Epigenetic Alterations in Fanconi Anaemia: Role in Pathophysiology and Therapeutic Potential

Hélio Belo1,2, Gabriela Silva1,2, Bruno A. Cardoso1,2, Beatriz Porto3, Jordi Minguillon4, José Barbot5, Jorge Coutinho5, Jose A. Casado6, Manuela Benedito7, Hema Saturnino7, Emília Costa5, Juan A. Bueren5, Jordi Surralles4, Antonio Almeida1,2*

1 Unidade de Investigação em Patobiologia Molecular, Instituto Português de Oncologia de Lisboa Francisco Gentil, E.P.E., Lisboa, Portugal, 2 CEDOC, Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal, 3 Laboratório de Citogenética do Instituto de Ciências Biomédicas de Abel Salazar, Porto, Portugal, 4 Center for Biomedical Network Research on Rare Diseases (CIBERER) and Department of Genetics and Microbiology, Universitat Autonoma de Barcelona, Barcelona, Spain, 5 Unidade de Hematologia Pediátrica do Centro Hospitalar do Porto, Porto, Portugal, 6 HematoPOiesis and Gene Therapy Division, CIEMAT, Madrid, Spain, 7 Serviço de hematologia do Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal

* amalmeida@ipolisboa.min-saude.pt

Abstract

Fanconi anaemia (FA) is an inherited disorder characterized by chromosomal instability. The phenotype is variable, which raises the possibility that it may be affected by other factors, such as epigenetic modifications. These play an important role in oncogenesis and may be pharmacologically manipulated. Our aim was to explore whether the epigenetic profiles in FA differ from non-FA individuals and whether these could be manipulated to alter the disease phenotype. We compared expression of epigenetic genes and DNA methylation profile of tumour suppressor genes between FA and normal samples. FA samples exhibited decreased expression levels of genes involved in epigenetic regulation and hypomethylation in the promoter regions of tumour suppressor genes. Treatment of FA cells with histone deacetylase inhibitor Vorinostat increased the expression of DNM3Tβ and reduced the levels of CIITA and HDAC9, PAK1, USP16, all involved in different aspects of epigenetic and immune regulation. Given the ability of Vorinostat to modulate epigenetic genes in FA patients, we investigated its functional effects on the FA phenotype. This was assessed by incubating FA cells with Vorinostat and quantifying chromosomal breaks induced by DNA cross-linking agents. Treatment of FA cells with Vorinostat resulted in a significant reduction of aberrant cells (81% on average). Our results suggest that epigenetic mechanisms may play a role in oncogenesis in FA. Epigenetic agents may be helpful in improving the phenotype of FA patients, potentially reducing tumour incidence in this population.
Introduction

Fanconi anaemia (FA) is an inherited disorder characterized by developmental abnormalities, bone marrow failure, leukemic progression and solid tumours, especially head and neck. At the cellular level, FA is characterized by impaired DNA repair and increased chromosomal fragility, a feature used in its diagnosis [1]. Mutations in 17 genes have been described, all with similar phenotypes, suggesting that all FA proteins function in common DNA repair pathway [2].

However, the severity of the disease varies even amongst patients from the same family and with the same mutation [3, 4].

It is therefore plausible that, alongside genetic mutations in FA genes, other factors may contribute to disease severity and increase the risk of neoplastic transformation.

Epigenetic modifications are important mechanisms by which cells regulate gene expression. DNA methylation and posttranslational modifications of histones affect chromatin structure, modulating gene expression and changes in cellular physiology and behavior [5]. There is ample evidence implicating epigenetic changes in the pathophysiology of MDS, AML and solid tumours [6–11]. In these malignancies, abnormal DNA methylation and histone deacetylation have been shown to silence tumour suppressor genes, and change normal expression of oncogenes, tumour suppressor genes and genes associated with several key cellular functions like DNA damage repair, cell cycle regulation, adhesion, motility, apoptosis and also signaling pathways [6, 8, 12]. For example, in ovarian and cervical cancers, hypermethylation of FANCF leads to its inactivation and to the disruption of the FA-BRCA pathway [13].

These epigenetic changes may be pharmacologically manipulated with DNA hypomethylating agents and histone deacetylase inhibitors (HDACi) [12, 14–16].

Vorinostat is an HDACi approved for the treatment of cutaneous T-cell-lymphoma. Vorinostat promotes protein acetylation, leading to the activation of genes involved in the control of cell cycle progression, differentiation and apoptosis. It also affects the expression of epigenetic regulator genes, contributing to their normal expression. In clinical trials it has shown promising clinical activity against hematological and solid tumours [17].

The aim of this study was to investigate whether epigenetic mechanisms could play a role in the pathophysiology of oncogenesis in FA and explore the potential of HDACi to improve the phenotype in FA.

