Retinoid X receptor signaling pathway in leukemia

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[Abstract] Retinoid X receptor (RXR) acts as ligand-dependent transcription factors playing an important role in regulating a serial of physiological processes, such as embryo development and organ homeostasis. At the molecular level, RXRs exert their functions by inter-activating with multiple signal pathways to regulate target gene expression which control cell growth, differentiation, survival and death. The interference in the network of RXR and other signal pathways has turned RXR into an attractive drug target.

[Key words] Retinoid X receptor; Signal pathway; Leukemia; Bone marrow transplantation

Retinoic acid (RA) exerts their effects through their binding and activation of specific nuclear receptors, retinoid X receptors (RXRA, RXRB, RXRG) and retinoic acid receptors (RARα, RARβ, RARγ), which function as ligand-dependent transcription factors by binding, as homodimers or heterodimers, to specific hormone response elements, retinoid X response elements (RXRE) and retinoic acid response elements (RARE), in the promoters of retinoic acid-mediated target genes. The retinoid receptors are considered to be ligand-activated, deoxyribonucleic acid (DNA) binding, trans-acting, transcription-modulating nuclear proteins involved in a general molecular mechanism responsible for mutual interaction with other pathways by regulating gene networks that control cell growth, differentiation, survival and apoptosis. Among them, RXRs play a central role as nuclear transcription factors by homodimerization or through heterodimerization with many other members of the retinoid and steroid receptor family of transcription factors, such as vitamin D receptor (VDR), thyroid hormone receptor (TR), peroxisome proliferator activated receptor (PPAR), and orphan receptors, thereby modulating multiple signaling pathways involved in many developmental and metabolic processes. It has been demonstrated that RXRA and its interaction with the X-RARα fusion proteins including PML-RARα, PLZF-RARα, STAT5b-RARα, and R1A-RARα are essential for the oncogenic activity of their respective APL fusion proteins. The chemotherapy response is different depending on respective APL protein. As for APL patients, it is very important to evaluate the chemotherapy response and know whether the patient would benefit from the bone marrow transplantation. Here we introduced the RXR signaling pathways in leukemia (Figure 1).

1 RARα/RXR heterodimer regulate retinoid target gene transcription in APL

Acute promyelocytic leukemia (APL) is a rare disease accounting for approximately 10% of acute myeloid leukemia (AML). In over 95% of APL cases, the origin of APL is a t(15;17) (q22; q21) chromosomal translocation that fuses the promyelocytic leukemia gene PML and the RARα gene. In rare variant cases, alternative chromosomal translocations generate RARα fusion proteins in which PML is replaced with PLZF, NuMA, NPM, STAT5b, PRKAR1A respectively. As the most common fusion protein in APL, PML-RARα homodimerizes with themselves and heterodimerizes with RXRs to form a transcriptional complex which binds to DNA sequences and recruits the transcriptional corepressor, silencing mediator for retinoid and thyroid hormone receptors (SMRT), and also recruits histone deacetylases (HDAC) and DNA methyl transferases, which modify histone and DNA to repress transcription. This whole complex mediates the myeloid differentiation block that leads to APL. In other rare variant cases, such as NuMA-RARα, NPM-RARα, STAT5b-RARα, PRKAR1A-RARα, XR-RARα heterodimer were proved to play an important role in regulating target gene transcription. A molecular mechanism by which RXR-RAR heterodimers act as ligand-dependent transcriptional regulators by binding to the

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Cyclic adenosine monophosphate (cAMP) - protein kinase A (PKA) signal pathway activation induce leukemia cell apoptosis and differentiation. Janus kinase-signal transducer and activator of transcription (JAK-STAT) 3 signal pathway induce leukemia cells apoptosis. Activate MEK pathway show different effect on gene transcription mediated by RXRE.

**Fig. 1 Simplified diagram of the RXR/RAR signaling network**

Specific RARE DNA sequences to regulate transcription of target genes has been proposed[13]. In the absence of retinoic acid or RAR agonist, the RXR-RAR heterodimer recruits the corepressor proteins NCoR or SMRT and associated factors such as HDAC or DNA methyl transferases that may lead to an inactive condensed chromatin structure, preventing target gene transcription. Upon retinoic acid or RAR agonist binding, corepressors are released, and coactivator complexes such as histone acetyltransferases or histone arginine methyl transferases are recruited to activate target gene transcription[14-15]. The treatment of APL patients with all-trans retinoic acid (ATRA) result in recruiting coactivator complexes to induce retinoic acid target gene transcription and causing the malignant cells to differentiate towards mature granulocytes, which, combined with anthracyclins, cures 70%-80% of patients with APL[16]. In APL cells and patient’s blasts, RAR agonists induce not only differentiation but also activation a death-signaling pathway. These two pathways may include by the ability of RA to induce both pro-apoptotic and anti-apoptotic program. Altucci et al[17-18] showed that RARα-reactive retinoids induce both early and late anti-apoptotic signaling pathways to allow expression of the differentiated phenotype, and that the induction of the tumor-selective death agonist TRAIL (tumor necrosis factor-related apoptosis-inducing ligand), which has features of tumor suppressors, is the cause of post-maturation NB4-cell apoptosis.

