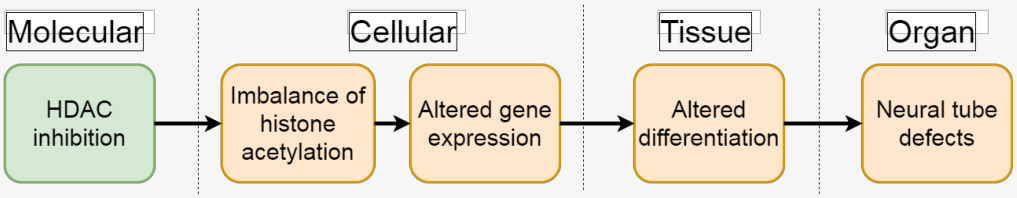
AOP ID and Title:

AOP 275: Histone deacetylase inhibition leads to neural tube defects
Short Title: HDAC inhibition leads to neural tube defects

Graphical Representation



Authors

Tanja Waldmann

Status

Author status	OECD status	OECD project	SAAOP status
Under Development: Contributions and Comments Welcome			

Abstract

The expression and function of histone deacetylases (HDAC) are well known during embryonic development and especially plays a pivotal role in the development of the nervous system. HDAC inhibition during embryonic development has been correlated to several congenital malformations mainly affecting neurodevelopment. However, the kind of malformation strongly depends on the timing of disturbance, i.e. when during embryonic development the exposure occurred. This AOP concentrates on disturbances by HDAC inhibition during the first weeks of neurodevelopment, before or around the time point of neural tube closure. Therefore, this AOP suggests a mechanism how HDAC inhibitors could lead to the observed neural tube defects. It assumes that HDAC inhibition leads to an imbalance of histone modifications and eventually to altered gene expression. In the next KE altered gene expression may lead to a wrong differentiation of neuroectodermal cells that cannot close the neural tube anymore and therefore leads to neural tube closure defects. This AOP is linked to case study 2 that investigates the effects of VPA and its structural analogs the EU-ToxRisk DART (development and reproductive toxicology) test methods.

Summary of the AOP

Events

Molecular Initiating Events (MIE), Key Events (KE), Adverse Outcomes (AO)

Sequence	Type	Event ID	Title	Short name
1	MIE	1502	Histone deacetylase inhibition	Histone deacetylase inhibition
2	KE	1503	Histone acetylation, increase	Histone acetylation, increase
3	KE	1239	Altered, Gene Expression	Altered, Gene Expression
4	KE	1560	Altered differentiation	Altered differentiation
5	AO	1561	Neural tube defects	Neural tube defects

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Histone deacetylase inhibition	adjacent	Histone acetylation, increase	Not Specified	Not Specified
Histone acetylation, increase	adjacent	Altered, Gene Expression	Not Specified	Not Specified
Altered, Gene Expression	adjacent	Altered differentiation	Not Specified	Not Specified

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Altered differentiation	adjacent	Neural tube defects	Not Specified	Not Specified

Overall Assessment of the AOP

References

Appendix 1

List of MIEs in this AOP

[Event: 1502: Histone deacetylase inhibition]

Short Name: Histone deacetylase inhibition

Key Event Component

Process	Object	Action
enzyme inhibitor activity	histone deacetylase 1	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:212 - Histone deacetylase inhibition leading to testicular atrophy	MolecularInitiatingEvent
Aop:274 - Histone deacetylase inhibition leads to impeded craniofacial development	MolecularInitiatingEvent
Aop:275 - Histone deacetylase inhibition leads to neural tube defects	MolecularInitiatingEvent

Stressors

Name
Methoxyacetic acid
Butyrate
Trichostatin A
Valproic acid
Suberoylanilide hydroxamic acid
MS-275
Apicidin
Rocilinostat / Ricolinostat