Materials and Methods

Blood samples

Anonymized blood samples were obtained from twelve confirmed Fanconi anemia (FA) patients following written informed consent. Blood from healthy blood donors was used for normal controls. The study was approved by the Ethical Committee of Instituto Português de Oncologia de Lisboa, Francisco Gentil, EPE and all samples treated according to the Declaration of Helsinki.

In vitro cell cultures

Peripheral blood mononuclear cells (PBMC) from blood samples were separated with Ficcol (Sigma) and cultured in RPMI—1640 medium supplemented with 10% fetal bovine serum (GIBCO), 2mM L-glutamine and 100μg/ml penicillin/streptomycin (all from Gibco). Treatments were performed with 1μM Vorinostat (Selleck Chemicals) or vehicle for the indicated time points.
Gene Expression analysis by real time PCR (qPCR)

Total RNA was isolated from cells using the RNeasy Mini Kit (Qiagen), treated with DNase (Qiagen) and reverse-transcribed into cDNA using RT² First Strand Kit (Qiagen) according to the manufacturer’s protocol. qPCR was performed on Roche LightCycler 480 with 84 gene specific primers for Human Epigenetic Chromatin Modification Enzymes (PAHS-085G, SABiosciences, Qiagen). Data was analyzed according to manufacturer’s instructions.

In silico analysis

Bioinformatic analysis of gene expression in FA was performed using the expression array data published in Vanderwerf I [18].

Analysis of DNA Methylation

Genomic DNA was extracted from primary cells (1x10⁷/ml) using Citogene kit (Citomed), treated with RNase (Citomed) and digested using EpiTect Methyl DNA Restriction Kit (Qiagen) according to the manufacturer’s protocol. qPCR was performed on Roche LightCycler 480 with 94 gene specific primers for Human Tumour Suppressor Genes (EAHS-3550ZG, Qiagen). Samples from 2 FA patients were compared with 2 Healthy donors. Data analysis was performed according to manufacturer’s instructions.

Chromosomal instability assay

Whole blood (0,5ml) was cultured in RPMI-1640 (supplemented as above and cultures were stimulated with 5μg/ml of phytohemaglutinin (GIBCO) during 24h. Thereafter, the cultures were treated with 1μM Vorinostat or vehicle for an additional 24h at which point 0.05 μg/ml of 1,2,3,4-diepoxybutane (DEB, Sigma) or vehicle was added to the cultures for 48h. After 96h of culture, cells were treated with 2μg/ml of colcemid (GIBCO) for 1h, spread on slides, subjected to hypotonic lysis with 75mM KCl [19, 20] and fixed in solution of 3:1 volumes of methanol: acetic acid [21]. Slides were stained with 0,5M Leishamn (Sigma) in phosphate buffer, pH 6.8. Fifty metaphases per sample were analysed for chromosome aberrations including chromosome and chromatid breaks, acentric fragments and chromosome and chromatid-type exchange. Gaps were excluded and rearrangements were scored as two breaks for the calculation of percentage of cells with aberrations.

Cellular viability assays. Viability was assessed by flow cytometry with Annexin-V- FITC (Biolegend) and Propidium Iodide (PI) (Sigma-Aldrich).

Statistical analysis. Populations were compared using unpaired 2-tailed Student’s t test or One-way ANOVA, when appropriate (a p < 0.05 was considered significant) using the GraphPad Prism version 5.00 for Windows (GraphPad Software).

Results

The clinical characteristics of the patients whose samples were used in these experiments are detailed in Table 1.

Fanconi anemia patients exhibit different expression of epigenetic genes compared to healthy donors

To evaluate the hypothesis that epigenetic alterations in Fanconi anemia could contribute to susceptibility to cancer, we used the Human Epigenetic Chromatin Enzymes PCR array to quantify the expression of 84 genes involved in epigenetic modification of DNA and histones...
in PBMC from 12 FA and compared these to PBMC from 14 healthy donors. We found that 13 genes were differentially expressed in FA as compared to normal cells (Fig 1). These included genes encoding DNA methyltransferases (DNMT1, DNMT3B) and genes encoding histone modifying enzymes: acetylases (CIITA), phosphorylases (PAK1), ubiquitinases (RNF20), deacetylases (HDAC2, HDCA8, HDAC9, HDAC10, HDAC11) and also methyltransferases (SETD6). This differential expression between normal and FA individuals was confirmed bioinformatically from data from Vanderwerf et al. [18] for PAK1, USP16, DNMT1, DNMT3B, HDAC2, HDAC9, CIITA, HDAC10 and HDAC11 (S1 Fig).

