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POSTER ABSTRACTS

617.ACUTE MYELOID LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

Trinary Fusion with Ligand Binding Domain Castration Is Crucial in *Rarg*-Driven APL and Render Unresponsive to ATRA Via Allosteric Disability Mechanism

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Introduction

All-trans retinoic acid (ATRA) has been used with great success in acute promyelocytic leukemia (APL) cases with *PML::RARA* fusion gene (FG). There are still *PML::RARA* negative cases that manifest as APL, and they often carry FGs involved in *RARA*, *RARB*, or *RARG*. *X::RARG* positive APL (*RARG*-APL) cases have been increasingly reported in recent years, thanks to the utilization of transcriptome sequencing (RNA-seq) and whole genome sequencing (WGS). Almost all evaluable *RARG*-APL cases were resistant to ATRA, and no mechanism has been elucidated. Intriguingly, cells transformed by artificial *X::RARG* were extremely sensitive to ATRA (PMID: 25510432), indicating intricate mechanisms unrevealed. This study first and systematically unveils the distinctive features and pivotal molecular mechanism of *RARG*-driven APL.

Methods

A total of 22 *RARG*-APL cases with RNA-seq and WGS data from 11 centers were enrolled (Fig. 1A). We used Arriba for routine FG calling and manually investigated the non-coding fusion sequence. Reverse transcription PCR (RT-PCR) and Sanger sequencing were used to validate the FGs and to determine whether the *RARG* 5′ and 3′ fusion events were located on the same cistron. The responsiveness of the fusion proteins to ATRA was evaluated using an optimized UAS/GAL4 reporter system.

Results

Among the cases enrolled, there were 15 males and 7 females, aged 0.9-69 (median 38) years. Morphological and immunophenotype analyses all showed features of APL. Routine FGs calling reported *RARG* fusion to a 5′ partner in each case, with variable *RARG* splicing sites (Fig. 1A). There were 7 *RARG* 5′ partner genes, with *CPSF6*, *HNRNPC*, and *NUP98* observed in multiple cases.

In the 3 index cases, we identified that they all had *RARG* 3′ fusion events besides the 5′ one (Fig. 1A). RT-PCR confirmed that the 5′ and 3′ *RARG* fusion events were in the same cistron in each case. Such as, the fusion transcript is the insertion of *RARG*-e4_9 (e refers to exon) between *NPM1*-e4 and e11 in case #G1, rather than two separate transcripts of *NPM1*-e4:: *RARG*-e4 and *RARG*-9:: *NPM1*-e11. We named this novel form of tandem splicingtrinary fusion.

Analysis of the 19 validation cases further confirmed that all *RARG*-APL cases had *RARG* 3′ fusion events (Fig. 1A). We also validated that the 5′ and 3′ *RARG* fusion events were in the same cistron in 3 more cases, of which archived cDNA were available. Remarkably, the 3′ splicing sits of all cases were consistently at the end of *RARG*-e9, leading to *RARG*-e10 truncation. The *RARG* 3′ partner gene was the same as its 5′ partner in 11 cases. But in the other 11 cases, the *RARG* 3′ partner was a transposon element (TE) sequence, the most common being a *LINE-L2a* (8 cases). Sequences analysis indicated that the committed locus of the involved *LINE-L2a* was at 4.2 Kbp upstream of the *RARG* gene. Gene expression analysis confirmed the aberrant activation of the involved TEs. All TEs involved in the *RARG* 3′ fusions confer a poly_A signal sequence to the fusion transcript, which was essential for a mRNA. In-depth analysis indicated that TEs participate in the formation of *RARG*-FGs through a transposition mechanism rather than a translocation mechanism.

RARG-e10 encodes helix 11_12 (H11_12) of its ligand binding domain (LBD), which plays a pivotal role in allosteric response to ATRA (PMID: 31178221). The trinary fusion protein with RARG LBD-H11_12 castration will lose responsiveness to ATRA via an allosteric disability mechanism (Fig. 1B, 1C). Experiments confirmed that cells transfected with *X::RARG* fusion with intact LBD responded well to ATRA, while fusion with LBD-H11_12 castration showed no response to physiological and pharmacological concentrations of ATRA (Fig. 1D, 1E).

Conclusions

This study clearly revealed that trinary fusion and LBD castration are crucial molecular etiologies in *RARG*-APL. The trinary fusion orchestrates the aberrant activation of *RARG* (5′ splicing) and the complete allosteric disability in responding to ATRA (3′ splicing). Thus, this study elegantly explains the mechanism of leukemogenesis and the extensive ATRA resistance of *RARG*-APL. The formation of trinary fusion requires two steps of genome splicing or transposition events, which explains the rarity of *RARG*-APL. This also clarifies that *RARG*-APL has a significant disparity in molecular mechanism from *PML::RARA*-positive APL, and we suggest that it should be considered as a separate entity of acute myeloid leukemia.

Disclosures No relevant conflicts of interest to declare.

