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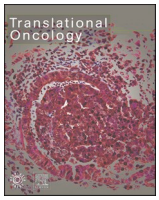

New insights into possible HDAC inhibitor resistance in DLBCL - Comment on 'defining cellular responses to HDAC-selective inhibitors reveals that efficient targeting of HDAC3 is required to elicit cytotoxicity and overcome naïve resistance to pan-HDACi in diffuse large B cell lymphoma' by Havas et al.

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Introduction

Over the past decades, the role of epigenetics and the related processes of histone acetylation (HAT) and histone deacetylation (HDAC) in human solid tumours and haematological malignancies has been extensively studied in vitro and in vivo in order to identify new therapeutic options. The key findings from these basic experimental studies were (i) that the HAT/HDAC ratio is unbalanced in tumours, (ii) that the expression pattern of HATs/HDACs is tumour-specific, and (iii) that drug-induced inhibition of HATs and/or HDACs can lead to anti-proliferative and/or pro-apoptotic as well as anti-vascular and pro-inflammatory effects in vitro and in vivo [1]. Although the portfolio of chemically designed HDAC inhibitors has increased dramatically in recent years, there has been a lack of translation into daily clinical use [2]. As shown in Fig. 1A, there are only four global FDA-approved HDAC inhibitors that (i) are not HDAC class-specific and (ii) have been used in truly rare and potentially pre-treated haematological diseases, leaving a definite lack of indication for HDAC inhibitors in the major haematological disease groups. Nevertheless, new approaches are being pursued to increase the therapeutic efficacy of such HDAC inhibitors alone or in combination with standard treatment regimens in various human malignancies, as reviewed in detail by Karagiannis and Rampias [3].

HDAC and (diffuse large B-cell) lymphomas

The lymphoma type of DLBCL is one of the most common forms of non-Hodgkin's lymphoma, representing an aggressive disease with an urgent need for therapy, as recently reviewed by Sehn and Salles [5]. As summarised in Fig. 1B, the characteristics of these cases diagnosed with DLBCL are highly heterogeneous in terms of morphology, immunohistochemical and molecular phenotype, genetic features and their biological pathways, which are associated with different prognosis as well as outcome due to the possibility of treatment failure to the standard chemotherapy protocol with R-CHOP immunochemotherapy (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone).

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Therefore, there is still a need for new therapeutic approaches for DLBCL [5].

It is known that HDACs are essential for B cell differentiation [8]: for example, specific knockouts of HDAC1, HDAC2 and HDAC3 in mice impaired B cell maturation. Furthermore, HDAC3, HDAC4 and HDAC9 are co-repressors of BCL6, an important transcriptional repressor of B cell differentiation. In particular, the HDAC6 and HDAC9-Bcl6 complex is often aberrant in DLBC, providing evidence for a pathogenic role in DLBCL. When analysing available biodata from the Gene Expression Profiling Interactive Analysis (GEPIA) platform ([6], http://gepia.cancer-pku.cn, last accessed on 2023–10–02) using data from The Cancer Genome Atlas (TCGA) project [9], the RNA expression of HDACs in DLBCL is highly heterogeneous as shown in Fig. 1C: Overall, HDAC class I, III and IV members were partially significantly over-expressed in the tumour compartment of DLBCL compared to normal control tissues, whereas only the two HDAC class II members HDAC4 and HDAC5 were under-expressed in DLBCL compared to normal control tissues. The highest levels of HDACs were found for HDAC3/7 and the lowest levels for HDAC4. Interestingly, the data were mostly comparable to previous protein expression analyses of HDACs in DLBCL [10] indicating their consistency and reproducibility . Furthermore, a protein-protein-interaction analysis (using the online available Database STRING [7] with default settings, https://string-db.org/, last accessed on 2023–10–02) reports intensive HDAC—HDAC-interactions (as demonstrated in Fig. 1D) whereby additional cluster analysis integrating biological pathway information (such as KEGG, UniProt, GeneOntology etc.) reveals no HDAC class-dependency of the HDAC isoforms whereby structural and functional similarities of some HDAC isoforms cannot be excluded, as already experimentally demonstrated [11]. Overall, it is not really surprising that - as described in detail by Chen et al. [8] - the HDAC inhibitors Vorinostat (SAHA), Belinostat (PXD101), Mocetinostat used in clinical trials as monotherapy for the DLBCL achieve only sobering overall response rate (ORR) up to 5.6 % for Vorinostat, 10.5 % for ORR for Belinostat and 18.9 % for Mocetinostat.