FA cells exhibit DNA hypomethylation of tumour suppressor genes

In order to ascertain whether the findings from the gene expression assays translated into a difference in epigenetic patterns in FA compared to normal subjects, we studied the pattern of DNA methylation in FA PBMC. For this we used the Human Tumour Suppressor gene PCR Array to assess promoter DNA methylation of 94 tumour suppressor genes in 2 PBMC of FA patients and 2 healthy donors. This revealed a global aberrant hypomethylation of tumour suppressor genes in FA cells as compared to healthy donors. Six of 94 genes were differentially methylated in FA relative to healthy donors. These included genes whose function are related to apoptosis (CADM1, SFRP1), cell cycle (ING1), motility (CDH13), oxidative stress (LOX) and angiogenesis/transcription (CDX2) (Fig 2).

Vorinostat modifies the expression of epigenetic genes

Having observed differences in epigenetic regulator gene expression and in epigenetic patterns between FA and normal subjects, we tested whether these could be normalized using epigenetic agents. We chose Vorinostat to test this effect as it is a wide HDACi which able to modulate epigenetic and gene expression patterns[8] and with proven clinical efficacy. We tested its
effect on the expression of epigenetic chromatin modification genes which expression was altered in PBMC from FA patients (Fig 1). Following treatment of FA PBMC with Vorinostat for 8h and 16h (Fig 3) there was an increase in the expression levels of the $DNMT3\beta$ gene. Interestingly, Vorinostat treatment reduced the expression of $CIITA$ and $HDAC9$, involved in
the immune response, PAK1, regulator of the MAPK signaling pathway, and USP16, involved in regulating the activity of histone H2A. The expression levels of HDAC10, HDAC11, HDAC2, SETD6, RNF20 and DNMT1 genes were not significantly altered by Vorinostat.

**Vorinostat reduces chromosomal breaks in FA cells**

Given the capacity of Vorinostat to modulate the expression of epigenetic regulator genes in FA samples, we investigated its effect on the *in vitro* phenotype of FA cells. This functional effect of Vorinostat in FA was tested by assessing its effect on chromosome breaks induced by DEB. The percentage of aberrant cells induced by DEB was assessed on metaphases obtained from peripheral blood lymphocytes of patients with FA. Vorinostat reduced the percentage of aberrant cells (81% ± 21%, p = 0.06) in 6 patients with Fanconi anemia (Table 2, Fig 4A, 4B, 4C, 4D and 4E). There was no reduction in the number of spontaneous chromosomal breaks in FA cells following treatment with Vorinostat (Table 2).

In this experimental system Vorinostat did not significantly reduce the viability of cells (S2 Fig).

**Discussion**

Fanconi anaemia is an inherited disease caused by defective DNA repair. Whereas the skeletal, genitourinary and morphological abnormalities are rarely life-threatening, the development of bone marrow failure, leukemia and solid cancers are frequently lethal. The aggressiveness of these complications are compounded by the low tolerance FA patients have to chemotherapy and radiotherapy [6]. Therefore, a treatment option which could improve the DNA repair
defect and reduce the incidence of mutations and secondary malignancies would be highly desirable.

Epigenetic modifications consist in the addition or removal of small molecules onto DNA or DNA-associated proteins, i.e. histones, and enable eukaryotic cells to alter their gene expression without altering the DNA sequence [8, 22]. The molecules most commonly implicated in
epigenetic regulation are methyl and acetyl groups and DNA methylation status is closely related and plays a role in regulating histone acetylation [7, 10, 22]. In addition to the regulation of gene expression, these modifications, in particular DNA methylation, play an important role in maintaining DNA and chromatin stability [17].

Table 2. Number of breaks per cell in cultured lymphocytes from FA patients.

| Patients | Spontaneous breaks (n) | Vor 1μM | % reduction | DEB 0.05μM (n) | DEB 0.05μM + Vor 1μM (n) | % reduction |
|----------|------------------------|---------|-------------|----------------|-------------------------|-------------|
| FA1      | 0.28 (50)              | 0.32 (50) | 0.00        | 2.04 (50)      | 0.24 (50)               | 88.24       |
| FA2      | 0.08 (50)              | 0.14 (50) | 0.00        | 0.88 (50)      | 0.14 (50)               | 84.09       |
| FA3      | 0.18 (50)              | 0.18 (50) | 0.00        | 1.38 (50)      | 0.7 (50)                | 49.28       |
| FA4      | 0.8 (50)               | 0.32 (50) | 60.00       | 7.96 (50)      | 0.24 (50)               | 96.98       |
| FA5      | 0.44 (50)              | 0.34 (50) | 22.72       | 1.58 (50)      | 0.88 (50)               | 44.3        |
| FA6      | 0.12 (50)              | 0.25 (50) | 0.00        | 0.74 (50)      | 0.16 (50)               | 79.38       |
| Mean±SD  | 0.23±0.269             | 0.28±0.083 | 1.48±2.750 | 0.24±0.3151    |                         |             |

The effect of the Vorinostat was calculated by the percentage of reduction of the number of breaks per cell in the Vorinostat treatments relatively to Spontaneous breaks and DEB.

