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MECHANISM-BASED THERAPY FOR LEUKEMIA: A LESSON FROM ATRA THERAPY

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ABSTRACT

In the past two decades, there has been a tremendous increase in our understanding of the molecular mechanism of human leukemias. Leukemias are now recognized as a deregulated state of cell proliferation, differentiation and apoptosis, which is induced by gene alterations, including chromosomal translocations. Many of the mechanisms are potentially exploited as new targets for drug development. All-*trans* retinoic acid therapy for acute promyelocytic leukemia, which was initially developed as a differentiation therapy in an experienced-based manner, is currently known to be the first successful oncoprotein-directed therapy. Basic and clinical research into ATRA-resistance provides new directions for acute myeloid leukemia therapy. Anti-leukemia therapy will continue to lead the field of chemotherapy in the coming decades.

INTRODUCTION

Our comprehension of the molecular biology and pathophysiology of leukemia has advanced tremendously over past two decades. Non-random chromosomal translocations, found in nearly half of all leukemia cases, are closely linked to leukemogenesis.[1)-3)] Some of the oncogenes and anti-oncogenes found in solid tumors are also associated with leukemia.[4)-6)] These gene alterations cause deregulated states of proliferation, differentiation and apoptosis, resulting in malignant hematopoiesis. Clinically, the molecular alterations are used as markers for diagnosis, detection of minimal residual disease and prediction of prognosis.[7),8)]

On the other hand, therapeutic advances in leukemia have been independent of the above. Not only the chemotherapeutic concept, but also most anti-leukemia agents were established more than 20 years ago. Dose-escalation, modification of the schedule, and combinations of the agents were developed as treatments for leukemia.[9),10)] In childhood acute lymphoblastic leukemia (ALL), over 80% patients are now cured.[11)] However, the prognosis of adult patients with acute leukemia has not significantly improved over the last decade.[12),13)]

Notably, the combination of all-*trans* retinoic acid (ATRA) increased the complete remission rate and the number of long-term survivors.[14)] The clinical use of ATRA for APL was first reported by the Shanghai group,[15)] and was developed in an experience-based manner. The molecular studies later showed that ATRA directly targets the PML-RARα oncoprotein generated by t(15;17) and modulates its function, resulting in differentiation and extinction of APL cells.[16)] This clinical success emphasized the importance of developing new anti-leukemic therapy based on a different concept. Here, the author revisits ATRA therapy, and reviews the future directions of post-ATRA therapy.

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PARADIGM SHIFT IN THE TREATMENT OF APL

In 1988, the Shanghai group reported the first clinical study of ATRA in 23 patients with APL. This treatment induced differentiation of blasts without bone marrow hypoplasia, followed by complete remission in 96% of the patients.[15] The effectiveness of ATRA therapy was confirmed in France[17] and Japan.[14] In the USA, a prospective randomized study showed a definitive advantage of ATRA-combined therapy.[18] However, it was unknown why ATRA was specifically effective against APL, although ATRA was known to be a non-specific inducer of differentiation. In 1990, two groups independently cloned the APL-specific chromosomal translocation t(15;17).[19),20] The translocation generates a chimeric gene between the PML gene on chromosome 15 and the retinoic acid receptor α (RARα) gene on chromosome 17. It was soon believed that the resultant PML-RARα products influenced both the RARα and PML functions in a dominant negative manner.[21),22] Although the role of RARα in myeloid differentiation remained unclear at this time, PML-RARα was thought to block differentiation.[16] Actually the above function of PML-RARα was confirmed by molecular studies *in vitro* and *in vivo*.[23)-25] Then, the next question was why pharmacological concentrations of ATRA relieved the dominant negative function of PML-RARα. Since PML-RARα has a retinoic acid (RA)-binding region, ATRA was thought to directly bind to PML-RARα and modulate its function. Two important findings have accounted for the mechanism of modulation; First, immunohistochemical studies of PML showed that PML-RARα and PML are localized in diffuse microgranular patterns in the nucleus and cytoplasm.[26] This localization is restored to a nuclear microspeckled pattern by ATRA, which is caused by the degradation of PML-RARα.[27] Second, biochemical studies indicated that PML-RARα recruits a co-repressor complex including histone deacetylases (HDACs), which represses ATRA-dependent transcription.[28),29] At pharmacological concentrations of ATRA, PML-RARα binds the co-activator complex including CBP/p300 instead of the co-repressor complex, and undergoes transcription.[28),29] These two mechanisms seem to cooperatively relieve the dominant negative character of PML-RARα.

