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WWOX Drives T Leukemia Cell Maturation via IκBα/WWOX/ERK Signal Pathway

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Abstract

Forced differentiation therapy has been shown to be effective in suppressing promyelocytic leukemia cell growth and other types of cancer cells in patients. A combination of calcium ionophore and phorbol ester is effective in inducing terminal maturation of T-cell acute lymphoblastic leukemia (T-ALL), in which it requires the signaling of IκBα/WWOX/ERK. While WWOX is generally regarded as a tumor suppressor, this editorial view addresses the critical role of WWOX phosphorylation at Ser14 and de-phosphorylation at Tyr33 that drives T-ALL maturation.

Keywords: T-ALL; Forced maturation; WWOX; IκBα; ERK

Editorial

Numerous approaches have been developed to force maturation of leukemia cells or the so-called differentiation therapy. Conceptually, a single chemical, or a combination of two or three chemicals, is used to treat patients suffering leukemias. Differentiation therapy has first been successfully utilized to treat acute promyelocytic leukemia (APL). APL occurs as a result of fusion of chromosomes 15 and 17 that generates a fusion gene, designated PML/RARa for promyelocytic leukemia (PML) and retinoic acid receptor alpha (RARa) genes, respectively. The resulting aberrant protein deters myelocytic cell maturation, but leads to an uncontrolled proliferation. All-trans retinoic acid (ATRA) and anthracycline-based chemotherapy, plus arsenic trioxide (ATO), have been used to treat APL [1-3]. Forced maturation by ATRA allows APL cells to reach a terminal differentiation stage, and arsenic trioxide accelerates the cell death. Promising outcomes show that the cure rates are greater than 80%, although there is a risk regarding death rate caused by severe infections and occurrence of secondary leukaemias [1-3]. Differentiation therapy has also been applied to solid tumors. For example, microRNA MiR-148a-mimetic treatment is known to suppress liver cancer growth and liver fibrosis and induce hepatocytic differentiation [4]. The underlying signaling is via the IκB kinase alpha (IKKα)/NUMB/NOTCH signaling [4]. Indeed, NOTCH proteins may block the differentiation effect of ATAR and phorbol ester [5,6]. Furthermore, ATRA restores IKKα expression *in vivo* that forces the differentiation of nasopharyngeal carcinoma cells and decreases tendency in tumorigenesis [5]. Expression of IKKα is low in nasopharyngeal carcinoma cells. This is due to epigenetic suppression by enhancer of zeste homologue 2 (EZH2) [5].

Alterations of oncogenes and tumor suppressors are known to affect thymocyte maturation, and an imbalanced cooperation among these tumor suppressors and oncogene proteins drives the formation of T-cell acute lymphoblastic leukemia (T-ALL). ATRA and ATO have also been used in treating T-ALL and adult T-cell leukemia lymphoma [7,8]. ATAR blocks the activity of c-Jun N-terminal kinase (JNK) and thereby induces growth inhibition of Tax-activated human Jurkat leukemia T cells [7]. Interestingly, combination of ATO, interferon-α and zidovudine shifts the phenotype of adult T-cell leukemia/lymphoma (ATL) from a T(reg)/Th2 cytokine profile toward a normal Th1 phenotype [8]. That is, the microenvironment of local immunocompetent T cells can be restored via forced differentiation. ATO alone can achieve complete remission in treating refractory and relapsed T-ALL patients [9]. Differentiation therapy is feasible in treating both leukemia cells and solid tumors [10]. In addition to ATRA and ATO, numerous chemicals drive terminal differentiation of cancer cells, including histone deacetylase inhibitors (HDACI), PPARγ agonists, active form of vitamin D3, phorbol myristate acetate, hexamethylene-bis-acetamide, transforming growth factor-beta, butyric acid, cAMP and vesnarinone [10,11]. Drug-initiated signaling is critical for the terminal maturation of cancer cells. However, a specific signal pathway for each drug, which is needed for inducing cancer terminal maturation, is largely unknown and has yet to be established.

By utilizing calcium ionophore A23187 and phorbol myristate acetate (IoP), Huang et al. demonstrated the terminal maturation of immature acute lymphoblastic leukemia MOLT-4 T cells in less than 24 h [12]. During the induced maturation process, there are changes in the endogenous complex of inhibitor of nuclear factor κB (IκBα), extracellular signal–regulated kinases (ERK) and WW domain-containing oxidoreductase (WWOX). A portion of the IκBα/ERK/WWOX complex is present in the mitochondria, suggesting its role in the mitochondrial homeostasis. Tyr33 phosphorylation in WWOX is needed for its interaction with ERK and IκBα in MOLT-4, as determined by co-immunoprecipitation, yeast two-hybrid analysis, and time-lapse Förster resonance energy transfer (FRET) microscopy [12]. pY33-WWOX binds and stabilizes IκBα from being degraded by ubiquitination and proteosomal degradation. WWOX binds to the non-PEST area of IκBα. That is, the binding involves the *N*-terminus of IκBα possessing an ankyrin domain and the *N*-terminal Tyr33-