Moreover, researchers are interested in the specific effects of RAR-RXR heteromeric complexes on APL pathogenesis. Zeisig et al[9] focused on a rare RARα fusion protein, STAT5b-RARα. They indicated that intrinsic homo-oligomeric DNA binding is not crucial for transformation by RARα fusion proteins *in vitro*. Heterodimerization with RXRα was essential for efficient DNA binding, and for interactions with chromatin modifiers that mediate RARα fusion protein suppression of RARα target gene expression. Furthermore, disruption of RXRα function with RXRα short hairpin ribonucleic acid (shRNA) or a RXRα agonist, SR11237, suppresses STAT5b-RARα fusion protein transformation. They concluded that recruitment of RXR by homotetrameric RARα fusion proteins is essential for transformation. Zhu et al[19] interested in more common fusion between PML and RARα. They made a PML-RARα mutant which could not form heterodimer with RXRα, but could still form homo-oligomeric complexes. They found that this mutant defective for RXR binding failed to trigger APL development in transgenic mice, but it still transformed primary hematopoietic progenitors *ex vivo*. They explained that in PML/RARA-expressing cells, RXR underwent a specific modification, sumoylation, which could further enhance transcriptional repression and contributes to several critical aspects of *in vivo* transformation. To identify the impact of RXR heterodimer with PRKAR1A-RARα on APL development, we introduced of point mutations within the RARα portion of R1A-RARα, previously demonstrated to eliminate RXRα interaction or treatment of
transduced cells with RXRα shRNA or a RXRα agonist, SR11237, reduced transformation capability of transduced cells. Thus, leukemic transformation by APL fusion protein PRKAR1A-RARα is critically dependent on RXRα. Therefore, drugs that target RXRα or that can reduce RARα fusion protein complexes to homodimers are potential future treatments for APL. Interestingly, Tanaka et al. have shown that high ligand-activated RAR upregulates p21 expression at the transcription level via RXR homodimer binding to the two consecutive RXRE in the p21 promoter region, leading to an induction of cell cycle arrest, followed by apoptosis. They further pointed out the RAR to RXR ratio may be a determinant of effectiveness of retinoid in p21 transcription and tumor suppression, i.e., high RXR to RAR expression level is required for p21 induction and high RAR to RXR to prevent p21 expression and lead to apoptosis. They also showed that the different RXR subcellular localization is another mechanism leading to retinoid resistance. However, molecules that have RAR, RXR selectivity, and specific agonist or antagonist features were designed with a focus on anticancer and metabolic diseases therapy. The corresponding challenges and opportunities of RXR target therapy have been evaluated.

2 Activation retinoid-cAMP signal crosstalk inducing leukemia cell differentiation

As one of the second messengers, cyclic adenosine monophosphate (cAMP) plays an important role in cell proliferation, differentiation and apoptosis. cAMP also differentiates many AML cell lines and cooperates with other differentiating agents, such as ATRA, As2O3. cAMP greatly enhances the RA-induced differentiation of many cell lines derived from embryonal carcinoma or myeloid leukemia, including APL. In APL cells, cAMP can increase the concentrations of RA required for differentiation. A rapid increase in cAMP levels and protein kinase A (PKA) activities were observed during ATRA-induced NB4 cell differentiation, but no such effect in NB4-R1 cells, suggesting that retinoid-cAMP crosstalk is essential for RA-induced differentiation. Combination of RXR and PKA agonists efficiently induced AML cell lines (HL60, PLB985, U937, NB4, and NB4-R2) differentiation, growth arrest, and apoptosis. AML patients’ blasts responded to retinoid-cAMP combination treatment with induction of maturation and apoptosis. Elevated cAMP levels, RXR-selective agonists differentiate and kill blasts derived from AML patients with retinoic acid-insensitive disease. PLZF-RARα-positive blasts and non-AML AML that are normally unresponsive to retinoids, fully differentiate and apoptosis upon RXR-PKA treatment. Except for the induction differentiation effect of activation retinoid-cAMP crosstalk on cell lines or AML patients’ blasts in vitro, identical results were also observed in vivo. Using an animal model of APL derived from PML/RARα transgenic mice, cAMP induces major cell growth arrest together with differentiation and cooperates with both As2O3 and RA to clear RA-sensitive or RA resistant APL. Moreover, addition of theophylline, an inhibitor of cAMP intracellular degradation, induced these two APL model clinical remissions.

At the molecular level, raising cyclic AMP levels allows RXR-selective ligands to induce differentiation of RA-resistant APL cells. cAMP activates PKA, which dissociates RARα from the SMRT co-repressor, allowing transcriptional activation by retinoid-induced recruitment of the co-activator complex. Retinoid-PKA induces cell surface expression of TRAIL receptor DR5 and caspase-dependent cell death, which is superinduced by TRAIL. Therefore, the crosstalk between the RAR-RXR and cAMP-activated pathways could lead to differentiation and activation of the TRAIL pathway. This crosstalk was also considered to enhanced APL cells differentiation and maturation by combining RA with a PDE4 inhibitor, or theophylline which by clinical data from a relapsed RA and arsene-resistant patient who has treatment response to theophylline.

