Abstract 1523

SY-1425 (tamibarotene) Induces Profound Transcriptional Changes in AML Tumors with High Retinoic Acid Receptor Alpha

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Abstract

Retinoic acid receptor alpha (RARα) regulates myeloid differentiation and proliferation through the regulation of specific sets of genes. When unbound by a ligand, RARα is a repressive transcription factor while in its ligand-bound state it functions as a transcriptional activator. Previously, blast cells from a subset of individuals with non-APL AML were found to have a super-enhancer (SE), as revealed by H3K27 acetyl ChIP-Seq, associated with the RARA locus (hereafter called RARA-high), suggesting that tumor cell proliferation may have a dependency on RARA that can be exploited for therapeutic benefit. SEs are exceptionally large, highly active chromatin regions that are densely occupied by transcription factors and have been implicated in oncogene expression. Indeed, RARA-high non-APL AML cell lines showed >1000-fold increased sensitivity compared to RARA-low cells to the potent and selective RARα agonist SY-1425 (tamibarotene) as well as efficacy in non-APL AML patient derived xenograft models with a dependency on RARA.

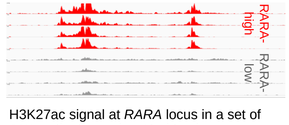
Since RARα is a transcription factor and the direct target of SY-1425, we investigated the change SY-1425 had on the transcriptional program of non-APL AML cell lines and the mechanism underlying those changes. Expression profiling on a panel of AML cell lines revealed that RARA-high AML cell lines had profound transcriptional changes in response to SY-1425, with 437 genes significantly changed, while RARA-low cell lines did not show significant gene expression changes. Gene set enrichment analysis (GSEA) of three RARA-high AML cell lines revealed that the genes upregulated by SY-1425 in the RARA-high cells are associated with immune signaling, interferon induction, protein secretion, and pathways associated with complement, MHC and integrin functions, all pathways indicative of more differentiated blood cells. Signatures downregulated by SY-1425 include MYC target genes. These findings are consistent with SY-1425 increasing the expression of genes involved in differentiation and decreasing those involved in proliferation. Genome-wide ChIP-Seq analysis revealed an increase in H3K27 acetylation at loci found to have strong RARα peaks as well as increased expression of those genes upon treatment with SY-1425. Together, these data support a model in which RARα binding nucleates functional enhancers in response to SY-1425 thereby upregulating proximal target gene expression and promotion of differentiation.

The gene expression and epigenomic responses of RARA-high AML cell lines to SY-1425 were found to be similar to the responses of an APL cell line (NB-4) to retinoids or SY-1425. Gene sets identified in response to either retinoid treatment or genetic perturbation, such as forced expression or RAR-fusions or knockdown, matched the gene sets identified in RARA-high AML cell lines. Furthermore, the quantitative response of both NB-4 and RARA-high AML cell lines to SY-1425 was found to be similar. Across the genome, RARα binding was highly conserved between NB-4 and RARA-high AML cell lines with less overlap with the RARA-low cell lines. For example, the transcriptional and H3K27 acetylation alterations at the known PML-RARα target gene TGM2 following retinoid treatment was similar in NB-4 and the RARA- high cell lines. This locus also had a strong RARα binding site that is conserved among the cell lines and co-localized with a strong H3K27 acetylation peak. Consistent with the pattern of occupancy of RARα on the genome, the transcriptional response of the RARA enhancer-high cell lines to SY-1425 treatment was similar to the response of APL ex-vivo patient samples to retinoic acid treatment. These data support a model of a common biological response to retinoids between cells with the RARA-PML translocation in APL and cells with the RARA SE in AML. The mechanistic studies described here support the therapeutic potential of SY-1425 in myeloid leukemia patients who have a SE associated with RARA. A biomarker directed clinical trial of SY-1425, a potent and selective RARα agonist, in a subset of AML and MDS patients with an altered RARA locus (clinicaltrials.gov, NCT02807558) is supported by these data.

Sensitivity to SY-1425 is associated with *RARA* SE

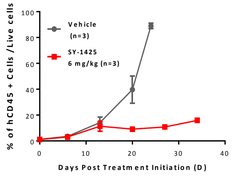
- SY-1425 (tamibarotene) developed to overcome liabilities associated with ATRA
  - Very potent and selective for RARα
    - 0.26 nM binding to RARα
    - Greater than 100x selectivity over RARβ and RARγ
  - Not metabolized by Cyp26A1; high sustained blood levels
- Approved (as tamibarotene) in Japan since 2005 for relapsed/refractory APL
  - Over 1400 patients treated
  - Oral drug with well-characterized safety profile
  - High single-agent CR rates in patients who have failed to respond to ATRA
  - Improved CR and molecular CR rates in APL head-to-head studies vs. ATRA

A subset of non-APL AML patients have a super-enhancer (SE) at *RARA*

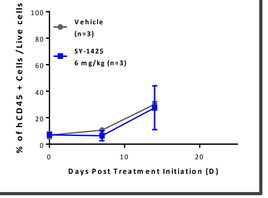


H3K27ac signal at *RARA* locus in a set of AML patient samples. Red tracks are RARA-high and grey tracks are RARA-low.