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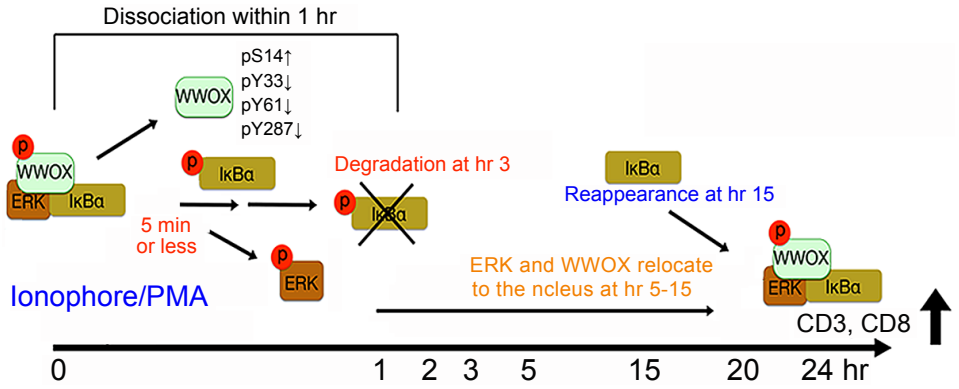

Figure 1: WWOX drives T-ALL maturation. A sequential molecular event is depicted for the dissociation of the WWOX/ERK/IκBα complex during ionophore/PMA (IoP)-induced T-ALL cell maturation. IoP rapidly induces ERK and IκBα phosphorylation of an endogenous IκBα/ERK/pY33-WWOX complex in 5 min or less and meanwhile WWOX undergoes de-phosphorylation at Tyr33 and Tyr287 and phosphorylation at Ser14. WWOX is released from the p-ERK/p-IκBα complex in 1 h, p-IκBα then degraded and ERK phosphorylation reduced, and ERK/WWOX in the nucleus (to induce gene transcription for cell maturation) in 5-15 h. Finally, expression of CD3 and CD8 and reappearance of the IκBα/ERK/WWOX complex occur in the cells in 15-24 h [adapted from reference #12]. (This research was originally published in Journal of Biological Chemistry, by Huang SS, Su WP, Lin HP, Kuo HL, Wei HL, Chang NS, Role of WW Domain-containing Oxidoreductase WWOX in Driving T Cell Acute Lymphoblastic Leukemia Maturation.Year 2016; 291(33):17319-31.(c) the American Society for Biochemistry and Molecular Biology).

phosphorylated first WW domain of WWOX. The domain/domain binding between ERK and WWOX remains to be investigated.

In a sequential reaction, IoP rapidly induces phosphorylation of endogenous ERK and IκBα in 5 min or less, along with WWOX de-phosphorylation at Tyr33 and Tyr287 and phosphorylation at Ser14 in 1-2 h (Figure 1). The de-phosphorylation at Tyr33 in WWOX appears to be critical as WWOX departs from the p-IκBα/p-ERK complex. However, the significance of Ser14 phosphorylation in WWOX is largely unknown. Then, degradation of p-IκBα and de-phosphorylation of ERK starts to occur in the next 3-5 h and the event lasts for the next 12 h. ERK de-phosphorylation causes its dissociation from p-IκBα, which then undergoes ubiquitination and proteosomal degradation. Meanwhile, a portion of WWOX and ERK re-associates and relocates to the nucleus to manipulate gene transcription and expression. When IκBα returns to a normal level, up-regulation of T cell maturation antigens CD3 and CD8 and re-formation of the IκBα/ERK/pY33-WWOX complex occur in 15-24 h (Figure 1). Inhibition of ERK phosphorylation by U0126, or blocking IκBα degradation by MD132, prevents MOLT-4 maturation. The signaling event has been confirmed by a designed time-lapse FRET microscopy for tri-molecular interactions [12]. IoP increases the binding of IκBα/ERK/pY33-WWOX complex by 1-2-fold after exposure for 15-24 h. Meanwhile, a portion of ERK and WWOX relocates to the nucleus, suggesting their role in the induction of CD3 and CD8 expression in MOLT-4.

What's intriguing is that Ser14 phosphorylation of WWOX is up-regulated during forced T-ALL cell maturation. The functional role of this regard remains to be elucidated. Conceivably, designed WWOX peptides with Ser14 phosphorylation may be of therapeutic efficacy in forcing maturation of T-ALL. In addition to its role in tumor suppression, WWOX participates in metabolism, neurodegeneration, ataxia, epilepsy, neural disorders, neuronal damages and interacts with oncogenic viruses [13]. That WWOX drives T-ALL cell maturation would be of great therapeutic considerations in curing the disease.

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