Biological Context

Level of Biological Organization
Molecular

Cell term

Cell term
cell

Organ term

Organ term

organ

Evidence for Perturbation by Stressor

Overview for Molecular Initiating Event

HDIs are classified according to chemical nature and mode of mechanism: the short-chain fatty acids (e.g., butyrate, valproate), hydroxamic acids (e.g., suberoylanilide hydroxamic acid or SAHA, Trichostatin A or TSA), cyclic tetrapeptides (e.g., FK-228), benzamides (e.g., N-acetyldinaline and MS-275) and epoxides (depeudecin, trapoxin A) [Richon et al., 2003; Ropero and Esteller, 2007; Villar-Garea et al., 2004]. There is a report showing that TSA and butyrate competitively inhibit HDAC activity [Sekhavat et al., 2007]. HDIs inhibit preferentially HDACs with some selectiveness [Hu et al., 2003]. TSA (Trichostatin A) inhibits class I and II of HDACs, while butyrate inhibits class I and IIa (HDACs 4, 5, 7, 9) of HDACs [Ooi et al., 2015; Park and Sohrabji, 2016; Wagner et al., 2015]. TSA inhibits HDAC1, 2, and 3 [Damaskos et al., 2016], whereas MS-27-275 has an inhibitory effect for HDAC1 and HDAC3 (IC_{50} value of ~0.3 microM and ~8 microM, respectively), but no effect for HDAC8 (IC_{50} value >100 microM) [Hu et al., 2003].

Rocilinostat / Ricolinostat

Rocilinostat / Ricolinostat is the first oral selective HDAC6 inhibitor.

Ricolinostat plus lenalidomide, and dexamethasone in relapsed or refractory multiple myeloma: a multicentre phase 1b trial

By: Yee, Andrew J.; Bensinger, William I.; Supko, Jeffrey G.; Voorhees, Peter M.; Berdeja, Jesus G.; Richardson, Paul G.; Libby, Edward N.; Wallace, Ellen E.; Birrer, Nicole E.; Burke, Jill N.; et al

Lancet Oncology (2016), 17(11), 1569-1578 | Language: English, Database: CAplus and MEDLINE DOI: 10.1016/s1470-2045(16)30375-8

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	High	NCBI
human	Homo sapiens	High	NCBI
mouse	Mus musculus	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	Moderate

Sex Applicability

Sex	Evidence
Unspecific	High

The inhibition of HDAC by HDIs is well conserved between species from lower organisms to mammals.

- HDAC inhibition restores the rate of resorption of subretinal blebs in hyperglycemia in brown Norway rat and HDAC activity was inhibited with HDIs in human ARPE19 cells [Desjardins et al., 2016].
- Treatment of HDIs inducing HDAC inhibition showed anti-tumor effects in human non-small cell lung cancer cells [Ansari et al., 2016; Miyanaga et al., 2008].
- HDAC acetylation level was increased by HDIs in the MRL-lpr/lpr murine model of lupus splenocytes [Mishra et al., 2003].
- SAHA increased histone acetylation in the brain and spleen of mice [Hockly et al., 2003].
- MAA inhibits HDAC activity in HeLa cells and spleens from C57BL/6 mice [Jansen et al., 2004].
- It is also reported that MAA inhibits HDAC activity in testis cytosolic and nuclear extract of juvenile rats (27 days old) [Wade et al., 2008].
- VPA and TSA inhibit HDAC enzymatic activity in the mouse embryo and human HeLa cell nuclear extract [Di Renzo et al., 2007].
- The treatment with HDAC inhibitors, phenylbutyrate (PB) (2 mM) and TSA (200 nM), inhibits HDAC in adjuvant arthritis synovial cells derived from rats, causing higher acetylated histone [Chung et al., 2003].

Key Event Description

Nucleosomes consist of eight core histones, two of each histone H2A, H2B, H3, and H4 [Damaskos et al., 2017]. DNA strands

(about 200 bp) wind around the core histones, which can be modified on their N-terminal ends. One possible modification is the acetylation of lysine residues, which decreases the binding strength between DNA and the core histone. Histone deacetylases (HDACs) hydrolyze the acetyl residues [Damaskos et al., 2017]. HDACs remove the acetyl groups from the lysine residues leading to the formation of a condensed and transcriptionally silenced chromatin. Thus, the inhibition of HDAC blocks this action and can result in hyperacetylation of histones associated mostly with increases in transcriptional activation. Histone deacetylase inhibitor (HDI) inhibits HDAC, causing increased acetylation of the histones and thereby facilitating binding of transcription factors [Taunton et al., 1996].