RARA-high AML PDX model is sensitive to SY-1425

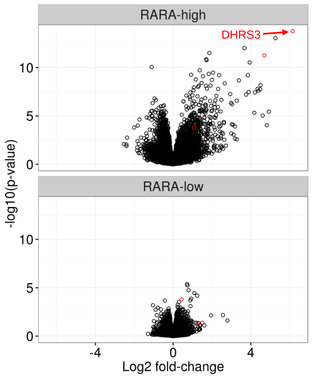


RARA-low AML PDX model is insensitive to SY-1425



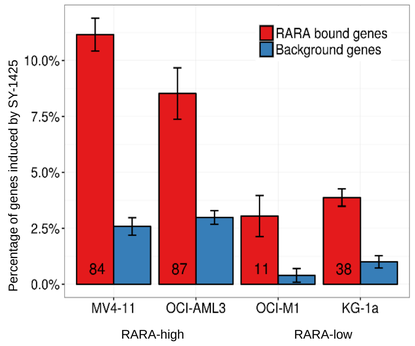
SY-1425 transcriptional response depends on RARA level

Gene expression response to SY-1425



Volcano plots of gene expression response to SY-1425 by Affymetrix array in RARA-high cell lines (OCI-AML3, MV4-11, and Sig-M5) and RARA-low cell lines (OCI-M1, KG-1a, Kasumi-1). Red points map to the DHRS3 gene.

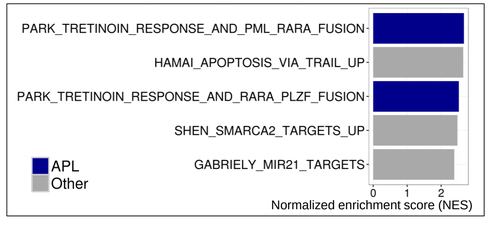
RARα-bound genes are enriched for SY-1425 response



Percentage of genes in each set that are up-regulated by SY-1425 (FDR<0.05 and log2 fold change >1) in each cell line. RARα bound genes contain a RARα ChIP-seq peak near the promoter. Numbers in the RARα bound bars indicate the number of genes up-regulated and bound by RARα in that cell line.

Gene expression changes induced by SY-1425 in RARA-high AML are similar to those of APL cells treated with retinoids

APL genes sets are in the top 5 gene sets (of out 2775) identified by RARA-high AML response to SY-1425

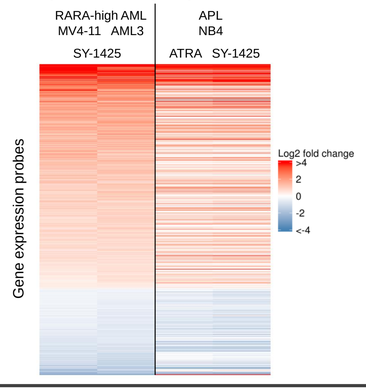


(Top left) Top 5 gene sets from MSigDB C2.CGP (perturbations, n=2775) that are enriched by GSEA (Subramanian et al., 2005) in SY-1425 response in RARA-high cell lines. Gene sets related to APL are in blue.

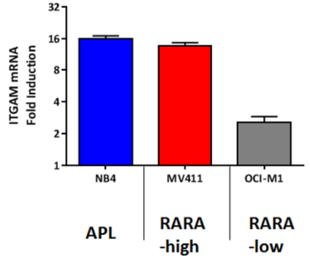
(Bottom left) Gene expression response to SY-1425 (log2 fold-change) by Affymetrix array. Probes with FDR<0.01 in joint group of OCI-AML3 and MV4-11 (n=575) are shown. Probes are sorted by log2 fold-change in joint group of OCI-AML3 and MV4-11.

(Bottom right) Fold change of ITGAM (CD11b) expression upon SY-1425 treatment.

