, demonstrating a differentiation level comparable to 1μM ATRA. Representative flow cytometry graphs are shown in Supplementary Figure 1B.

ATRA and type I IFNs impair colony formation capacity in TR murine cells

To further decipher the role of IFNs in TR-induced leukemia, bone marrow cells obtained from TR mice were treated with different drugs for serial CFAs. In the first-generation (Figure 4A), we found the colony-forming capacity of myeloid leukemia cells derived from TR mice was further impaired with increasing doses of ATRA, 1μM ATRA showed the least colony formation units (CFU). Though ATO elicited certain reduction on the number of colonies, the size of colonies was greater. Type I IFNs reduced the colony formation, yet surprisingly, IFNγ significantly increased the number and size of leukemic colonies in TR
mice. Typical images of first-generation colonies are shown in Figure 4A (right panel). Cells were then collected from groups with significant decrease of CFU as well as control group in first-generation, and the second- and third-generation CFA were performed (Figure 4B). Consistent with results in first-generation, TR mouse leukemia cells treated with 1μM ATRA maintained weakened colony formation capacity. In contrast, ATO-treated group showed a significant increase in both the number and size of colonies. Type I IFNs significantly inhibited colony formation capacity in the serial plating assays. Representative images of second- and third-generations of colonies are shown in Figure 4C.

Targeting IFN pathway confers survival benefit in TR mice

Finally, in vivo experiments were carried out in TR mice. Since we found increasing doses of ATRA promoted oncoprotein degradation, cell differentiation and colony formation ability in TR leukemic cells, we first challenged TR mice with different doses of ATRA in vivo. Clinically, 45mg/m\(^2\) ATRA are used to treat APL patients, which is equal to 14.8mg/kg in mice converted by body surface area (BSA)[7]. In our previous studies, we found 2.5mg/kg and 12.5mg/kg ATRA failed to benefit TR mice[4]. Hence, in this study we selected 15mg/kg ATRA (RA\(^{\text{Low}}\)), the human equivalent dose (HED) as the lowest dose, with 25mg/kg (RA\(^{\text{Medium}}\)) and 50mg/kg ATRA (RA\(^{\text{High}}\)) included as the middle and high dose treatment groups. Considering single-drug IFNs could neither promote cell differentiation nor degrade oncoprotein in TR-induced leukemia, single agent of IFNs group were not include in the treatment regimen. Instead, 5000U IFNs combining with RA\(^{\text{Low}}\) were added to identify the synergistic efficacy of IFNs and ATRA. TR mice were administered subcutaneously on day 7 after transplantation for seven days. The schematic diagram of treatment regimens is shown in Figure 5A.

Body weight of each group was examined 14 days post treatment (Figure 5C). TR mice in RA\(^{\text{High}}\) group experienced robust weight loss and began to die 14 days post treatment, possibly due to toxicity. As is shown in survival curves, on the one hand, both RA\(^{\text{Low}}\) and RA\(^{\text{Medium}}\) group significantly prolonged the survival of mice compared to the DMSO group (Figure 5D). On the other hand, though the combinational regimen of RA\(^{\text{Low}}\) and 5000U type I IFNs conferred certain survival benefit, there was no significant advantage over single-agent RA\(^{\text{Low}}\) (Figure 5E), which may be related to the dose of IFNs administered. As predicted, IFN\(\gamma\) combining with RA\(^{\text{Low}}\) failed to display survival benefit, consistent with results of in vitro CFAs, suggesting that IFN\(\gamma\) may impart aberrant increase in self-renewal capacity of TR leukemia cells.

To determine whether increasing doses of IFNs could further benefit TR mice, the administration of RA\(^{\text{Low}}\) with 10000U tpe I IFNs were challenged in the second-round in vivo assays. Recent studies have unveiled the role of stimulator of IFN genes (STING) as the upstream trigger to product type I IFNs[8], therefore we added single dose of mouse STING activator DMXAA intraperitoneally on day 7 after transplantation (Figure 5B). DMXAA group showed great weight loss 14 days post treatment (Figure 5F), which recovered spontaneously another one week later (Figure 5G). It was reported that DMXAA injection in influenza mouse models could induce significant weight loss and followed by gradual recovery[9], which was similar to what we observed in TR leukemia mice. As is shown in Figure 5H, increasing the dose of type I
IFNs to 10000U combining with RA_{low} exhibited a survival advantage, while there was still no statistically significant difference from single-agent ATRA. Encouragingly, single agent of DMXAA could significantly prolong the survival with delayed onset of leukemia in TR mice, implying its role as a potential therapeutic target in TR-driven leukemia.