It is known that eukaryotic HDAC isoforms are classified into four classes: class I HDACs (isoforms 1, 2, 3, 8), class II HDACs (isoforms 4, 5, 6, 7, 9, 10), class III HDACs (the sirtuins), and HDAC11 [Gregoretti et al., 2004; Weichert, 2009; Barneda-Zahonero and Parra, 2012]. HDACs 1, 2, and 3 are ubiquitously expressed, whereas HDAC8 is predominantly expressed in cells with smooth muscle/myoepithelial differentiation [Weichert, 2009]. HDAC6 is not observed to be expressed in lymphocytes, stromal cells, and vascular endothelial cells [Weichert, 2009]. Class III HDACs, sirtuins, are widely expressed and localized in different cellular compartments [Barneda-Zahonero and Parra, 2012]. SirT1 is highly expressed in testis, thymus, and multiple types of germ cells [Bell et al., 2014]. HDAC11 expression is enriched in the kidney, brain, testis, heart, and skeletal muscle [Barneda-Zahonero and Parra, 2012]. The members of classes 1, 2, and 4 are dependent on a zinc ion and a water molecule at their active sites, for their deacetylase function. The Sirtuins of class 3 depend on NAD^+ and are considered impervious to most known HDAC inhibitors [Drummond et al., 2005].

Several structurally distinct groups of compounds have been found to inhibit HDACs of class 1, 2, and 4, among others short-chain fatty acids (e.g. butyrate and VPA), hydroxamic acids (e.g. TSA and SAHA), and epoxyketones (e.g. Trapoxin) [Drummond et al., 2005]. The hydroxamic acids seem to exert their inhibitory action by mimicking the acetyl-lysine target of HDACs, chelating the zinc ion in the active site, and displacing the water molecule [Finnin et al., 1999]. Several high-resolution crystal structures support this mode of inhibition [Decroos et al., 2015; Luckhurst et al., 2016]. The mode of inhibition of epoxyketones seems to function in the formation of a stable transition state analog with the zinc ion in the active site [Porter and Christianson, 2017]. The inhibitory actions of the short-chain fatty acids are less well defined, but it has been speculated that VPA blocks access to the binding pocket [Göttlicher et al., 2001]. It has been shown that VPA exerts similar gene regulatory effects to TSA, on a panel of migration-related transcripts in neural crest cells [Dreser et al., 2015], supporting a mode of action similar to hydroxamic-acid type HDAC inhibitors. Some *in silico* methods including molecular modeling, virtual screening, and molecular dynamics are used to find the common HDAC inhibitor structures [Huang et al., 2016; Yanuar et al. 2016].

How it is Measured or Detected

The measurement of HDAC inhibition monitors changes in histone acetylation. HDAC inhibition can be detected directly by the measurement of HDAC activity using commercially available colorimetric or fluorimetric kits or indirectly by the increase of histone acetylation as the detection of global histone acetylation changes by Western blot or mass spectrometry (MS)-based proteomics methods or as detection of site-specific histone acetylation changes using chromatin immunoprecipitation (ChIP) or ChIP-on-Chip. The measurement methods include the immunological detection of histone acetylation with anti-acetylated histone antibodies [Richon et al., 2004]. The histones are isolated from pellets of cells treated with HDIs, followed by acid-urea-triton gel electrophoresis, western blotting, and immunohistochemistry [Richon et al., 2003]. The HDAC activity is measured directly with ultra-high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UHPLC-ESI-MS/MS) by calculating the ratio of deacetylated peptide and acetylated peptide [Zwick et al., 2016].

