Abstract 1511

AML patient clustering by super-enhancers reveals an RARA associated transcription factor signaling partner

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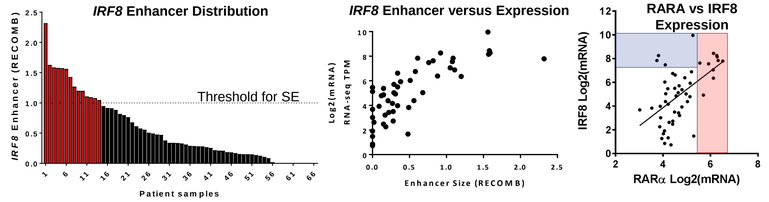
Abstract

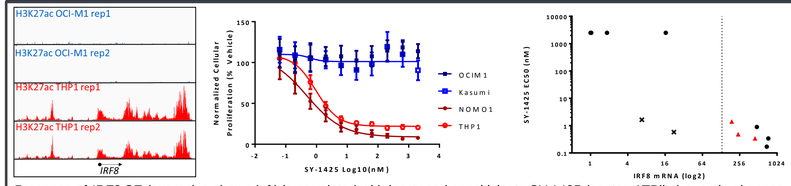
Prior studies have shown that the *RARA* gene is associated with a super-enhancer (SE) and has upregulated mRNA expression in a subset of AML patients. Furthermore, this has been found to confer increased sensitivity to SY-1425, a potent and selective RARα agonist. We sought to better characterize the cell state and transcription factor circuitry in these RARA-high AML cells. Clustering of 66 primary AML patient samples based on their genome wide SE maps identified six discrete clusters. RARA-high patients partitioned principally into cluster 2, and to a lesser extent 1, suggesting that RARA upregulation is associated with a specific transcription factor (TF) network and cell state. To start unraveling the TF circuitry in the RARA-high cluster, we investigated which other TFs were SE associated with clusters 1 and 2. In particular, interferon regulatory factor 8 (IRF8) was found to be strongly associated with clusters 1 and 2 by SE and mRNA expression, similar to RARA. Moreover, the expression of both genes is correlated in primary patient samples. IRF8 is involved in interferon signaling and previous studies have shown crosstalk between interferon and retinoic acid signaling. Furthermore, aberrant IRF8 pathway signaling is implicated in AML and CML pathogenesis. The tight clustering of RARA and IRF8 in patient subgroups defined by genome wide enhancer maps suggests RARα and IRF8 may form an integrated transcriptional circuit. Indeed, treatment with SY-1425 was found to strongly induce interferon-like gene expression changes in AML cells with high RARA or IRF8 levels, including the tumor suppressive IFN responsive gene IRF1. While RARA-high AML cell line models have been previously shown to respond to SY-1425, we found that models with high IRF8 expression and low levels of RARA were also found to respond to SY-1425. Such IRF8-high, RARA-low AML cell lines showed activation of similar transcriptional pathways as RARA-high cell lines in response to SY-1425 based on GSEA. IRF8-high AML also had comparable low nM EC50 anti-proliferative effects following SY-1425 treatment. In addition, SY-1425 was found to elicit differentiation in both RARA-high and IRF8-high AML cell lines based on flow cytometry. While RARA and IRF8 expression appear correlated, this data suggests that IRF8 levels may predict for sensitivity to SY-1425 in addition to RARA levels, particularly in cases of AML with high IRF8 expression but low RARA levels. Insights derived from enhancer analysis, transcriptional profiling and differentiation response in preclinical models support the recently initiated Phase 2 trial of SY-1425 (NCT02807558) in which we are evaluating the SE based patient selection strategies and gene circuitry derived pharmacodynamics clinical measurements, including differentiation markers, in patients with AML and MDS.

