Histone Deacetylase 3 Protein: towards a unified structural-functional approach

Leyla M. Sánchez-Palancar

Abstract— The inhibition procedure of histone deacetylase 3 (HDAC3) has been intensively studied because of the important roles that this protein plays in the regulation of gene expression, epigenetic repression, transcription, replication, recombination, and repair. HDAC3 is required for the proper balance of acetylation/deacetylation dynamics of genes, and it is involved in cell growth and the apoptotic process of all cell types via the regulation of pro-apoptotic genes. The overexpression of HDAC3 protein might lead to uncontrolled cell proliferation and inhibition of both differentiation and apoptosis. Therefore, downregulating HDAC3 seems to be critical for the treatment of a wide range of diseases, with special emphasis on cancer. In the present work, the main structural-functional characteristics of HDAC3 are exhaustively described. A first feature set consisted of the nucleotide sequence and from the primary to the quaternary structures of HDAC3. The knowledge of the conformations and coupling of this protein in different complexes determines the functionality of HDAC3. Therefore, a second feature set was defined to elucidate the interactions, ligands, homologies, and functions of HDAC3. Summarizing most of the characteristics of HDAC3 into a single structural-functional approach, including histone deacetylase inhibitors (HDIs) to regulate its overexpression as a possible practical application, is the key contribution of this review.

Keywords— HDAC3, nuclear receptor co-repressor (NCOR/SMRT), deacetylation, HDI, inositol tetraphosphate (IP4), cancer.

1. Introduction

Histone deacetylases (HDACs) are part of an extensive family of enzymes that have crucial roles in numerous biological processes, largely through their repressive influence on transcription, cell differentiation, apoptosis, and survival. Transcriptional control occurs through regulators that bind specific DNA sequences, leading to the histone acetylation/deacetylation modifications in chromatin structure. The state of amino acid residues within histone tails is the main factor influencing chromatin structure. Histone tails serve as targets for a variety of reversible post-translational modifications that modulate nucleosome structure and gene transcription, both positively and negatively. Acetylation and deacetylation, which are the most widespread modifications of histones, provide a mechanism for coupling extracellular signals with the genome [1].

The HDAC superfamily is wide and ancient, dating back to prokaryotes. This superfamily is divided into four families (class I, IIa, IIb and IV), classified by their differences in structure, enzymatic function, subcellular localization, and expression patterns. Additionally, mammalian genomes encode another group referred to as class III HDACs or sirtuins. Class I HDAC family involves zinc-dependent enzymes and consists of HDAC1, 2, 3 (or RPD3), and 8. These enzymes possess the conserved deacetylase domain. Class I HDACs are widely expressed in organisms, whereas HDACs of other classes are tissue-specific [1].

From the structural point of view, HDAC3 protein is 428 amino acids long. Although other class I HDACs are found mainly in the nucleus of the different cells, HDAC3 is located in both the nucleus, the cytoplasm as well as at the plasma membrane. The HDAC3 gene in humans is located in chromosome 5 [2]. The main function of HDAC3 resides in its catalytic activity, in which HDAC3 removes acetyl groups from N(6)-acetyl-lysine residues on a histone, resulting in chromatin condensation. HDAC3 is overexpressed in some tumors including leukemia, colon cancer, lung, and maxillary carcinoma, among others. Thus, inhibitors precisely targeting HDAC3 could be therapeutic drug options [3]. In the present study, the most important structural-functional characteristics of HDAC3 are reviewed and exhaustively described, paying special attention to HDAC3's nucleotide sequence, its structures from primary to quaternary, the main interactions with other proteins, the different types of ligands, its homologies with other proteins in different organisms, and its most relevant functions.

2. Results

2.1. Nucleotide Sequence

The HDAC3 gene is located in chromosome 5 (location 5q31.3) of Homo Sapiens and the mRNA transcribed from it is between 1920 [4] and 1934 nucleotides long (different untranslated region length according to different sources, so the 3' end is filled with adenine) [5], [6]. However, the coding region is constant and consists of 1287 nucleotides (from 23 to 1309 with 1920 nucleotides), (see Fig. 1).