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List of Key Events in the AOP

Event: 1503: Histone acetylation, increase

Short Name: Histone acetylation, increase

Key Event Component

Process	Object	Action
regulation of protein modification process	histone	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:212 - Histone deacetylase inhibition leading to testicular atrophy	KeyEvent
Aop:275 - Histone deacetylase inhibition leads to neural tube defects	KeyEvent

Biological Context

Level of Biological Organization

Cellular

Cell term

Cell term

cell

Organ term

Organ term

organ

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	High	NCBI
human	Homo sapiens	High	NCBI
mouse	Mus musculus	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Not Otherwise Specified	Moderate

Sex Applicability

Sex	Evidence
Unspecific	High

The histone acetylation increase by HDIs is well conserved between species from lower organisms to mammals.

MAA, an HDAC inhibitor, induces acetylation of histones H3 and H4 in Sprague-Dawley rats (*Rattus norvegicus)* [Wade et al., 2008].

It is also reported that MAA promotes acetylation of H4 in HeLa cells (*Homo sapiens*) and spleens from C57BL/6 mice (*Mus musculus*) treated with MAA [Jansen et al., 2014].

VPA, an HDAC inhibitor, induces hyperacetylation of histone H4 in protein extract of mouse embryos (*Mus musculus*) exposed *in utero* for 1 hr to VPA [Di Renzo et al., 2007a].

Apicidin, MS-275 and sodium butyrate, HDAC inhibitors, induce hyperacetylation of histone H4 in homogenates from mouse embryos (*Mus musculus*) after the treatments [Di Renzo et al., 2007b].

MAA acetylates histones H3K9 and H4K12 in limbs of CD1 mice (*Mus musculus*) [Dayan and Hales, 2014].

Key Event Description

Gene transcription is regulated with the balance between acetylation and deacetylation. A dynamic balance of histone acetylation and histone deacetylation is typically catalyzed by enzymes with histone acetyltransferase (HAT) and HDAC activities. Histone acetylation relaxes chromatin and makes it accessible to transcription factors, whereas deacetylation is associated with gene silencing. The balance can be disturbed also by targeting HAT, not only HDACs. At least theoretically, an activation of HAT might lead to an increase in histone acetylation. The acetylation and deacetylation are modulated on the NH_3^+ groups of lysine amino acid residues in histones. HDAC inhibition promotes hyperacetylation by inhibiting the deacetylation of histones with classes of H2A, H2B, H3, and H4 in nucleosomes. [Wade et al., 2008]. The inhibition of HDACs results in an accumulation of acetylated proteins such as tubulin or histones.

How it is Measured or Detected

Histone acetylation is measured by the immunological detection of histone acetylation with anti-acetylated histone antibodies [Richon et al., 2004]. Histone acetylation on chromatin can be measured using the labeling method with sodium [^{3}H]acetate [Gunjan et al., 2001]. The histone acetylation increase is detected as global histone acetylation changes by Western blot or mass spectrometry (MS)-based proteomics methods or as site-specific histone acetylation changes using chromatin immunoprecipitation (ChIP) or ChIP-on-Chip.

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Event: 1239: Altered, Gene Expression

Short Name: Altered, Gene Expression

Key Event Component

Process	Object	Action
gene expression		abnormal

AOPs Including This Key Event

AOP ID and Name	Event Type

AOP ID and Name	Event Type
Aop:200 - Estrogen receptor activation leading to breast cancer	KeyEvent
Aop:275 - Histone deacetylase inhibition leads to neural tube defects	KeyEvent

Biological Context

Level of Biological Organization
Molecular

Cell term

Cell term
eukaryotic cell

Key Event Description

It is well documented that alterations of histone acetylation have an impact on gene expression. Therefore if the acetylation status of the epigenetic set-up at the regulatory sequences of genes is altered, this leads to changes in gene expression.

How it is Measured or Detected

Gene specific alterations in histone acetylation at gene regulatory seqences can be measured by chromatin immunoprecipitation (ChIPs) and gene expression analysis by RT-qPCR or whole transcriptomics (RNAseq, gene chips).

