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): enhancer in a subset of AML patients. Furthermore, this has been found to confer increased sensitivity to SY-1425, a potent and selective RARA agonist. We sought to better characterize the cell state and transcription factor circuitry in these RARA-high AML cells. Clustering of 84 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 specific transcription factor (TF) network and cell state. To start unraveling the TF circuitry in the RARA-high cluster, we investigated whether the TFs were SE-associated clusters 1 and 2. In particular, interferon regulatory factor (IRF) 8 (IRF8) was found associated with cluster 2. Importantly, IRF8 has been previously found to be frequently upregulated in both genes in primary patient samples. IRFs are involved in interferon signaling and previous studies have shown that IRF8 and RARα mediate antiproliferative activity. Furthermore, disruption of the IRF8 pathway signaling is implicated in AML. CML pathogenesis. The tight clustering of RARα and IRF8 in patient subgroups defined by genome-wide enhancer maps suggests RARα and IRF8 may form an integrated transcriptional circuit. Indeed, increased IRF8 expression was found to strongly induce interferon-like gene expression changes in AML cells with high RARA or IRF8 levels, including the tumor suppressive IRF9 expression gene. While RARA-high AML cell 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, including IRF9 expression. GSEA IRF8-high AML high activity, also had comparable low mR mRNA expression and cellular effects following SY-1425 treatment. In addition, SY-1425 was found to elicit differentiation in both RARA-high and IRF8-low AML cell lines, based on flow cytometry. While RARA and IRF8 synergize to increase cellular sensitivity to SY-1425 in AML cell models, levels, particularly in cases of high AML cell model activity lower in high but low levels. Increased interest from enhancer analysis further highlights the potential of RARA and IRF8 integration in the disease-making circuitry for regulatory control. The shape of IRF8 as an SE partner point suggests that SY-1425 is an SE partner point, with the IRF8 activity vector (SEIRF8) sensitivity to AML cell lines, which are characteristic of the SE4A patient selection strategy in gene clusters derived from enhancer-promoter co-expression networks. Additionally, this study characterized RARA and IRF8 enhancer activity in AML cell lines and primary patient samples, thus potentially capturing additional patients and allowing for the incorporation of the exploratory IRF8 biomarker in our clinical study potentially captures additional patients and allowing for the incorporation of the exploratory IRF8 biomarker in our clinical study.

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

Flow cytometry showing induction of the CD38+ maturation marker relative to SY-1425 (all 34 AML, RARA-high) and SY-1425 high cell line but not in a RARA-low, IRF8-low cell line (OCCI-M1). SY-1425 treatment causes differentiation in IRF8-high AML cell lines but not in RARA-low, IRF8-low cell lines. Flow cytometry showing induction of the CD38+ maturation marker relative to SY-1425 (all 34 AML, RARA-high) and SY-1425 high cell line but not in a RARA-low, IRF8-low cell line (OCCI-M1).

SY-1425 Treats Causes Differentiation in RARA-high AML Patient Sample

Whole blood has a high expression of SY-1425 high/RARA-high AML patient sample enriched with RARα SV-1425 ex vivo for 48 hours. Rady plot (left) and top right (right) of data indicates a reduction in blast, immature blast, and CD41+ monocyte counts. Strong induction of CD38+ maturation marker and gain of CD56 is observed after SY-1425 treatment indicating a strong loss of disease phenotype.

Conclusions

SY-1425 has been identified as an SE driven transcription factor in AML. The development of a novel and selective RARA agonist (in addition to RARα-knockout (a) predictive of sensitivity to SY-1425, on an oral agent and selective RARA agonist AML models with high RARA, a component of the retinoic acid signaling pathway, show increased sensitivity to SY-1425 and RARA agonists but not to SY-1425 and RARA agonists but not to SY-1425. RARA mediated response to SY-1425 shown cross talk with the interferon pathway by mRNA expression and chromatin circuitry. RARα and RARA have been developed as a patient selection strategy in a biomarker-driven Phase 2 trial of SY-1425 in genetically defined, RARA and RARα patients (including those with NCOA2T270M). The incorporation of the exploratory IRF8 biomarker in our clinical study potentially captures additional patients with RARA pathway-dependent cancers sensitive to SY-1425.

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