**Discussion**

Despite the textbook rationale of ATRA and ATO has turned most APL patients with PR fusion gene from highly fatal to highly curable\(^\text{[10]}\), TR-driven APL did not yield long-term remission both in murine models and patients, which underpins the necessity to dissect the mechanism of therapy unresponsiveness and explore novel strategies. In this study, we found IFN pathway was specifically attenuated in TR- rather than PR-induced APL. When ATO failed to initiate oncoprotein degradation and loss of self-renewal in TR-induced APL, type I IFNs impaired colony formation capacity and conferred potential survival benefit in TR murine models. Based on previous findings, this study further elaborated the variability in drug responses imposed by the canonical PR and the rare TR fusion gene, attempted potential remedies targeting IFN pathway specifically downregulated in TR-driven APL, as well as proposed possible underlying mechanisms.

In our study, we first established in vitro doxycycline-inducible leukemia cell models to identify the molecular pathway directly regulated by TR fusion gene, which was confirmed to be reversible, flexible and reproducible\(^\text{[11]}\). With transcriptomic sequencing analysis, IFN pathway was found to be substantially suppressed by TR fusion gene, indicating an oncogene-driven specific regulatory mechanism. Then a series of in vitro and in vivo assays were utilized to confirm and compare drug responses between TR- and PR-induced APL, yielding major observations summarized as follows. First, ATO is unresponsive in TR-driven APL, which failed to elicit oncoprotein degradation, apoptosis and loss of self-renewal. Second, lower doses of ATRA are inadequate in TR-driven APL. Although a low dose of ATRA was sufficient to trigger differentiation, increasing doses of ATRA further promoted the degradation of oncoprotein and reduced the colony formation capacity of TR leukemic cells, which ultimately conferred survival benefits. Third, type I IFNs is promising in TR-driven APL. Although both type I IFNs and IFN\(_\gamma\) had dismal effect on U937 cell itself without dox induction, which may be associated with the endogenous inhibited state of IFN pathway in U937 leukemia cells, the expression of TR and PR fusion protein did increase the sensitivity of U937 cells to the pro-apoptotic effect of type I IFNs and IFN\(_\gamma\). In addition to apoptosis promotion, type I IFNs induced apoptosis, cooperated with ATRA to boost differentiation, and exhibited potential to reduce self-renewal, reflecting anti-leukemia efficacy. Last but not least, type I IFNs combining with ATRA displayed survival benefit to TR mice. Intriguingly, murine STING agonist DMXAA, which acted upstream to produce type I IFNs, significantly endowed TR mice with extended survival time.

Canonically, mechanisms of leukemogenesis in PR-driven APL are based on differentiation blockage and self-renewal enhancement, with the latter mainly attributed to p53 block triggered by PML nuclear bodies (NBs) disruption, which is due to the binding of PR fusion protein to wild-type PML\(^\text{[12]}\). Clinically, single
agent of ATO could lead to disease cure in 70% of patients with PR-driven APL[13]. The key to the potent efficacy of ATO resides in its selective binding to PML, degradation of PR fusion protein as well as restoration and reformation of PML NBs, which subsequently reactivates p53 signal and impairs self-renewal[14]. In TR-driven APL, ATO failed to attenuate self-renewal capacity, which was attributed, at least in part, to the failure of oncoprotein degradation. Besides, previous studies showed ATRA/ATO could not bring survival benefit in Pml<sup>−/−</sup> APL mice, suggesting the association between drug resistance and PML expression[14]. According to our results, the expression of TR did downregulate the expression of PML protein (data not shown), potentially pointing to the resistance of ATRA/ATO. Conversely, studies have found that IFNs could transcriptionally induce the expression of PML[15], which usually acts as a tumor-suppressor[16], implying the efficacy of type I IFNs in TR-driven APL.