Event: 1560: Altered differentiation

Short Name: Altered differentiation

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:275 - Histone deacetylase inhibition leads to neural tube defects	KeyEvent

Stressors

Name
Ionizing Radiation

Biological Context

Level of Biological Organization
Cellular

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Mus musculus	Mus musculus	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
Birth to < 1 month	
Pregnancy	Moderate

Sex Applicability

Sex	Evidence
Unspecific	Moderate

Embryonic development

Key Event Description

Proper differentiation during embryonic development is regulated by the expression of genes at the right time and space. If key regulator genes are not expressed or wrongly expressed this leads to a different cell type.

How it is Measured or Detected

Differentiation can be measured e.g. by in vitro hESC or iPSC based differentiation systems. Pre-requisite for this is a well characterized and homogenous cell population. Then it can be measured by the analysis of altered genes by gene set enrichment analysis (GEA) comparing control with potentially disturbed differentiation.

In the context of embryonic brain development, immunofluorescence on brain sections can be used with antibodies against neuronal differentiation markers such Tuj1, NeuN and betaIII-tubulin.

List of Adverse Outcomes in this AOP

Event: 1561: Neural tube defects

Short Name: Neural tube defects

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:275 - Histone deacetylase inhibition leads to neural tube defects	AdverseOutcome

Biological Context

Level of Biological Organization
Tissue

Key Event Description

Wrongly differentiated cells may not be able to perform the process of neural tube closure.

How it is Measured or Detected

In vitro assays that follow rosettes formation
In vivo animal models

Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 1709: Histone deacetylase inhibition leads to Histone acetylation, increase

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Histone deacetylase inhibition leading to testicular atrophy	adjacent	High	Moderate

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Histone deacetylase inhibition leads to neural tube defects	adjacent	Not Specified	Not Specified

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI
Rattus norvegicus	Rattus norvegicus	High	NCBI
Mus musculus	Mus musculus	High	NCBI
Oryctolagus cuniculus	Oryctolagus cuniculus	Moderate	NCBI
Brassica napus	Brassica napus	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	Moderate

Sex Applicability

Sex	Evidence
Unspecific	High

The relationship between HDAC inhibition and increase in histone acetylation is conceivably well conserved among various species including mammals.

- Hyperacetylation by HDIs such as SAHA and Cpd-60 are observed in mice (*Mus musculus*) [Schroeder et al., 2013].
- TSA induces acetylation of histone H4 in a time-dependent manner in mouse cell lines (*Mus musculus*) [Alberts et al., 1998].
- AR-42, a novel HDI, induces hyperacetylation in human pancreatic cancer cells (*Homo sapiens*) [Henderson et al., 2016].
- SAHA and MS-275 lead to the hyperacetylation of lysine residues of histones in human cell lines of epithelial (A549) and lymphoid origin (Jurkat) (*Homo sapiens*) [Choudhary et al., 2009].
- SAHA treatment induces the H3 and H4 histone acetylation in human corneal fibroblasts and conjunctiva from rabbits after glaucoma filtration surgery (*Homo sapiens*, *Oryctolagus cuniculus*) [Sharma et al., 2016].
- TSA induces the acetylation of histones H3 and H4 in *Brassica napus* microspore cultures (*Brassica napu*) [Li et al., 2014].

Key Event Relationship Description

The HDAC inhibitors (HDIs) inhibit deacetylation of the histone, leading to the increase in histone acetylation and gene transcription. HDACs deacetylate acetylated histone in epigenetic regulation [Falkenberg and Johnstone, 2014].

Histone acetylation is one of the major epigenetic mechanisms and belongs to the posttranslational modifications of histones. Histone acetyltransferase is setting the mark, and deacetylase (HDAC) is responsible for removing the acetyl group from specific lysine residues of the histones. It has been shown that the inhibition of HDACs leads to a hyperacetylation of histones and in general to an imbalance of other histone modifications.