doi:10.1371/journal.pone.0139740.t002

Fig 4. Effect of vorinostat on DEB-induced chromosome fragility of FA lymphocytes. (A) Lymphocytes metaphase without treatment. (B) Lymphocyte metaphase treated with DEB. (C) Lymphocyte metaphase treated with DEB after Vorinostat treatment. The red arrows indicate aberrant chromosomes characteristic of FA cells. (D) Number of breaks per cell after treatment with Vorinostat. (E) Number of breaks per cell after treatment with Vorinostat on DEB-induced breaks. The p values are indicated.

doi:10.1371/journal.pone.0139740.g004
Aberrant epigenetic patterns have been implicated in the pathophysiology of a variety of haematological and solid tumours [23]. These have been successfully manipulated pharmacologically with promising therapeutic results [8, 24, 25].

Our aim was to investigate whether the epigenetic machinery in FA differs from that of unaffected individuals and whether its manipulation could somehow affect the FA phenotype.

In the present study, we show that FA cells present decreased levels of several genes involved in epigenetic regulation as compared to cells from healthy subjects (Fig 1) Gene expression studies revealed that DNMT1 and DNMT3β expression, involved in DNA methylation, were significantly reduced in FA patients both in PBMCs and BM. DNMT1 is responsible for copying DNA methylation patterns established during embryonic development and their subsequent maintenance. DNMT3β is a tumour-suppressor gene with a critical role in DNA methylation playing a major role in the establishment and maintenance of genomic methylation patterns. Reduced activity of DNMT1 and DNMT3β may lead to DNA hypomethylation, inducing genomic instability and disruption of proto-oncogenes and is directly associated with tumour formation [26–28]. Abnormal expression of DNMT1 and DNMT3β has been reported in several tumours, including lung, liver, breast, ovarian, colorectal, meningiomas and lymphomas [29, 30]. Reduced expression of CIITA was observed in PBMCs but not in BM. This gene regulates the expression of MHCII gene by recruiting the transcriptional machinery of basic proteins, acetyltransferases, histone deacetylases (HDAC), and other proteins involved in chromatin remodeling. CIITA expression is decreased in several types of hematological and solid tumours and is associated with decreased tumour immune recognition [31].

The expression of SETD6, a gene that may also impair anti-tumour immune response by dysregulation of the NF-κB pathway [32], was also reduced in FA PMBCs but remain roughly the same in BM.

RNF20, which is essential for the regulation of normal levels of p53 [33], is also underexpressed in FA. Its reduced expression causes chromosome instability and has been described in several types of tumours [34–48]. The decreased expression of RNF20 enhances the transcriptional effects of EGF, leading to an increase in transformation, migration and metastasis of cancer cells and tumourigenesis [5, 23, 49].

We also found reduced expression of histone deacetylases HDAC 2, 8, 9, 10 and 11 in FA PMBCs and confirmed HDAC2 and HDAC9 reduced expression in FA BM samples. HDACs regulate chromatin remodeling and regulate many genes involved both in the initiation and progression of a variety of cancers.

Despite some discrepancy between our data, obtained from blood samples and the bioinformatics analysis of gene expression data, obtained from bone marrow samples, both concur that there is altered expression of epigenetic regulating genes in FA. A larger number of samples would be required to reach firm conclusions.

The reduced expression of these genes in FA, suggests that these patients have altered epigenetic regulation, which may be involved in the neoplastic complications of this disease. This hypothesis is corroborated by our finding showing increased DNA hypomethylation at tumor suppressor gene loci in FA as compared to normal cells (Fig 2). Liu et al have described aberrant expression of tumor suppressor and tumor-related genes in FA, corroborating our data [50]. It is possible that this hypomethylation may increase genomic instability and have an additive effect on the DNA repair defect of FA, increasing the oncogenic potential in FA tissues.

Our initial results show that epigenetic regulation and DNA methylation are altered in FA. These findings suggested that epigenetic manipulation in FA may have a beneficial effect on this disease phenotype.
In fact, treatment of FA cells with Vorinostat induced expression of the DNMT3β, involved in the maintenance of physiological DNA methylation (Fig 3). The suppression of expression of CIITA and HDAC9 are consistent with a reduction in inflammatory response, which may play a role in oncogenesis in the context of DNA repair defects.

Of particular relevance was our finding that Vorinostat treatment reduced chromosomal breaks in FA cells (Fig 4). This may have been due to DNA stabilization through induction of DNMT3β or mediated by another mechanism yet to be investigated. Whichever mechanism at play, our findings are very suggestive that Vorinostat exerts a protective effect on the chromosomal breaks induced by cross-linking agents. This effect may have an important clinical counterpart as it may translate into greater tolerability to chemotherapeutic agents in FA patients.