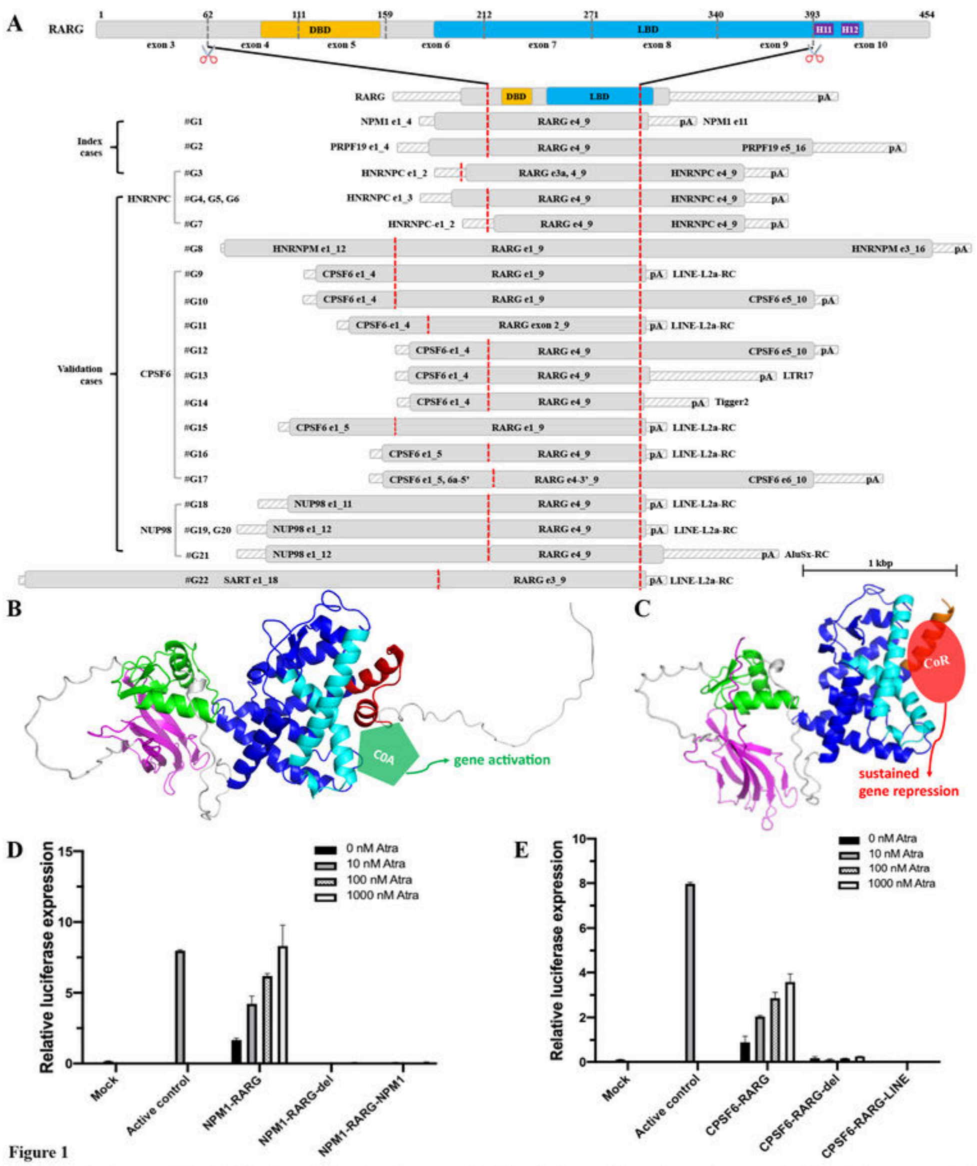

Figure 1

A. Schematic diagram of the RARG trinary fusion. Broad gray or colored bars indicate coding regions and narrow striped bars indicate untranslated regions. DBD, DNA binding domain. LBD, ligand binding domain. H11, H12, helices of LBD. The RARG 5′ splicing sites were variable. Remarkably, the RARG 3′ splicing sites were highly consistent at the end of RARG exon 9 (e9), which led to e10 truncation, including the H11_12 coding region.

B. C. 3D model of NPM1::RARG fusion protein with intact RARG-LBD (B) and NPM1::RARG::NPM1 trinary fusion as in case #G1 with LBD H11_12 castration (C). Magenta, beta sheets coding by NPM1. Green helices, RARG-DBD. Blue, cyan (H3, H4), and red (H11, H12), helices of RARG-LBD. Orange helix, a short helix coding by NPM1 e11. Green translucent pentagon, co-activator. Red translucent oval, co-repressor.

B. In response to ATRA, the highly dynamic H11_12 region turned in helical conformation and composed a co-activator interaction surface with H3 & H4. LBD then dissociates with co-repressor and recruits co-activator, thereby activating the RARG-targeted genes.

C. The trinary RARG fusion protein loses the allosteric ability to respond to ATRA due to the loss of LBD H11_12. The fusion protein will thus sustainedly bind with the co-repressor and repress RARG-targeted genes.

D. E. UAS/GAL4 reporting experiment in vitro. NPM1-RARG & CPSF6-RARG, fusion with intact RARG e9. NPM1-RARG-del & CPSF6-RARG-del, fusion with RARG e9 truncation. NPM1-RARG-NPM1 & CPSF6-RARG-LINE, trinary fusion as in cases studied. Results showed that cells transfected with RARG fusion with intact LBD responded well to ATRA, while fusion with LBD-H11_12 castration showed no response to physiological and pharmacological concentrations of ATRA.

Figure 1

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