Evidence Supporting this KER

Biological Plausibility

HDACs are important proteins in the epigenetic regulation of gene transcription. Upon the inhibition of HDAC by HDIs, lysine in histone remains acetylated which leads to transcriptional activation or repression, changes in DNA replication, and DNA damage repair [Wade et al., 2008].

In all eukaryotes, the DNA containing the genetic information of an organism is organized in chromatin. The basic unit of chromatin is the nucleosome around which the naked DNA is wrapped. A nucleosome consists of two copies of each of the core histones H2A, H2B, H3, and H4 [Luger et al., 1997]. In order to dynamically regulate this highly complex structure several mechanisms are involved, including the posttranslational modifications of histones (reviewed in [Bannister and Kouzarides, 2011; Kouzarides, 2007]. For a long time, it is known that histones get acetylated and that this reaction is catalyzed by histone acetyltransferases (HAT) whereas the acetyl groups are removed by histone deacetylases (HDAC). Inhibition of HDACs by small-molecule compounds leads to hyperacetylation of the histones as the homeostasis of acetylation and deacetylation is disturbed (reviewed in [Gallinari et al., 2007]).

Empirical Evidence

The major empirical evidence came from the incubation of cell culture cells with small molecule compounds that inhibit HDAC enzymes followed by western blots or acid urea gel analysis. The first evidence was shown by Riggs et al. who showed that

incubation of HeLa cells with *n*-butyrate leads to an accumulation of acetylated histone proteins [Riggs et al., 1977]. Later, it was shown that *n*-butyrate specifically increases the acetylation of histone by the incorporation of radioactive [^{3}H]acetate and analysis of the histones on acid urea gels that allow the detection of acetylated histones [Cousens et al., 1979]. TSA was shown to be an HDAC inhibitor by acid urea gel analysis in 1990 [Yoshida et al., 1990] and good evidence for VPA as an HDAC inhibitor *in vitro* and *in vivo* was shown using acetyl-specific antibodies and western blot [Gottlicher et al., 2001].

There exist several pieces of evidence showing the link between histone deacetylase inhibition and increase in histone acetylation as follows:

- Exposure of mouse embryos *in utero* to VPA and TSA (two well-known HDAC inhibitors) showed an increased histone acetylation level in whole embryo extracts and was also shown *in situ* immuno-stainings [Menegola et al., 2005].
- HDAC inhibition by HDIs leads to hyperacetylation of histone and a large number of cellular proteins such as NF-kappaB [Falkenberg and Johnstone, 2014; Chen et al., 2018].
- The concentrations of half-maximum inhibitory effect (IC_{50}) for HDAC enzyme inhibition of 20 valproic acid derivatives correlated with teratogenic potential of the compounds, and HDAC inhibitory compounds showed hyperacetylation of core histone 4 in treated F9 cells [Eikel et al., 2006].
- HDIs increase histone acetylation in the brain [Schroeder et al., 2013].
- More acetylation sites on the histones H3 and H4 are responsive to SAHA than to MS-275 indicating that an HDI selectivity exists [Choudhary et al., 2009].
- HDI AR-42 induces acetylation of histone H3 in a dose-response manner in human pancreatic cancer cell lines [Henderson et al., 2016].
- MAA treatment in rats (650 mg/kg, for 4, 8, 12, and 24 hrs) induced hyperacetylation in histones H3 and H4 of testis nuclei [Wade et al., 2008].
- HDAC inhibition induced by valproic acid (VPA) leads to histone hyperacetylation in mouse teratocarcinoma cell line F9 [Eikel et al., 2006].
- Hyperacetylation of histone H3 was observed in HDAC1-deficient ES cells [Lagger et al., 2002].
- The treatment of MAA induced histone acetylation in H3K9Ac and H4K12Ac, as well as p53K379Ac [Dayan and Hales, 2014].