These results are the first report of a potential improvement in FA phenotype with an epigenetic agent. They warrant further pre-clinical testing in animal models with a view to initiating clinical trials in FA if the results remain promising.

Supporting Information

S1 Fig. FA patients have altered expression of epigenetic chromatin modification enzymes. Each panel (A-J) represents the expression of selected genes of interest in FA and control samples as described in materials and methods section. With the exception of DNMT3B, there is significant difference in the expression of these genes in FA compared to normal samples (* 0.05 > p; ** 0.01 > p; *** 0.001 > p).

S2 Fig. Vorinostat has no effect on the viability of DEB-treated cells. Viability PBMC of FA patients as determined by Annexin V/PI staining following treatment with Vorinostat and DEB in cell culture medium as used to test for chromosomal fragility.

Acknowledgments

The authors would like to thank volunteers that provided blood samples.

Author Contributions

Conceived and designed the experiments: HB GS BC AA JS. Performed the experiments: HB GS BC JM. Analyzed the data: HB GS BC AA JM. Contributed reagents/materials/analysis tools: BP JB JC JAC MB HS EC JB JS. Wrote the paper: HB GS BC JS AA.

References

1. Auerbach AD. Fanconi anemia and its diagnosis. Mutat Res. 2009; 668(1–2):4–10. Epub 2009/07/23. doi: 10.1016/j.mrfmmm.2009.01.013 S0027-5107(09)00053-0 [pii]. PMID: 19622403; PubMed Central PMCID: PMC2742943.

2. Sawyer SL, Tian L, Kahkonen M, Schwartzentruber J, Kircher M, Majewski J, et al. Biallelic mutations in BRCA1 cause a new Fanconi anemia subtype. Cancer Discov. 2015; 5(2):135–42. Epub 2014/12/05. doi: 10.1158/2159-8290.CD-14-1156 2159-8290.CD-14-1156 [pii]. PMID: 25472942; PubMed Central PMCID: PMC4320660.

3. Soulier J. Fanconi anemia. Hematology Am Soc Hematol Educ Program. 2011; 2011:492–7. Epub 2011/12/14. doi: 10.1182/asheducation-2011.1.492 2011/1/492 [pii]. PMID: 22160080.

4. Geiselhart A, Lier A, Walter D, Milsom MD. Disrupted Signaling through the Fanconi Anemia Pathway Leads to Dysfunctional Hematopoietic Stem Cell Biology: Underlying Mechanisms and Potential Therapeutic Strategies. Anemia. 2012; 2012:265790. Epub 2012/06/08. doi: 10.1155/2012/265790 PMID: 22675615; PubMed Central PMCID: PMC3366203.
5. Feinberg AP, Tycko B. The history of cancer epigenetics. Nat Rev Cancer. 2004; 4(2):143–53. Epub 2004/01/21. doi: 10.1038/nrc1279 nrc1279 [pii]. PMID: 14732866.

6. Ganesan A, Nolan L, Crabb SJ, Packham G. Epigenetic therapy: histone acetylation, DNA methylation and anti-cancer drug discovery. Curr Cancer Drug Targets. 2009; 9(9):963–81. Epub 2009/12/23. PMID: 20025605.

7. Issa JP. Epigenetic changes in the myelodysplastic syndrome. Hematol Oncol Clin North Am. 2010; 24 (2):317–30. Epub 2010/04/03. doi: 10.1016/j.hoc.2010.02.007 S0889-8588(10)00028-6 [pii]. PMID: 20359628; PubMed Central PMCID: PMC2848959.

8. Jones PA, Baylin SB. The epigenomics of cancer. Cell. 2007; 128(4):683–92. Epub 2007/02/27. S0092-8674(07)00127-4 [pii] doi: 10.1016/j.cell.2007.01.029 PMID: 17320506; PubMed Central PMCID: PMC3894624.

9. Plass C, Oakes C, Blum W, Marcucci G. Epigenetics in acute myeloid leukemia. Semin Oncol. 2008; 35(4):378–87. Epub 2008/08/12. doi: 10.1053/j.seminoncol.2008.04.008 S0093-7754(08)00118-8 [pii]. PMID: 18692688; PubMed Central PMCID: PMC3463865.

10. Seligson DB, Horvath S, Shi T, Yu H, Tze S, Grunstein M, et al. Global histone modification patterns predict risk of prostate cancer recurrence. Nature. 2005; 435(7046):1262–6. Epub 2005/07/01. nature03672 [pii] doi:10.1038/nature03672 PMID: 15988529.

11. Stintzing S, Kemmerling R, Kiesslich T, Alinger B, Ocker M, Neureiter D. Myelodysplastic syndrome and histone deacetylase inhibitors: “to be or not to be acetylated”? J Biomed Biotechnol. 2011; 2011:214143. Epub 2011/06/02. doi:10.1155/2011/214143 PMID: 21629744; PubMed Central PMCID: PMC3100562.