The HDAC3 gene spans 15.97 kilobases (kb) of genomic sequence on chromosome 5 (in minus-strand orientation). It consists of 15 exons and 14 introns in between (see Fig. 2). Alternative splicing is the process by which different regions of exons and introns are joined to produce mature mRNA transcripts, which often lead to unique proteins or isoforms [7].

Leyla M. Sánchez-Palancar. Faculty of Experimental Sciences (Degree in Biotechnology). Universidad Pablo de Olavide. lmsanpal@alu.upo.es.

Fig. 1. Nucleotide Sequence of the HDAC3 gene. In green it is shown the coding sequence of the gene that will be translated into a protein. Data collected and modified from: [4], [5], [6].

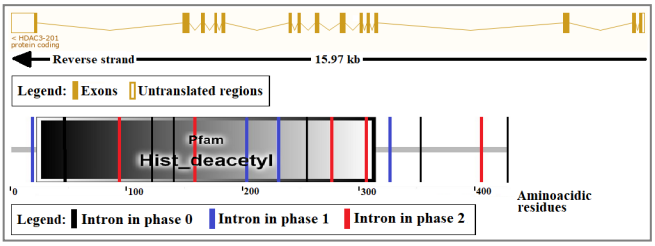

Fig. 2. Exons and introns along the HDAC3 gene. In the top image, the exons are marked (see color coding in the legend) throughout the reverse strand (marked with an arrow). In the bottom image, introns are marked (see color coding in the legend) along aminoacidic residues (from 0 to 428, amino to carboxy). Data collected and modified from: [5], [8].

2.2. Primary Structure and Isoforms

HDAC3 is a protein that consists of 428 amino acids, its aminoacidic sequence is illustrated below (see Fig. 3).

```
ORIGIN
  1 MAKTVAYFYD PDVGNFHYGA GHPMKPHRLA LTHSLVLHYG LYKKMIVFKP YQASQHDMCR
 61 FHSEDYIDFL QRVSPTNMQG FTKSLNAFNV GDDCPVFPGL FEFCSRYTGA SLQGATQLNN
121 KICDIAINWA GGLHHAKKFE ASGFCYVNDI VIGILELLKY HPRVLYIDID IHHGDGVQEA
181 FYLTDRVMTV SFHKYGNYFF PGTGDMYEVG AESGRYYCLN VPLRDGIDDQ SYKHLFQPVI
241 NQVVDFYQPT CIVLQCGADS LGCDRLGCFN LSIRGHGECV EYVKSFNIPL LVLGGGGYTV
301 RNVARCWTYE TSLLVEEAIS EELPYSEYFE YFAPDFTLHP DVSTRIENQN SRQYLDQIRQ
361 TIFENLKMLN HAPSVQIHDV PADLLTYDRT DEADAEERGP EENYSRPEAP NEFYDGDHDN
421 DKESDVEI
```

Fig. 3. Aminoacidic sequence of the HDAC3 protein. Data collected from: [4], [9].

There are two described isoforms of HDAC3 resulting from alternative splicing of the mRNA in the 5' region [2]. Isoform 1 has a length of 428 amino acids, a mass of 48848 Daltons (Da), and an isoelectric point (pI) of 4.98. On the other hand, Isoform 2 is 429 amino acids long, has a mass of 49111 Da and a pI of 5.11. The aminoacidic sequence of isoform 2 differs from the sequence of isoform 1 (canonical) as follows: 1-15 (5′ to 3′): MAKTVAYFYDPDVGN → MIVFKPYQASQHDMCR (see Fig. 4).

```
O15379     HDAC3_HUMAN   1   MA-KTVAYFYDPDVGNFHY
O15379-2   HDAC3_HUMAN   1   MIVFKPYQASQHDMCRFHY
                             *    .      : *: .***
```

Fig. 4. Alignment of Isoforms 1 & 2 of HDAC3. In green are shown the differences in the aminoacidic sequence of isoform 1 (top) compared to that of isoform 2 (bottom); from 5' to 3' (left to right). Data collected and modified form: [10].