Uncertainties and Inconsistencies

HDACs affect a large number of cellular proteins including histones, which reminds us the HDAC inhibition by HDIs hyperacetylates cellular proteins other than histones and exhibit additional biological effects. It is also noted that HDAC functions as the catalytic subunits of the large protein complex, which suggests that the inhibition of HDAC by HDIs affects the function of the large multiprotein complexes of HDAC [Falkenberg and Johnstone, 2014]. Related-analysis are usually indirect or in purified systems, therefore a direct cause-consequence relation is difficult to obtain.

Quantitative Understanding of the Linkage

To quantify acetylation by HDAC, stable isotope labeling with amino acids in cell culture (SILAC) method is used [Choudhary et al., 2009].

Response-response relationship

SAHA or MS-275 treatment leads to an increase in acetylation of specific lysine residues on histones more than two-fold [Choudhary et al., 2009]. Acetylation of the variant histone H2AZ-a mark for DNA damage sites- was upregulated almost 20-fold by SAHA, whereas a number of sites on the core histones H3 and H4 are several times more highly regulated in response to SAHA than by MS-275 [Choudhary et al., 2009].

TSA (100 ng/ml) treatment leads to accumulation of the acetylated histones in a variety of mammalian cell lines, and the partially purified HDAC from wild-type FM3A cells was effectively inhibited by TSA (K_i = 3.4 nM) [Yoshida et al., 1990].

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Relationship: 1806: Histone acetylation, increase leads to Altered, Gene Expression

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Histone deacetylase inhibition leads to neural tube defects	adjacent	Not Specified	Not Specified

Key Event Relationship Description

The structure of chromatin is a major component of gene regulation in eukaryotes by providing or preventing accessibility for the transcriptional machinery to the relevant regulatory DNA sequences. Histone acetylation is one of the major posttranslational modifications that are involved in the regulation of gene expression. Generally spoken, acetylation is correlated with actively transcribed genes, whereas hypoacetylation is involved in gene silencing.

Evidence Supporting this KER

Biological Plausibility

In all eukaryotes, the DNA containing the genetic information of an organism is organized in chromatin. The basic unit of chromatin is the nucleosome around which the naked DNA is wrapped. A nucleosome consists of two copies of each of the core histones H2A, H2B, H3 and H4 (Luger et al., 1997). In general, chromatin is a permissive structure for all DNA-dependent processes such as DNA replication, recombination, repair, and transcription and therefore also for gene expression. However, chromatin structure is very dynamically regulated and can be made accessible for the transcriptional machinery and is, therefore, an important mechanism of gene regulation. One mechanism of chromatin structural regulation is the post-translational modifications of the

histone proteins including the acetylation of lysine residues (reviewed in (Bannister and Kouzarides, 2011; Bannister et al., 2002; Kouzarides, 2007; Tessarz and Kouzarides, 2014)). These modifications serve as a docking station for further proteins and protein complexes that finally open or close the chromatin structure and allow or inhibit access of the transcriptional machinery (Musselman et al., 2012) or directly influence DNA histone interaction (reviewed in (Tessarz and Kouzarides, 2014). Histones get acetylated by histone acetyltransferases (HAT) and deacetylated by histone deacetylases (HDAC) (reviewed in (Gallinari et al., 2007; Bannister and Kouzarides, 2011; Kouzarides, 2007)). In general, it can be assumed, hyperacetylated histones are associated with actively transcribed genes, whereas hypoacetylation of histones is involved in gene silencing.

Empirical Evidence

The first direct evidence that histone acetylation has an impact on gene expression came from mutation studies in yeast. Using ChIP on chip analysis showed that mutation or deletions of HDAC enzymes lead to changed gene expression levels on a subset of genes (Xu et al., 2005; Bernstein et al., 2000; Robyr et al., 2002).

In the Drosophila cell line S2, it was shown that deregulation of transcription occurs only by know-down (RNAi) HDAC enzymes, that at least class 1 and 3 HDAC enzymes have an influence on gene expression (measured via gene chips). However, this study did not show a direct link between histone acetylation and gene expression (Foglietti et al., 2006).

In mice knockout of HDACs are mostly embryonically lethal. However, the use of embryonic stem cells and the expression and acetylation profiles shows that also in mice an imbalance of histone acetylation may lead to changes in gene expression (Zupkovitz et al., 2006).