12. Jain N, Rossi A, Garcia-Manero G. Epigenetic therapy of leukemia: An update. Int J Biochem Cell Biol. 2009; 41(1):72–80. Epub 2008/10/25. doi: 10.1016/j.biocel.2008.10.006 S1357-2725(08)00416-0 [pii]. PMID: 18948224; PubMed Central PMCID: PMC3833715.

13. Taniguchi T, Tischkowitz M, Ameziane N, Hodgson SV, Mathew CG, Joenje H, et al. Disruption of the Fanconi anemia-BRCA pathway in cisplatin-sensitive ovarian tumors. Nat Med. 2003; 9(5):568–74. Epub 2003/04/15. doi: 10.1038/nm852 nm852 [pii] PMID: 12692539.

14. Griffiths EA, Gore SD. DNA methyltransferase and histone deacetylase inhibitors in the treatment of myelodysplastic syndromes. Semin Hematol. 2008; 45(1):23–30. Epub 2008/01/09. doi: 10.1053/j.seminhematol.2007.11.007 S0037-1963(07)00164-3 [pii]. PMID: 18179966; PubMed Central PMCID: PMC2234265.

15. Narayanan G, Arias-Pulido H, Nandula SV, Basso K, Sugirtharaj DD, Vargas H, et al. Promoter hypermethylation of FANCF: disruption of Fanconi Anemia-BRCA pathway in cervical cancer. Cancer Res. 2004; 64(9):2994–7. Epub 2004/05/06. PMID: 15126331.

16. Wang Z, Li M, Lu S, Zhang Y, Wang H. Promoter hypermethylation of FANCF plays an important role in the occurrence of ovarian cancer through disrupting Fanconi anemia-BRCA pathway. Cancer Biol Ther. 2006; 5(3):256–60. Epub 2006/01/19. 2380 [pii]. PMID: 16418574.

17. Siegel D, Hussein M, Belani C, Robert F, Galanis E, Richon VM, et al. Vorinostat in solid and hematologic malignancies. J Hematol Oncol. 2009; 2:31. Epub 2009/07/29. doi: 10.1186/1757-8722-2-31 1756-8722-2-31 [pii]. PMID: 19635146; PubMed Central PMCID: PMC2731787.

18. Vanderwerf SM, Svingen J, Olson S, Rathbun RK, Harrington C, Yates J, et al. TLR8-dependent TNF-(alpha) overexpression in Fanconi anemia group C cells. Blood. 2009; 114(26):5290–8. Epub 2009/10/24. doi: 10.1182/blood-2009-05-222414 blood-2009-05-222414 [pii]. PMID: 19857403; PubMed Central PMCID: PMC2796134.

19. Silva G, Cardoso BA, Belo H, Almeida AM. Vorinostat induces apoptosis and differentiation in myeloid malignancies: genetic and molecular mechanisms. PLoS One. 2013; 8(1):e53766. Epub 2013/01/16. doi:10.1371/journal.pone.0053766 PONE-D-12-19513 [pii]. PMID: 23320102; PubMed Central PMCID: PMC3540071.

20. Richon VM, Garcia-Vargas J, Hardwick JS. Development of vorinostat: current applications and future perspectives for cancer therapy. Cancer Lett. 2009; 280(2):201–10. Epub 2009/02/03. doi: 10.1016/j.canlet.2009.01.002 S0304-8853(09)00002-0 [pii]. PMID: 19181442.

21. Kennedy RD, D’Andrea AD. DNA repair pathways in clinical practice: lessons from pediatric cancer susceptibility syndromes. J Clin Oncol. 2006; 24(23):3799–808. Epub 2006/08/10. 24/23/3799 [pii] doi:10.1200/JCO.2005.05.4171 PMID: 16896009.

22. Andreoli F, Barbosa AJ, Parenti MD, Del Río A. Modulation of epigenetic targets for anticancer therapy: clinicopathological relevance, structural data and drug discovery perspectives. Curr Pharm Des. 2013; 19(4):578–613. Epub 2012/09/29. CPD-EPUB-20120926-1 [pii] PMID: 23016851; PubMed Central PMCID: PMC3529403.

23. Rodriguez J, Frigola J, Vendrell E, Risques RA, Fraga MF, Morales C, et al. Chromosomal instability correlates with genome-wide DNA demethylation in human primary colorectal cancers. Cancer Res.
Chernikova SB, Shima K, Irahara N, Kure S, Baba Y, Kirkner GJ, et al. DNMT3B expression might contribute to CpG island methylator phenotype in colorectal cancer. Clin Cancer Res. 2009; 15(11):3663–71. Epub 2009/05/28. doi: 10.1158/1078-0432.CCR-08-2383 1078-0432.CCR-08-2383 [pii]. PMID: 19470733; PubMed Central PMCID: PMC2866637.