IRF8 has an SE and upregulation in AML correlated with RARA



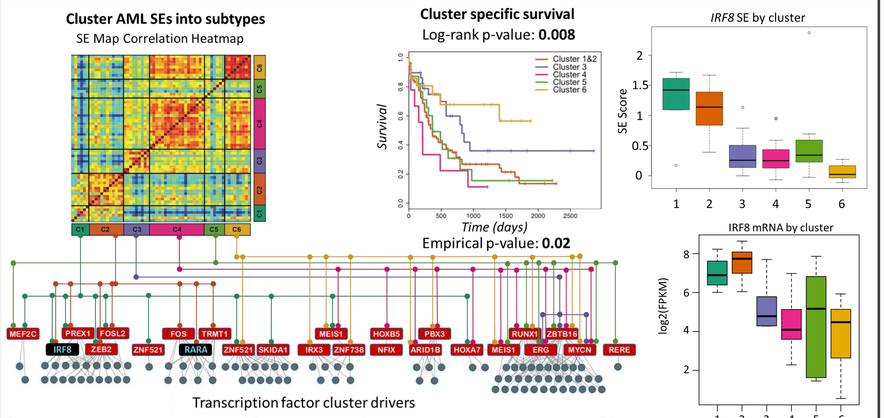
IRF8 has a super-enhancer in a subset of AML patients that correlates with upregulated IRF8 and RARA mRNA. Dashed line indicates SE cutoff (left). RARA versus IRF8 levels (right) with RARA-high in red box and IRF8-high in blue illustrating biomarker calling

IRF8 SE/Overexpression Predicts Response to SY-1425 *in vitro*



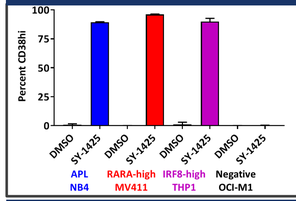
Presence of *IRF8* SE (examples shown left) is correlated with increased sensitivity to SY-1425 (center, ATPlite) even in absence of RARA-high phenotype. NOMO1 and THP1 AML cell lines are both RARA-low, IRF8-high while OCIM1 and Kasumi1 are double low. Figure on right illustrates IRF8 expression versus SY-1425 sensitivity in AML cell lines. IRF8-high/RARA-low shown as red triangles and APL in black x's. Other responsive black circles are high in both IRF8 and RARA.

IRF8 and RARA Cluster in Epigenomically Defined AML Subsets

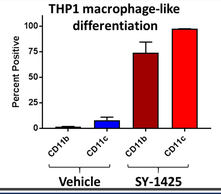


Clustering of pt samples by genome wide SE maps resulted in 6 clusters that are charcterized by distinct SE associated transcription factors. Individual clusters are associated with varying survival (based on TCGA data). IRF8 showed a strong enrichment (based on its own SE and mRNA) with clusters 1 & 2 similar to the RARA SE (highlighted in black)

SY-1425 Induces Differentiation in IRF8-high AML Cell Lines

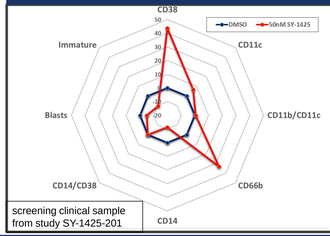


Flow cytometry showing induction of the CD38 maturation marker response to 50nM SY-1425 (24 h) in APL, RARA-high, and IRF8-high cell lines but not in a RARA-low, IRF-8 low cell line (OCI-M1).



THP1s show differentiation toward a macrophage phenotype in response to 24hr 50nM SY-1425 as measured by CD11b and CD11c expression.