Major gene expression changes were observed during the differentiation of hESC towards neuroectodermal progenitor cells. In these studies also the acetylation status of the deregulated genes was investigated by chromatin immunoprecipitation (Balmer et al., 2014; Balmer et al., 2012)

Uncertainties and Inconsistencies

All above-mentioned analysis are indirect or in purified systems.
Therefore a direct cause-consequence relation is difficult to obtain.

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Relationship: 1807: Altered, Gene Expression leads to Altered differentiation

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Histone deacetylase inhibition leads to neural tube defects	adjacent	Not Specified	Not Specified

Key Event Relationship Description

During early embryonic development of the nervous system-specific developmental genes need to be activated in a highly regulated and concerted manner. Genes need to be expressed (or silenced) at the right time and space. If this concerted regulation is disturbed this may lead to severe malformations in the later fetus. The type of malformation depends strongly on the timing of disturbance, i.e. during which developmental stage the disturbance happened. For the development of neural tube defects, especially neural tube closure, the disturbance must happen before neurulation at the stage when neuroepithelial cells arise. Therefore, for this AOP the imbalance of gene expression must occur before neural tube closure. However, compare AOP23 the inhibition of neural crest migration by HDAC inhibition happens later in embryonic development.

Evidence Supporting this KER

Biological Plausibility

Gene expression during embryonic development is a highly regulated process. The genes need to be expressed and silenced at the right time and place. Therefore, if cells that are determined to differentiate into e.g. NEP wrongly express neural crest marker genes this may lead to a failure of neural tube closure. One reason for this effect is that these cells lose their epithelial character and undergo an epithelial mesenchymal transition as neural crest cells do (Park and Gumbiner, 2010). In summary, imbalanced gene expression may lead to the differentiation of the wrong cell type at the wrong time and space.

Empirical Evidence

It was shown that HDAC inhibition changes the gene expression pattern during the differentiation of hESC towards NEP cells. Furthermore, the changed differentiation track points to a wrong differentiation towards neural crest cells (Balmer et al., 2014).

The differentiation track of hESC towards NEP is strongly disturbed in vitro by 6 different (structurally not related) HDAC inhibitors in a comparable manner (Rempel et al., 2015).

TSA treated chicken embryos showed a disturbed gene expression pattern of the posterior neural tube, that points to a wrong differentiation track towards neural crest cells (Murko et al., 2013).

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Relationship: 1808: Altered differentiation leads to Neural tube defects

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Histone deacetylase inhibition leads to neural tube defects	adjacent	Not Specified	Not Specified

Key Event Relationship Description

During the process of neural tube closure, the cells at the dorsal boundary a new cell type arises, the neural crest cells. These cells undergo an epithelial to mesenchymal transition that enables them to migrate away from the neural tube. If the neuroepithelial cells of the neural tube express the wrong regulator genes it may happen that they acquire such neural crest characteristics, which prevents them from performing the closure of the neural tube.

Evidence Supporting this KER

Biological Plausibility

Many HDAC inhibitors used as drugs have been shown to induce congenital malformations, including neural tube closure defects in humans and model organisms (Menegola et al., 1996; Nau, 1994). Most studies show that after treatment with HDAC inhibitors these malformations occur in the experimental animals, however direct evidence that neural tube closure defects result from wrongly differentiated neural tube cells.

Empirical Evidence

TSA treated chicken embryos showed a disturbed gene expression pattern of the posterior neural tube, that points to a wrong differentiation track towards neural crest cells. This was shown by in situ immune staining using neural crest-specific antibodies (Murko et al., 2013).

Further Menegola et al. showed direct evidence that HDAC inhibition is occurring in vivo in the neural tube of mice (Menegola et al., 2005).

Uncertainties and Inconsistencies

Due to lacking studies directly showing that the neural tube cells are wrongly differentiated and that this may causative for closure defects the uncertainty of this KER is very high and based on correlation studies.

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