Expression changes upon treatment

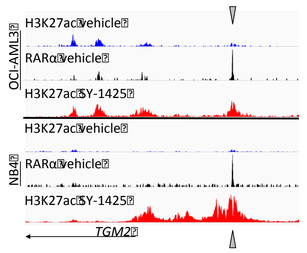


CD11b (ITGAM) induction by SY-1425



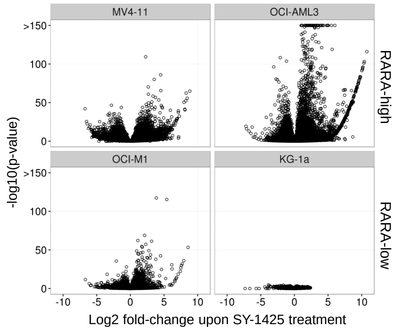
SY-1425 causes enhancer formation at RARα-bound loci

Enhancer response to SY-1425 at TGM2 locus



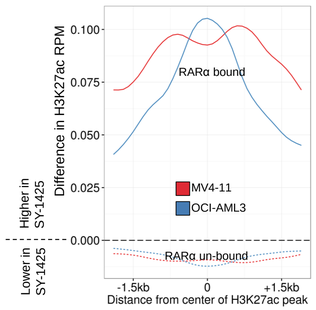
H3K27ac and RARα ChIP-seq tracks at the TGM2 locus in the RARA-high cell line OCI-AML3 and the APL cell line NB4.

Global changes in enhancer size upon SY-1425 treatment



Volcano plots of response to SY-1425 vs. vehicle for H3K27ac peaks RARA-high (MV4-11 and OCI-AML3) and RARA-low (OCI-M1, KG-1a) cell lines.

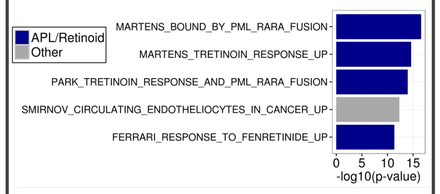
SY-1425 induces RARα bound enhancers to become stronger



H3K27ac meta-peak in RARA-high cell lines, showing difference between SY-1425 treatment and vehicle treatment. MV-4-11 is in red, and OCI-AML3 is in blue. RARα bound peaks overlap a RARα ChIP-seq peak.

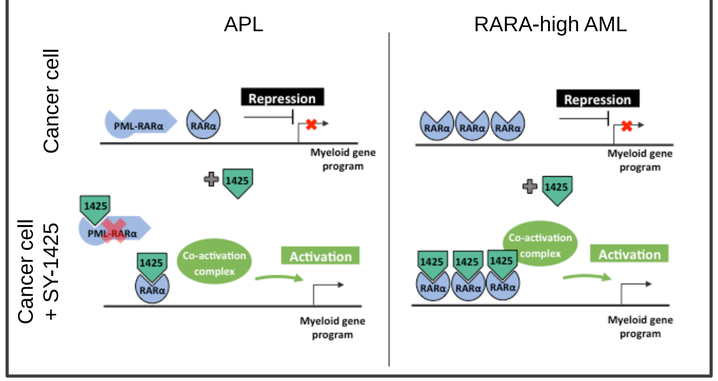
Induced enhancers in RARA-high AML match APL gene sets

APL genes sets are in the top 5 gene sets (of out 2775) identified by enhancers induced by SY-1425 in RARA-high AML



GREAT (McLean et al. 2010) analysis of gene sets enriched in H3K27ac peaks that are up-regulated by SY-1425 in the RARA-high cell lines (OCI-AML3 and MV4-11). Top 10 (by FDR) perturbation gene sets (MSigDB C2.CGP, n=2775) are shown. Gene sets related to APL are in blue.

Proposed mechanism



Conclusions

- SY-1425 is a first-in-class, potent and selective RARα agonist with favorable PK properties and is approved in Japan for the treatment of R/R APL, which is characterized by fusions between *RARA* and other transcription factor genes.
- Sensitivity of non-APL AML cells to SY-1425 is correlated with *RARA* enhancer strength, as *RARA*-high AML cells are sensitive and *RARA*-low are not.
- SY-1425 induces the expression of genes in *RARA*-high non-APL AML cell lines that are also induced by retinoids in APL cells.
- These mechanistic similarities, including transcriptional and epigenomic responses to SY-1425, support the clinical potential of SY-1425 in *RARA*-high AML.
- Based on SY-1425's well-established safety and efficacy profile in R/R APL and our strong preclinical data, we have initiated a biomarker-directed Phase 2 clinical trial in genomically defined subsets of AML and MDS patients with high levels of *RARA* gene expression (clinicaltrials.gov, NCT02807558).
- See ASH poster #2898 on Sunday, December 4, 2016 for more details on clinical pharmacodynamic markers and the combination potential with SY-1425.

ASH Annual Meeting and Exposition 2016