It is well known that RARα could be phosphorylated by cAMP-activated PKA on Ser369 by cdk7-cyclin H. Retinoic acid resistance of PML-RARαS873A (corresponding to S69A) APL implies that, cAMP-induced or retinoic acid-induced phosphorylation of Ser873 is crucial for the response of APL to retinoic acid and for retinoic acid-induced PML-RARα degradation and leukemia-initiating cells (LiC) clearance both in vitro and in vivo. They also indicated that activation of cAMP signaling enhances LiC loss by retinoic acid. Therefore, phosphorylation controls nuclear receptor signaling in vivo and identifies cAMP signaling as another candidate for targeted APL therapy.

3 JAK-STAT pathway in leukamogenesis

Signal transducers and activators of transcription (STAT) comprise a family of several transcription factors that are activated by a variety of cytokines, hormones and growth factors. The Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway is prominent both in normal hematopoiesis and hematological malignancies. STATs are activated through tyrosine phosphorylation, mainly by JAK kinases, which lead to their dimerization, nuclear translocation and regulation of target genes expression. STAT proteins mediate cell growth, differentiation, apoptosis, transformation, and other
fundamental cell functions. Recently, mutations in the JAK2 gene driving the proliferation of the neoplastic clone have been identified in myeloproliferative disorders [28-29]. In addition, constitutive activation of the JAK-STAT pathway has been reported in various types of leukemia such as acute myelogenous leukemia, Down syndrome acute lymphoblastic leukemia, T-LGL leukemia, and multiple myeloma [30-31]. Like PML-RARα, STAT5b- RARα binds RAREs both as a homodimer and as a heterodimer with RXRα resulting in the recruitment of corepressor SMRT and inhibition of RARα/RXRα transcriptional activity. STAT5b-RARα and other APL fusion proteins including PML-RARα and PLZF-RARα could enhance STAT3 transcriptional activity, which may contribute to leukemogenesis by interaction with the STAT3 oncogene pathway. It was demonstrated that NuMA-RARα binds RARE as either a homodimer or as a heterodimer with RXRα, inhibits RARα transcriptional activity, augments transcriptional activity of the oncogene STAT3. Thus the activation of STAT3 pathway may be another leukaemogenic mechanism of APL.

4 JNK and ERK pathways influence the expression of RXR in T lymphocyte leukemia cells

As nuclear receptor, phosphorylation of RXRs by kinases facilitates the recruitment of coactivators or components of the transcription complex, and therefore, cooperates with the ligand to influence transcription activation, which may impact their action in certain diseases or cancers [32-35]. Phosphorylated RXRα implicated in the regulation of the cell cycle. Mouse RXRα can be hyperphosphorylated by stress kinases at four additional residues, three being located in the AF-1 domain (S61, S75 and T87) and one (S265) in the AF-2 domain upon activation of e-Jun N-terminal kinase (JNK) [36]. The ligand-binding domain (LBD) can also be phosphorylated at tyrosine residues (Y248 and Y397) by mitogen-activated protein kinase (MAPK) -kinase kinase 4 (MEKK4). Ishaq et al. [37] reported that T lymphocyte activation signals regulate the expression and transactivation function of RXRα through an interplay of complex signaling cascades. Unlike resting immature peripheral blood T (PBT), activation of cycling human mature PBT lymphocytes, and T lymphocyte leukemia cell lines, (Jurkat and SupT13), is accompanied by the accumulation of RXRα messenger ribonucleic acid (mRNA) and protein, which is found to be transcriptionally inactive, and results in the silencing of RXRE-mediated gene transcription. They proved that the activation of JNK pathway inhibits the RXRE-mediated gene transcription, while the activation of ERK pathway is found to increase the RXRE-mediated gene transcription. When both were activated simultaneously, JNK pathway was dominant over ERK pathway and resulted in inhibition of RXRE mediated gene activation. Moreover, they show that cellular Ser/Thr protein phosphatases (PPs) play an important role in the regulation of RXRα expression and RXRα-dependent transcriptional activation in T lymphocytes, and that calcineurin synergizes with protein kinase C (PKCα) θ in regulating RXRE-dependent transcriptional activation, a cooperative function that is antagonized by activated PKCα [38].

5 Summary

RXR participates in multiple signal transduction pathways which relate to leukemogenesis. In addition to application of RA-based therapy in APL, novel therapeutic paradigms originating from interrupting the RXR and other signaling pathways crosstalk act as another exciting pharmacological target for leukemia therapies.

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维 A 酸 X 受体信号通路与白血病

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【摘要】 维 A 酸 X 受体 (RXR) 是配体依赖的转录因子，在调节一系列生理病理过程中起重要作用。在分子水平上，RXR 与多个信号通路相互作用，调控细胞生长、分化、生存与死亡等靶基因的表达。RXR 信号通路与其他信号通路之间的相互作用使 RXR 成为抗肿瘤药物作用的新靶点。本文就 RXR 信号通路在白血病发生发展中的作用进行简要综述。

【关键词】 维 A 酸 X 受体；信号通路；白血病；骨髓移植

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