Bai X, Song Z, Fu Y, Yu Z, Zhao L, Zhao H, et al. Clinicopathological significance and prognostic value of DNA methyltransferase 1, 3a, and 3b expressions in sporadic epithelial ovarian cancer. PLoS One. 2012; 7(6):e40024. Epub 2012/07/07. doi: 10.1371/journal.pone.0040024 PONE-D-12-04122 [pii]. PMID: 22768205; PubMed Central PMCID: PMC3386927.

Nosho K, Shima K, Irahara N, Morimoto Y, Itoh T, Minamino M, et al. Deficiency in mamalian histone H2B ubiquitin ligase Bre1 (Rnf20/Rnf40) leads to replication stress and chromosomal instability. Cancer Res. 2012; 72(8):2111–9. Epub 2012/05/09. doi: 10.1158/0008-5472.CAN-11-2209 0008-5472.CAN-11-2209 [pii]. PMID: 22354749; PubMed Central PMCID: PMC3328627.

Levy D, Kuo AJ, Chang Y, Schaefer U, Kitson C, Cheung P, et al. Lysine methylation of the NF-kappaB subunit RelA by SETD6 couples activity of the histone methyltransferase GLP at chromatin to tonic repression of NF-kappaB signaling. Nat Immunol. 2011; 12(1):29–36. Epub 2010/12/07. doi: 10.1038/ni.1968 10.1038/ni.1968 [pii]. PMID: 21131967; PubMed Central PMCID: PMC3074206.

Karim M, Cao Y, Greten FR, Li ZW. NF-kappaB in cancer: from innocent bystander to major culprit. Nat Rev Cancer. 2002; 2(4):337–47. Epub 2002/03/30. doi: 10.1038/sj.bjc.6601602 6601602 [pii] PMID: 12081440.

Milde-Langosch K, Janke S, Wagner I, Schroder C, Streichert T, Bamberger AM, et al. Role of Fra-2 in breast cancer: influence on tumor cell invasion and motility. Breast Cancer Res Treat. 2008; 107(3):337–47. Epub 2007/03/30. doi: 10.1007/s10549-007-9559-y PMID: 17393299.

Chernikova SB, Razorenova OV, Higgins JP, Sishc BJ, Nicolau M, Dorth JA, et al. Deficiency in mamalian histone H2B ubiquitin ligase Bre1 (Rnf20/Rnf40) leads to replication stress and chromosomal instability. Cancer Res. 2012; 72(8):2111–9. Epub 2012/02/23. doi: 10.1158/0008-5472.CAN-11-2209 0008-5472.CAN-11-2209 [pii]. PMID: 22354749; PubMed Central PMCID: PMC3328627.

Shema E, Tirosch I, Aylon Y, Huang J, Ye C, Moskovits N, et al. The histone H2B-specific ubiquitin ligase RNF20/NBRE1 acts as a putative tumor suppressor through selective regulation of gene expression. Genes Dev. 2008; 22(19):2664–76. Epub 2008/10/04. doi: 10.1101/gad.1703008 17/0308 22/19/2664 [pii]. PMID: 18832071; PubMed Central PMCID: PMC2559905.

Glozak MA, Seto E. Histone deacetylases and cancer. Oncogene. 2007; 26(37):5420–32. Epub 2007/08/19. 1210610 [pii] doi: 10.1038/sj.onc.1210610 PMID: 17694083.

Gallinari P, Di Marco S, Jones P, Pallao R, Steinkühler C. HDACs, histone deacetylation and gene transcription: from molecular biology to cancer therapeutics. Cell Res. 2007; 17(3):195–211. Epub 2007/02/28. 7310149 [pii] doi: 10.1038/sj.cr.7310149 PMID: 17325692.

Jung KH, Noh JH, Kim JK, Eun JW, Bae HJ, Xie HJ, et al. HDAC2 overexpression confers oncogenic potential to human lung cancer cells by deregulating expression of apoptosis and cell cycle proteins. J Cell Biochem. 2012; 113(6):2167–77. Epub 2012/04/12. doi: 10.1002/jcb.24090 PMID: 22492270.

Noh JH, Jung KH, Kim JK, Eun JW, Bae HJ, Xie HJ, et al. Aberrant regulation of HDAC2 mediates proliferation of hepatocellular carcinoma cells by deregulating expression of G1/S cell cycle proteins. PLoS One. 2011; 6(11):e28103. Epub 2011/12/02. doi: 10.1371/journal.pone.0028103 PONE-D-11-13259 [pii]. PMID: 22132221; PubMed Central PMCID: PMC3223227.

Waltregny D, Glenisson W, Tran SL, North BJ, Verdin E, Collge A, et al. Histone deacetylase HDAC8 associates with smooth muscle alpha-actin and is essential for smooth muscle cell contractility. FASEB J. 2005; 19(8):966–8. Epub 2005/03/18. 04-2303fje [pii] doi: 10.1096/fj.04-2303fje PMID: 15772115.