RETINOIDS-RESISTANCE IN APL

As the clinical usefulness of ATRA-therapy was confirmed, limitations of the sole or second ATRA-therapy became evident. If APL is treated by ATRA alone, the treated APL easily gains resistance to ATRA.[30] The mechanism of the ATRA-resistance *in vivo* remains to be clarified. The major reason is reportedly the altered pharmaco-kinetics of ATRA; decreased absorption from the gastro-intestinal tract, sequestration in liver or other organs due to induction of cellular retinoic acid-binding protein-II, and rapid oxidation of ATRA by the p450 system.[30] Notably, mutations of PML-RARα transcripts within its ligand-binding domain have been reported in relapsed APL,[31),32] although the cases are infrequent. Similar mutations have been also found in ATRA-resistant APL cell lines established *in vitro*.[33),34] Several different reported point mutations are localized in the E-domain, a ligand-binding domain, which is similar to ATRA-resistant HL 60 cells. Importantly, these mutations cluster at two particular regions within the E-domain. The crystal structure model of the RAR family suggests that these two cluster regions surround the ligand in the ligand-binding form. Studies of ATRA-resistance clarified that ATRA directly targets PML-RARα. Importantly, it should be noted that the mechanism of resistance in molecule-targeted therapy might be different from that in conventional chemotherapy, represented by multi-drug resistance.

TREATMENT OF ATRA-RESISTANT APL

ATRA-resistant APL has stimulated research for additional therapies. One approach is arsenic trioxide (As_2O_3). Clinical use of As_2O_3 for APL began in North Eastern China in 1971,[35] and was introduced to the world in 1997. *In vitro* experiments support the clinical efficacy of As_2O_3.[36] That is, treatment with 1 μM As_2O_3, a concentration that is clinically achievable, induces apoptosis in an APL cell line.[37] A lower concentration of As_2O_3 causes morphological and immunophenotypic changes, although it does not induce terminal differentiation.[38]

Since As_2O_3 has been proven effective against APL with t(15;17) *in vivo*, PML-RARα was first speculated to be associated with the sensitivity to As_2O_3. Furthermore, PML-RARα is down-regulated by As_2O_3 more rapidly than by ATRA.[37] However, degradation of PML-RARα and changes in PML-subcellular localization were similarly induced by As_2O_3 in As_2O_3-sensitive and -resistant APL cell lines, suggesting that their contribution to apoptosis is small.[39] As_2O_3-treatment activated caspase 8 in a CD95-independent manner, but reduced glutathione concentration-dependently, which is different from the ATRA pathway.[39]

Another approach is a histone deacetylase inhibitor. The acetylated and deacetylated histones are regarded as the key machinery of transcriptional activation and repression, respectively.[40] The p300/CBP and other coactivators have histone acetyltransferase activity associated with transcription.[41] Many investigators have also shown that histone deacetylases (HDACs) interact with inactive and/or non-liganded transcription factors via co-repressors such as mSin3A, N-CoR and SMRT to repress transcription in mammalian cells.[42),43] On the basis of molecular background, HDAC inhibitors (HDACI) such as butyrate, trichostatin A (TSA) and trapoxin A (TPX) were shown to block the repression, resulting in transcription of the target genes.[44] Recently, three independent groups demonstrated *in vitro* that HDACI blocked the repression by PML-RARα. The combined therapy using ATRA and HDACI is effective in inducing differentiation in ATRA-resistant APL cells and cell lines.[28),29] Thus HDACI is considered as a promising agent for "differentiation therapy" in APL.

CHIMERIC TRANSCRIPTIONAL FACTORS

Differentiation therapy is theoretically applicable to all types of AML, because differentiation-block is one of the most important pathophysiological events in AML. Recent studies showed that chimeric transcriptional factors, generated by chromosomal translocations, are frequently associated with a differentiation-block. For example, in t(8;21), the fusion protein AML1-ETO recruits a co-repressor/HDAC complex. HDACs are also responsible for transformation by AML1-ETO, suggesting that HDAC is a common target for myeloid leukemias.[42),43] Strikingly, AML1-ETO expression blocks retinoic acid (RA) signaling in myelopoiesis.[45] Accordingly, activation of the RA signaling pathway and inhibition of HDAC activity might represent a general strategy for differentiation induction in AML.

FUTURE DIRECTIONS

In the development of mechanism-based therapy, one of the most important issues is how to predict clinical efficacy. So far, leukemia cell lines have been used for screening chemotherapeutic agents. However, there are significant differences between *in vitro* and *in vivo* activities Transplanted or genetically modified mouse models of human leukemia are important not only for elucidating the mechanism of leukemia, but also for evaluating the *in vivo* efficacy.

After the success of ATRA, encouraging results emerged in the treatment of chronic myeloid leukemia. The effectiveness of a tyrosine kinase inhibitor (STI571), developed to inhibit abl kinase, was seen on clinical studies.[46] This is the first example of a medicine developed in a mechanism-based and molecule-directed manner. The author believes that anti-leukemia therapy, which has led the field of chemotherapy from the middle of the 20th century onwards, will continue to be at the forefront of advances in cancer treatment.

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