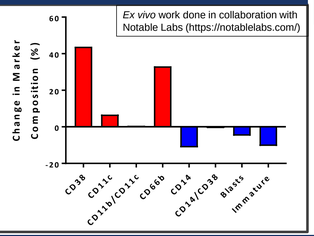
SY-1425 *ex vivo* Treatment Causes Differentiation in IRF8-high AML Patient Sample



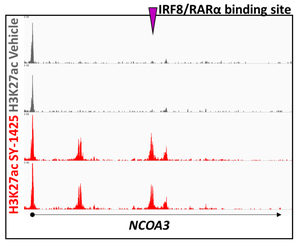
screening clinical sample from study SY-1425-201

White blood cells from an IRF8-high/RARA-low AML patient sample treated with 50nM SY-1425 *ex vivo* for 48 hours. Radar plot (left) and bar plot (right) of data indicates a reduction in blast, immature blast, and CD14+ monocyte composition. Strong maturation indicated by CD38 induction and gain of CD66b is found suggesting granulocytic lineage differentiation

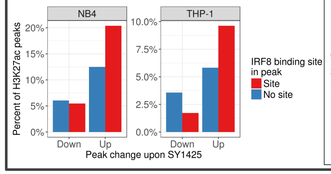
Ex vivo work done in collaboration with Notable Labs (https://notablelabs.com/)



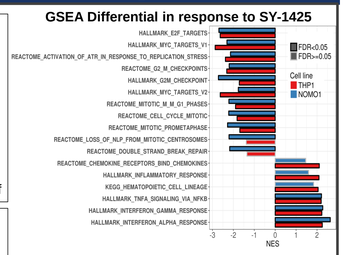
IRF8 binding and RARα Agonism Activate IRF Target Genes

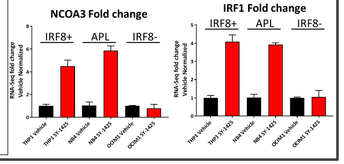


Left, top: The NCOA3 gene is bound by both IRF8 and RARα but minimally activated in the absence of SY-1425. Treatment induces acetylation and activation of the gene locus.
Left, bottom: SY-1425 increases acetylation of loci with IRF8 binding motif

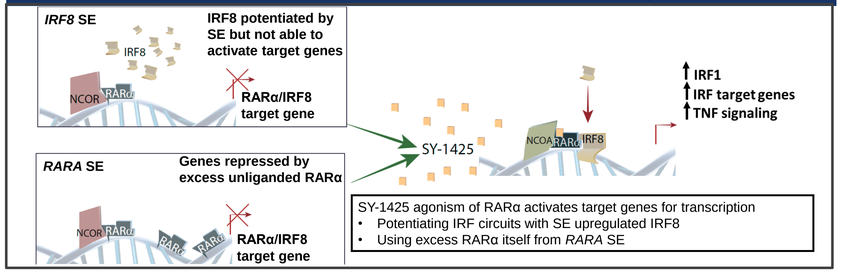


Right, top: SY-1425 leads to a reduction in proliferation and cell cycle pathways and strong upregulation of IRF target genes and related signaling
Right, bottom: IRF8/RARα target gene mRNA for NCOA3 and IRF1 are upregulated in response to SY-1425





Proposed mechanism of SY-1425 in IRF8-high AML



SY-1425 agonism of RARα activates target genes for transcription
- Potentiating IRF circuits with SE upregulated IRF8
- Using excess RARα itself from *RARA* SE

Conclusions

- *IRF8* has been identified as an SE driven transcription factor in a subset of AML
- The level of this enhancer and consequent mRNA expression [in addition to RARA biomarker] is predictive of sensitivity to SY-1425, an oral potent and selective RARα agonist
- AML models with high IRF8, a component of the retinoic acid signaling pathway, show 1000x increased sensitivity to SY-1425 and induced differentiation
- RARα mediated response to SY-1425 shows cross talk with the interferon pathway by mRNA expression and chromatin circuitry dynamics
- IRF8 and RARA have been developed as a patient selection strategy in a biomarker-directed Phase 2 trial of SY-1425 in epigenomically defined subsets of AML and MDS patients (clinicaltrials.gov, NCT02807558)
- The incorporation of the exploratory IRF8 biomarker in our clinical study potentially captures additional patients with RARA pathway dependent cancers sensitive to SY-1425
- Also see posters 5103 and 5575 for information on SY-1425 drug combination studies

AACR Annual Meeting 2017