2.2.1. Post-translational modifications

Post-translational modification (PTM) is a term that entails dynamic modification of proteins after their translation. PTMs are not only involved in homeostasis but also pathologic conditions and diverse diseases. Histone deacetylases (HDACs), which are known to act as transcriptional regulators, are one example of post-translational modifiers with diverse roles in human physiology and diseases. Since they cause the deacetylation of histones, the regulation and inhibition of HDACs might be beneficial for the treatment of certain diseases. Class I HDACs have greater enzymatic activity than the other classes of HDACs and target various non-histone proteins as well as the histone-core complex [11]. Class I HDACs, in which HDAC3 is included, not only regulate PTMs in histones and other proteins, but they also undergo post-translational modifications such as phosphorylation and ubiquitination (see Fig. 5), sumoylation, and S-nitrosylation [8], [12].

The activity of HDAC3 is regulated by the phosphorylation of the Ser424 residue of the protein (Fig. 5) by Casein Kinase 2 and the same residue is dephosphorylated by protein serine/threonine phosphatase 4 (PP4). It is also possible to indirectly regulate the activity of HDAC3 by post-translational modification of its associated proteins [2]. HDAC3 can also be sumoylated *in vitro* [4], [13].

The most relevant post-translational modifications that HDAC3 undergo are present in both isoforms of the protein and are located at the same sites, which indicates that these post-translational modifications have specific purposes and functions in regulating the activity of the protein and the correct functioning of genes and proteins.

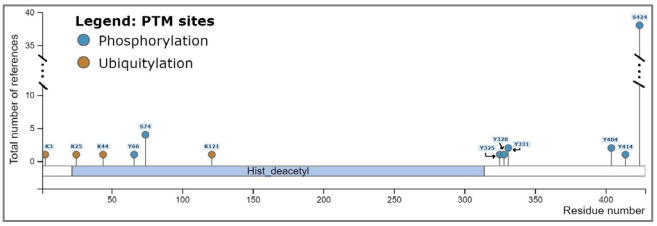

Fig. 5. Most relevant post-translational modifications of HDAC3. The graph represents the different PTMs along the HDAC3 aminoacidic sequence. The y-axis is the total number of references of HDAC3 and the x-axis shows the residue number. In the top-left of the figure the legend describes the color coding for the different PTMs. Data collected and modified from: [14].

2.3. Secondary Structure

The secondary structure of HDAC3 consists of alpha (α)-helixes and beta (β)-sheets linked by connecting segments and β-turns (see Fig. 6).

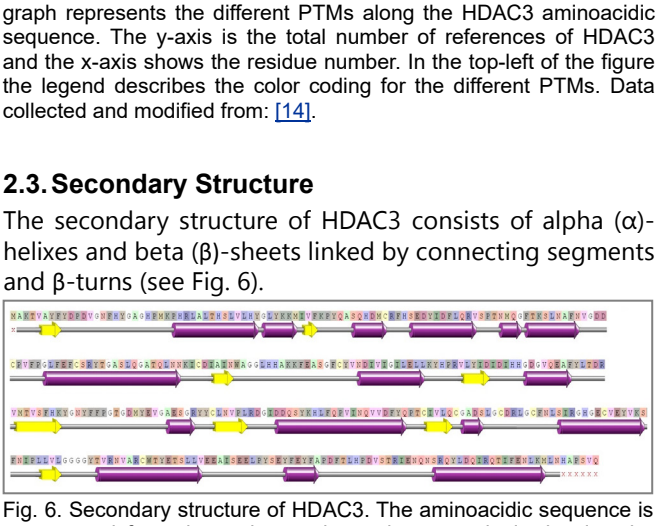

Fig. 6. Secondary structure of HDAC3. The aminoacidic sequence is represented from the amino to the carboxy terminal, showing the corresponding secondary structure below each region. β-sheets (yellow/rectangular arrows); α-helixes (purple/cylindrical arrows). Data collected and modified from: [15].

2.3.1. Supersecondary Structures (Motifs)

The main supersecondary structures or motifs that are present in HDAC3 are of α-β Class and α-β-α Sandwich Architecture, their Topology is that of Arginase and they are conserved in the HDAC domain superfamily (see Fig. 7).