The possible mechanism of HDAC resistance in DLBCL

Since the efficacy and clinical success rates of HDAC inhibitors as monotherapy in lymphomas and especially in DLBCL are very low, the underlying reason(s) for this clinical observation becomes a central

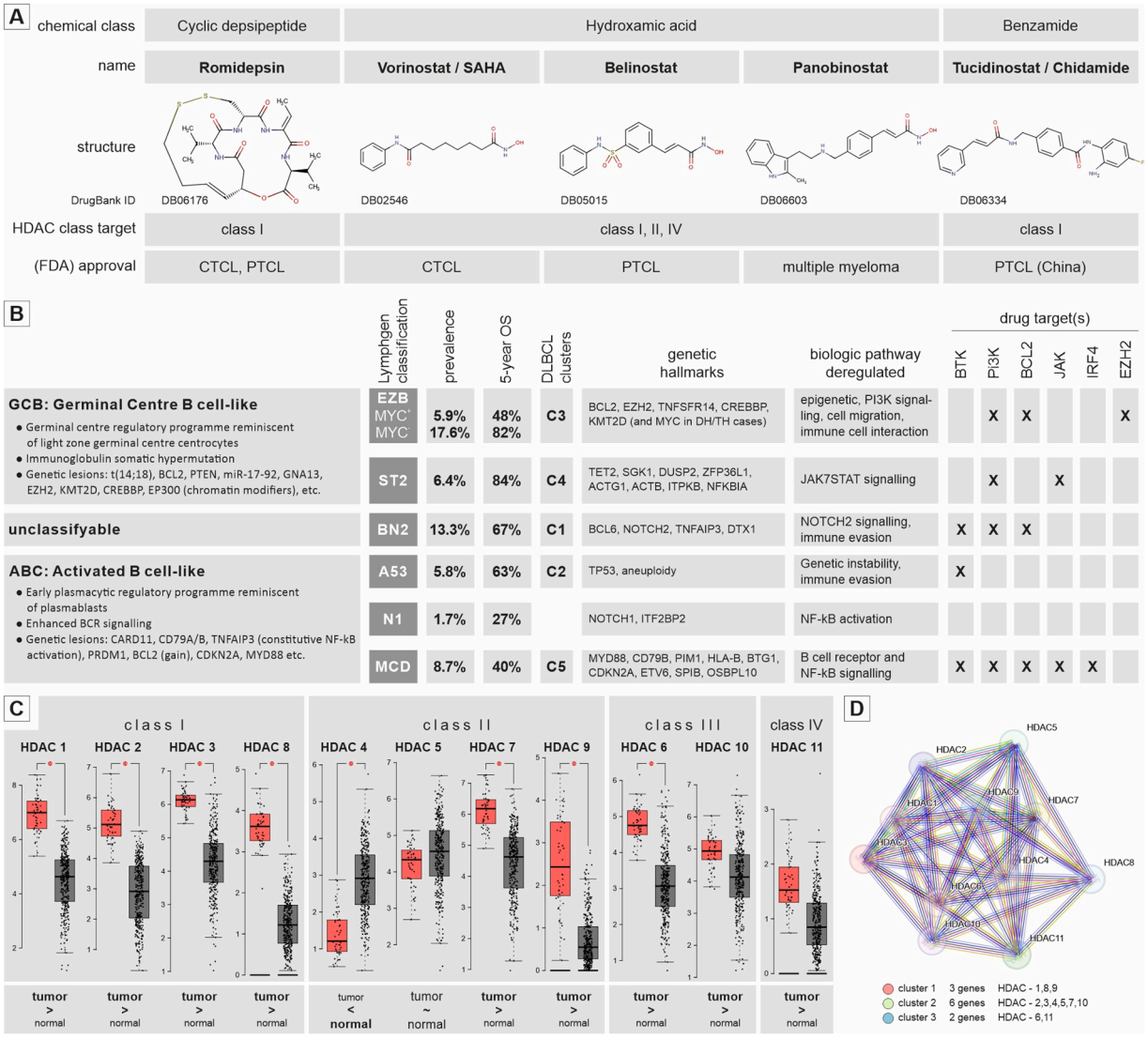

Fig. 1. (A) Approved HDAC inhibitors. Based on [3]; structures adopted from https://www.drugbank.com/. (B) Classification of B cell-like lymphomas. Based on [4, 5]. (C) Expression profiling of HDAC isoforms in DLBCL (red bars) versus non-tumour tissue (grey bars). Based on http://gepia.cancer-pku.cn, [6]. (D) Protein interaction model for HDAC isoforms. Adopted from https://string-db.org/ [7]. Abbreviations: CTCL = cutaneous T-cell lymphoma, PTCL = peripheral T-cell lymphoma, SAHA = suberoylanilide hydroxamic acid.