IFNs are a class of cytokines with antiproliferative and immunomodulatory effects. type I IFNs mainly include IFNα and IFNβ, while type II IFNs are specifically denoted as IFNγ. Loss of type I IFN signal induces an immunosuppressive tumor microenvironment (TME) and promotes tumor development, dissemination and metastasis. Treatment with IFNs or IFN activators can exert anti-tumor efficacy by restoring anti-tumor immunity or directly inhibiting the proliferation of tumor cells[17]. Some studies found IFNs could impact the biological function of hematopoietic stem and progenitor cells[18, 19]. Our results showed that type I IFNs conferred impaired serial colony formation ability of TR leukemic cells, as well as survival benefit on TR mice, inferring the role of type I IFNs in blunting self-renewal capacity of TR leukemic cells. As an important upstream regulator of type I IFNs[8], cGAS/STING pathway regulates innate immune response against tumor cell clearance. Some tumors can evade the immune response mediated by this signaling pathway, with abnormal cGAS/STING signaling involved in oncogenesis and metastasis[20]. STING-IFN signaling axis affects tumor progression and drug efficacy mainly by modulating TME[21]. One recent study revealed that the silencing of type I IFN signaling in AML may be related to STING inactivation[22]. In this study, though the knockout of the type I IFN receptor (IFNAR) in leukemic mice accelerated the onset of disease instead of survival time, STING activator significantly prolonged survival in leukemic mice, which was due to STING-triggered production of other cytokines beyond IFNs, thereby exerting stronger anti-leukemia effect than IFNs. In our study, murine STING activator displayed survival benefit with a single dose administration, supporting its role as a potential agent against leukemia cells.

Based on above analysis, we propose the following mechanistic hypothesis of TR-driven APL in comparison with the classic PR-driven APL (Fig. 6). In TR-driven APL, despite the unresponsiveness of ATO, we may utilize STING-IFN pathway agonist to upregulate PML, form PML NBs and activate p53 signal, which may obliterate self-renewal independent of ATO response based on initial NBs disruption, providing therapeutic implications for APL driven by rare RARα fusion genes.

**Abbreviations**

APL  
acute promyelocytic leukemia
AML  
acute myeloid leukemia  
PR  
PML-RARα  
TR  
TBLR1-RARα  
ATRA  
all-trans-retinoic acid  
ATO  
arSENic trioxide  
Dox  
doxyCycline  
IFN  
interferon  
DEG  
differential expression genes  
GO  
gene ontology  
IPA  
ingenuity pathway analysis  
CFA  
colony formation assay  
STING  
stimulator of IFN genes  
NBs  
nuclear bodies

Declarations

Conflict-of-interest: The authors declare no competing financial interests.

Availability of data and materials: Data and materials are available from the corresponding author upon reasonable request.

Funding statement: This work was supported by grants from the National Natural Science Foundation of China (81830005) and the National Key Research and Development Program (2021YFC2500300)

Conflict of interest disclosure: The authors declare no competing financial interests.

Ethics approval statement: Not applicable.
**Author contributions:** XY performed all experiments and wrote the manuscript; HYX helped with in vivo experiments; SWQ participated in the analysis of data; KJT and ZT. provided the study material; QR, MW and JXW conceived, designed and supervised the study, reviewed and approved the manuscript.

**References**


### Tables

**Table 1.** List of top 10 ISGs downregulated by TR in comparison with PR
<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Fold change in U937-TR_48h</th>
<th>Fold change in U937-PR_48h</th>
<th>Ratio TR/PR</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFIT1</td>
<td>-3.346</td>
<td>-0.789</td>
<td>4.241</td>
</tr>
<tr>
<td>IFIT3</td>
<td>-3.187</td>
<td>-1.002</td>
<td>3.181</td>
</tr>
<tr>
<td>ISG15</td>
<td>-3.181</td>
<td>-1.026</td>
<td>3.100</td>
</tr>
<tr>
<td>OAS1</td>
<td>-2.971</td>
<td>-0.355</td>
<td>8.369</td>
</tr>
<tr>
<td>IFITM1</td>
<td>-2.728</td>
<td>-0.891</td>
<td>3.062</td>
</tr>
<tr>
<td>IFI6</td>
<td>-2.540</td>
<td>-1.109</td>
<td>2.290</td>
</tr>
<tr>
<td>IFI35</td>
<td>-2.221</td>
<td>-0.306</td>
<td>7.258</td>
</tr>
<tr>
<td>STAT1</td>
<td>-1.604</td>
<td>-0.454</td>
<td>3.533</td>
</tr>
<tr>
<td>IRF9</td>
<td>-1.570</td>
<td>-0.140</td>
<td>11.214</td>
</tr>
<tr>
<td>STAT2</td>
<td>-1.354</td>
<td>-0.248</td>
<td>5.460</td>
</tr>
</tbody>
</table>