Oehme I, Deubzer HE, Wegener D, Pickert D, Linke JP, Hero B, et al. Histone deacetylase 8 in neuroblastoma tumorigenesis. Clin Cancer Res. 2009; 15(1):91–9. Epub 2009/01/02. doi: 10.1158/1078-0432.CCR-08-0684 15/1/91 [pii]. PMID: 19118036.

Dearolf MA, Bando M, Nakato R, Wattrin E, Itho T, Minamino M, et al. HDAC8 mutations in Cornelia de Lange syndrome affect the cohesin acetylation cycle. Nature. 2012; 489(7415):313–7. Epub 2012/
41. Harakalova M, van den Boogaard MJ, Sinke R, van Lieshout S, van Tuil MC, Duran K, et al. X-exome sequencing identifies a HDAC8 variant in a large pedigree with X-linked intellectual disability, truncal obesity, gynaecomastia, hypogonadism and unusual face. J Med Genet. 2012; 49(8):539–43. Epub 2012/08/15. doi: 10.1136/jmedgenet-2012-100921 jmedgenet-2012-100921 [pii]. PMID: 22889856.

42. Zhang CL, McKinsey TA, Chang S, Antos CL, Hill JA, Olson EN. Class II histone deacetylases act as signal-responsive repressors of cardiac hypertrophy. Cell. 2002; 110(4):479–88. Epub 2002/08/31. S0092867402008619 [pii]. PMID: 12202037.

43. de Zoeten EF, Wang L, Sai H, Dillmann WH, Hancock WW. Inhibition of HDAC9 increases T regulatory cell function and prevents colitis in mice. Gastroenterology. 2010; 138(2):583–94. Epub 2009/11/03. doi: 10.1053/j.gastro.2009.10.037 S0016-5085(09)01934-9 [pii]. PMID: 19879272; PubMed Central PMCID: PMC3369426.

44. Milde T, Oehme I, Korshunov A, Kopp-Schneider A, Remke M, Northcott P, et al. HDAC5 and HDAC9 in medulloblastoma: novel markers for risk stratification and role in tumor cell growth. Clin Cancer Res. 2010; 16(12):3240–52. Epub 2010/04/24. doi: 10.1158/1078-0432.CCR-10-0395 1078-0432.CCR-10-0395 [pii]. PMID: 20413433.

45. Lucio-Eterovic AK, Cortez MA, Valera ET, Motta FJ, Queiroz RG, Machado HR, et al. Differential expression of 12 histone deacetylase (HDAC) genes in astrocytomas and normal brain tissue: class II and IV are hypoxressed in glioblastomas. BMC Cancer. 2008; 8:243. Epub 2008/08/21. doi: 10.1186/1471-2407-8-243 1471-2407-8-243 [pii]. PMID: 18713462; PubMed Central PMCID: PMC2536671.

46. Osada H, Tatematsu Y, Saito H, Yatabe Y, Mitsudomi T, Takahashi T. Reduced expression of class II histone deacetylase genes is associated with poor prognosis in lung cancer patients. Int J Cancer. 2004; 112(1):26–32. Epub 2004/08/12. doi: 10.1002/ijc.20395 PMID: 15305723.

47. Villagrasa A, Cheng F, Wang HW, Suarez I, Gluzak M, Maurin M, et al. The histone deacetylase HDAC11 regulates the expression of interleukin 10 and immune tolerance. Nat Immunol. 2009; 10(1):92–100. Epub 2008/11/18. doi: 10.1038/ni.1673 n.1673 [pii]. PMID: 19011628; PubMed Central PMCID: PMC925685.

48. Bugilo D, Khaskhely NM, Voo KS, Martinez-Valdez H, Liu YJ, Younes A. HDAC11 plays an essential role in regulating OX40 ligand expression in Hodgkin lymphoma. Blood. 2011; 117(10):2910–7. Epub 2011/01/18. doi: 10.1182/blood-2010-08-303701 blood-2010-08-303701 [pii]. PMID: 21239696; PubMed Central PMCID: PMC3062301.

49. Costello JF, Plass C. Methylation matters. J Med Genet. 2001; 38(5):285–303. Epub 2001/05/23. PMID: 11333864; PubMed Central PMCID: PMC1734882.

50. Liu GH, Suzuki K, Li M, Qu J, Montserrat N, Tarantino C, et al. Modelling Fanconi anemia pathogenesis and therapeutics using integration-free patient-derived iPSCs. Nat Commun. 2014; 5:4330. Epub 2014/07/08. doi: 10.1038/ncomms5330 ncomms5330 [pii]. PMID: 24999918; PubMed Central PMCID: PMC4291073.