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# A novel approach to detect resistance mechanisms reveals FGR as a factor mediating HDAC inhibitor SAHA resistance in B-cell lymphoma

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ABSTRACT

Histone deacetylase (HDAC) inhibitors such as suberoylanilide hydroxamic acid (SAHA) are not commonly used in clinical practice for treatment of B-cell lymphomas, although a subset of patients with refractory or relapsed B-cell lymphoma achieved partial or complete remissions.

Therefore, the purpose of this study was to identify molecular features that predict the response of B-cell lymphomas to SAHA treatment. We designed an integrative approach combining drug efficacy testing with exome and captured target analysis (DETECT). In this study, we tested SAHA sensitivity in 26 B-cell lymphoma cell lines and determined

*Abbreviations:* BL, Burkitt lymphoma; CC, capture compound; CCMS, capture compound mass spectrometry; CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; DETECT, drug efficacy testing combined with exome and captured target analysis; DLBCL, diffuse large B-cell lymphoma; FGR, Gardner-Rasheed feline sarcoma viral (v-fgr) oncogene homolog; HDAC, histone deacetylase; $IC_{50}$, half maximal inhibitory concentration; NHL, Non-Hodgkin lymphoma; SAHA, suberoylanilide hydroxamic acid.

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SAHA-interacting proteins in SAHA resistant and sensitive cell lines employing a SAHA capture compound (CC) and mass spectrometry (CCMS). In addition, we performed exome mutation analysis. Candidate validation was done by expression analysis and knock-out experiments.

An integrated network analysis revealed that the Src tyrosine kinase Gardner-Rasheed feline sarcoma viral (v-fgr) oncogene homolog (FGR) is associated with SAHA resistance. FGR was specifically captured by the SAHA-CC in resistant cells. In line with this observation, we found that FGR expression was significantly higher in SAHA resistant cell lines. As functional proof, CRISPR/Cas9 mediated *FGR* knock-out in resistant cells increased SAHA sensitivity. *In silico* analysis of B-cell lymphoma samples (n = 1200) showed a wide range of FGR expression indicating that FGR expression might help to stratify patients, which clinically benefit from SAHA therapy.

In conclusion, our comprehensive analysis of SAHA-interacting proteins highlights FGR as a factor involved in SAHA resistance in B-cell lymphoma.



## 1. Introduction

During the past decades, epigenetics has emerged as an important area in drug discovery. So called “epidrugs” are defined as drugs that inhibit or activate disease-associated epigenetic proteins to ameliorate or cure the disease (Ivanov et al., 2014). Especially inhibitors of histone deacetylases (HDAC) are considered to be promising anticancer agents. By regulating acetylation states of histone and several non-histone proteins, they directly induce modifications in the cancer epigenome. Thereby, epigenetically silenced genes are re-expressed eventually resulting in growth arrest or apoptosis of cancer cells (Carew et al., 2008). Due to this anti-tumor activity, HDAC inhibitors had a rapid phase of clinical development as monotherapy and in combination with other anticancer drugs (Budde et al., 2013; Kirschbaum et al., 2011; Morschhauser et al., 2015; Ogura et al., 2014; Oki et al., 2013; Straus et al., 2015; Watanabe et al., 2010). However, a broad clinical response has been observed in patients with hematologic or solid malignancies ranging from complete remissions to no response (Chun, 2015).

Suberoylanilide hydroxamic acid (SAHA) (Vorinostat, Zolinza® (Merck and Co., Inc.)) was the first HDAC inhibitor approved by the American Food and Drug Administration in October 2006 for the treatment of a subset of patients with cutaneous T-cell lymphoma (Mann et al., 2007). Furthermore, SAHA is also used in clinical trials for patients with other types of Non-Hodgkin lymphoma (NHL) (Budde et al., 2013; Kirschbaum et al., 2011; Ogura et al., 2014; Straus et al., 2015; Watanabe et al., 2010).

NHL is a heterogeneous group of lymphoproliferative neoplasms. Two aggressive subtypes of B-cell NHLs are diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL), which account for 40% and 2% of all NHLs, respectively (Siegel et al., 2015; Swerdlow et al., 2008). Although survival of patients with B-cell lymphoma has improved by the addition of targeted therapies to conventional chemotherapy regimens, a considerable proportion relapse leading to adverse clinical outcome (Coiffier et al., 2010; Hoelzer et al., 2014; Reeder and Ansell, 2011; Sweetenham et al., 1996). Thus, novel approaches are urgently needed to improve disease control and patient’s survival. More specific and therefore less toxic treatment opportunities would be highly desirable (Chun, 2015). Preclinical and clinical studies with SAHA mono- or combination therapies showed promising results for the treatment of patients with B-cell lymphoma (Kirschbaum et al., 2011; Ogura et al., 2014; Richter-Larrea et al., 2010; Watanabe et al., 2010). Remarkably, response rates of up to 30% were achieved in patients with relapsed or refractory B-cell lymphoma upon SAHA monotherapy, comprising also complete remissions (Kirschbaum et al., 2011; Ogura et al., 2014; Watanabe et al., 2010). 70% of patients, however, do not respond to this treatment. Therefore, it is essential to identify those patients who will not benefit from HDAC inhibitor therapy in order to prevent ineffective treatment.

These clinical data stimulated us to seek for molecular features that help to stratify upfront B-cell lymphoma patients for SAHA treatment. To this end, we hypothesized (i) that proteins, which directly interact with SAHA, may be involved in SAHA response and (ii) that the expression and mutation status of these direct SAHA targets might be of relevance for SAHA response.

Direct SAHA interaction partners can be identified by Capture Compound Mass Spectrometry (CCMS). CCMS is based on a trifunctional small molecular probe called Capture Compounds (CC). SAHA is attached as selectivity function to a CC-scaffold that can interact with the target proteins in homogeneous phase under equilibrium conditions. The reactivity function of the CC-scaffold covalently binds the target proteins through photo-induced cross-linking. The sorting function of the CC-scaffold (e.g. biotin) enables the isolation of CC-proteins conjugates from complex protein mixtures (Supplemental Figures. S1 and S2). Finally, captured proteins are identified using high-resolution mass spectrometry (Fischer et al., 2011b; Koster et al., 2007).

To comprehensively identify the molecular mechanisms leading to SAHA resistance we designed an approach that applies **d**rug **e**fficacy **t**esting with **e**xome and **c**aptured **t**arget