Table 2. Summary of protein degradation elicited by ATRA, ATO and IFNs

<table>
<thead>
<tr>
<th>Protein</th>
<th>ATRA</th>
<th>ATO</th>
<th>IFN-I/II</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBLR1-RARα</td>
<td>↓</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>PML-RARα</td>
<td>↓</td>
<td>↓</td>
<td>ns</td>
</tr>
</tbody>
</table>

Figures
Figure 1

Establishment of inducible leukemia cell lines and transcriptomic analyses

(A) Structure of Tet-On advanced system and doxycycline-controlled regulation of RARα fusion protein. (B) TBLR1-RARα(TR) and PML-RARα(PR) expression detected by Western blot in U937 cells with or without doxycycline induction. (C-D) Volcano plots of overall gene expression in U937-TR(C) and U937-PR(D) compared to controls.
PR(D) with 48-hour doxycycline induction. Significant upregulated and downregulated DEGs were shown in red and green, respectively. (E-F) GO enrichment of DEGs in U937-TR (E) and U937-PR (F) were analyzed by ClusterProfiler, exhibiting the Top 5 significant GO terms. The bar chart represents significance of gene enrichment for GO term. The orange lines indicate the percentage of enrichment ratio. (G) Pathway analysis was performed on downregulated genes of U937-TR by IPA, displaying enrichment in both Type-I (IFN-α/β) and Type-II (IFNγ) IFN pathways.

![Figure 2](image)

**Figure 2**

**ATRA, ATO and IFN elicit distinct responses to TR and PR in terms of oncoprotein degradation**

(A-B) Oncoprotein degradation of TR(A) and PR(B) elicited by ATRA, ATO, type I IFNs and IFNγ in U937-TR and U937-PR. Each sample was normalized to β-actin intensity. RI was quantified with Image J and calculated against control group (normalized intensity=1). (C) The expression of TR fusion protein with increasing doses of ATRA and ATO.
Figure 3

In vitro apoptosis and differentiation assays in U937-TR and U937-PR with ATRA, ATO and IFN treatment

(A-B) The apoptosis level of U937-TR(A) and U937-PR(B) administered with different drugs.

(C-D) The differentiation level of U937-TR(C) and U937-PR(D) administered with different drugs. All experiments were done in triplicates and significance was indicated with asterisks (*P<0.05; **P<0.01; ***P< 0.001).
Figure 4

Colony formation capacity of TR murine cells with ATRA, ATO and IFN treatment

(A) First-generation colony formation assay performed in TR murine leukemia cells with different drugs. Colonies were counted and displayed by bar plot (left panel). Typical images of first-generation colonies are shown in Figure 4A (right panel). (B) A summary of colonies counted in serial plating. Assays were
done in triplicates and significance was indicated with asterisks (*P<0.05; **P<0.01; ***P<0.001). (C) Representative images of second- and third-generations of colonies.

Figure 5

**In vivo treatment of TR mice**

(A) Schematic diagram of transplantation and treatment schedule with ATRA and IFNs. $\text{RA}^{\text{Low, Medium, High}}$ refers to 15mg/kg, 25mg/kg and 50mg/kg, respectively. SC, subcutaneous. (B) Schematic diagram of transplantation and treatment schedule with $\text{RA}^{\text{Low}}$, increasing dose of type I IFN(IFN-I) and DMXAA. IP, intraperitoneal administration. (C-E) Body weight (C) and survival plot (D-E) of TR mice with treatment listed in schedule (A). (F-H) Body weight (F-G) and survival plot (H) of TR mice with treatment listed in schedule (B).
Figure 6

Mechanistic hypothesis of TR-driven APL in comparison with the PR-driven APL

In PR-driven APL, in addition to terminal differentiation elicited by ATRA, oncprotein degradation is orchestrated by higher doses of ATRA and ATO. Then PR-triggered PML NBs disruption are reformed and enhanced by ATO, with the downstream p53 pathway activated, which ultimately lead to loss of self-renewal and disease cure (upper panel). In TR-driven APL, only ATRA rather than ATO could induce differentiation and oncprotein degradation, which fails to entail definitive cure. It is hypothesized that type I IFNs and its upstream activator STING may promote PML NBs formation by upregulating the expression of PML, which then initiates p53-mediated loss-of-self-renewal and brings survival benefit (lower panel).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigureS1.docx