issue. Therefore, Catharine Smith's research group has been investigating different aspects of a possible resistance mechanism of HDACs in DLBCL in recent years. In 2013, they classified seven DLBCL-derived cell lines (germinal centre B-cell-like type: SUDHL6, OCI-Ly19, SUDHL4, DB and SUDHL8; activated B-cell-like type: OCI-Ly3 and U2932) as sensitive (DB, SUDHL6, OCI-Ly3 and OCI-Ly19) or resistant (SUDHL4, SUDHL8 and U2932) toward HDACis (HDAC inhibitors). The experimental in vitro results showed that the sensitivity of DLBCL to HDACi is associated with G2/M arrest and apoptosis, whereas resistance of DLBCL to HDACi is associated with reversible G1 growth arrest [12]. In particular, this HDACi resistance response mechanism of DLBCL cells appears to be mediated by sustained HDACi-induced over-expression of the cyclin-dependant kinase inhibitors p21 and p27 and their inhibition of cyclin E/cdk2. Subsequently, Catharine Smith's research group observed synergistic cytotoxicity of low-dose vincristine or paclitaxel in combination with belinostat in those HDACi-resistant DLBLC cell lines. The authors demonstrated a positive cytotoxic 'handshake' between microtubule-targeting therapies (such as vincristine or paclitaxel) and epigenetic modifiers (such as HDAC): (i) vincristine or paclitaxel induces mitotic arrest to sensitize DLBCL cells to the cytotoxic effects of belinostat, and, (ii) belinostat prevents polyploidy to avoid vincristine resistance [13]. Based on these earlier experimental findings, Havas et al. focused on defining cellular responses to isoform-selective HDACi in their recent study published in this issue of *Translational Oncology* to characterize in detail HDACs that may possibly mediate cellular responses to the pan-HDACi, belinostat, as summarized in the following [14]:

- Treatment of DLBCL cell lines with apicidin, a selective inhibition of HDAC1–3, recapitulates the observed belinostat-sensitive and -resistant phenotypes of DLBCL cell lines.
- Treatment with RGFP966, a selective inhibitor of HDAC 3, leads to apoptosis only in pan-HDACi sensitive DLBCL cell lines without initial induction of mitotic arrest via DNA damage (measure by γ-H2AX protein levels). Furthermore, siRNA-mediated transient knockdown of HDAC3 produces a longer-lived population of HDAC3 protein in DLBCL cells.
- Treatment with BRD2492, a highly selective inhibitor of HDAC1 and HDAC2 (> 100 fold over HDAC3), leads to sufficient G1 cell cycle arrest in both pan-HDACi sensitive and resistant cell lines.
- Higher doses of the pan-HDACi belinostat induce DNA damage and apoptosis which is comparable to a cellular response elicited by a HDAC3-selective inhibitor.

Therefore, Havas et al. [14] postulate that (i) specific inhibition of HDACs −1 and −2 may be the cause of a pan-HDACi-resistant phenotype in the investigated DLBCL cells and that (ii) variable sensitivity of HDAC3 is a possible novel resistance mechanism to pan-HDACi as shown by dose-escalation experiments with pan-HDACi.

Possible new roads in DLBCL: HDACis and more…

The following aspects are relevant for future applications and role of HDACis in DLBCL:

- Which type of DLBCL is definitely a candidate for HDACitreatment? This question can't be answered yet due to a lack of data. However, the ongoing experimental investigations of Catharine Smith's research group [12–14] provide evidence that selective HDACis, rather than pan-HDACis, will open up new therapeutic options for the treatment of DLBCL. Furthermore, ex-vivo experiments on primary human cultured DLBCL cells could help to identify HDACi sensitivity and resistance as early as possible to define the further HDAC inhibitor strategy [15].
- Can we chemically improve HDACis? As recently published, the development of new and selective HDACis is challenging due to intensive chemical structural modifications in order to achieve satisfactory metabolic stability, desirable oral bioavailability, good anti-tumour efficacy without severe toxicity [16]. Furthermore, the development of dual-target inhibitors such as fimepinostat, targeting HDAC and PI3K, is promising to improve the therapeutic success rate of HDACi in DLBCL: a phase II study (NCT02674750) demonstrated prolonged response in patients with MYC-altered relapsed/refractory DLBCL treated with single-agent fimepinostat [17].
- Which combinations of HDACisandother new therapeutic options in DLBCL are promising? It has been shown that HDACis influence tumour inhomogeneity (i) to increase the anti-tumour immune response, and, (ii) to decrease the immunosuppressive tumour environment which is common in human tumours [18] and especially in DLCBL [19]. Due to a HDAC class- and isoform-dependency, the focus on the combination of selective HDACis and immunotherapy represents an innovative approach to improve the therapeutic outcome in DLBCL.

Concluding remarks on HDACis in DLBCL

The development of selective HDACis and dual inhibitors is an ongoing story in general, and, in particular, for DLBCL [20,21]. In the context of the present study by Havas et al., more efforts should be made to develop new and HDAC isoform-specific inhibitors as well as dual target inhibitors to exploit the full potential of such drugs targeting the dysregulated epigenetic machinery in human tumours and especially in the aggressive lymphoma type DLBCL [22].

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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