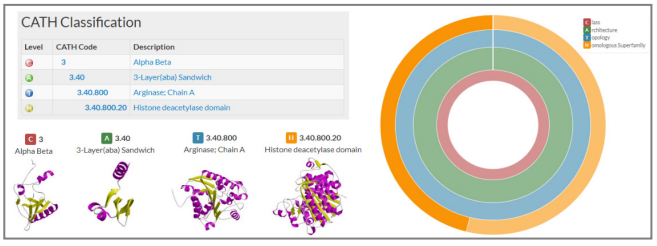

Fig. 7. CATH (Class, Architecture, Topology, Homologous Superfamily) classification of the main Supersecondary structures and domains of HDAC3. The chart and representations in the left refer to the Supersecondary structures and domains of HDAC3, color coded in the sunburst graph in the right. Data collected from: [16].

2.3.2. Domains

Class I HDACs possess simple structures, consisting of the conserved deacetylase domain with short amino- and carboxy-terminal extensions. As mentioned in the Supersecondary structures section (Fig. 7), the Arginase topology of the histone deacetylase domain is characteristic of HDAC3 as well as the rest of the HDACs and it consists of a 3-layer (α-β-α) sandwich. This domain spans throughout the majority of the HDAC3 chain (see Fig. 8), acting as the main structural and functional unit of the HDAC3 protein.

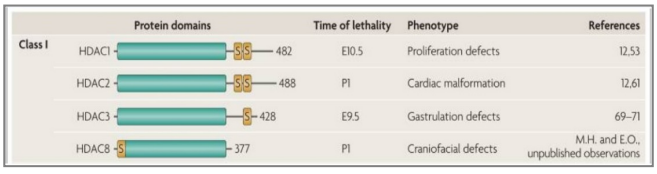

Fig. 8. Protein domains of the histone deacetylase (HDAC) superfamily, only Class I HDACs showing. The green rectangles (left) indicate the conserved HDAC domain; numbers following the HDAC domain indicate the number of amino acids in each class I HDAC; S represents serine phosphorylation sites. Data collected from: [1].

Depending on the references of each database, the domains are classified according to different features such as the beginning and the end of the domain throughout the aminoacidic sequence (see Fig. 9). This way, it is possible to represent and see their expansion through the protein's 3D structure, thus we are able to compare the CATH Arginase superfamily and its corresponding Pfam HDAC domain for example (see Fig. 10).

Domain source	Start	End	Description
CDD	3	383	HDAC3
PANTHER	3	421	HISTONE DEACETYLASE
PANTHER	3	421	HISTONE DEACETYLASE 2
PIRSF	1	427	HDAC_I_euk
PRINTS	24	41	HISDACETLASE
PRINTS	57	75	HISDACETLASE
PRINTS	88	105	HISDACETLASE
PRINTS	109	129	HISDACETLASE
PRINTS	150	166	HISDACETLASE
PRINTS	210	223	HISDACETLASE
PRINTS	227	245	HISDACETLASE
Pfam	22	313	Hist_deacetyl
Gene3D	1	376	Histone deacetylase domain superfamily
PRINTS	131	154	HDASUPER
PRINTS	163	178	HDASUPER
PRINTS	249	259	HDASUPER
SuperFamily	4	369	Arginase/deacetylase

Fig. 9. Domains of HDAC3 protein. Data collected from: [17].

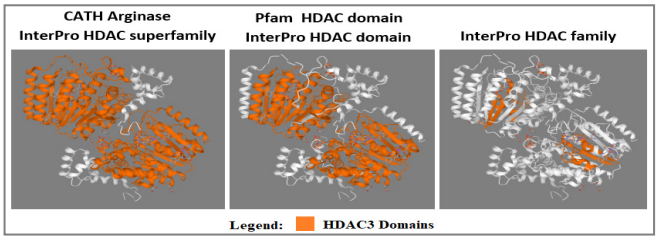

Fig. 10. HDCA3 Domains. The most relevant HDAC3 domains are shown in orange, in both chains of the protein, which in this case are bound to NCOR2. Data collected and modified from: [18].

2.3.3. Ramachandran Plot

The Ramachandran plot is used to deduce the secondary structure of a protein from the possible distribution of psi (Ψ) and phi (Φ) torsion angles found in it. Besides showing the common elements of secondary structures, Ramachandran plots reveal the regions of unusual arrangement of the peptide skeleton among other quality estimates (see Fig. 11).

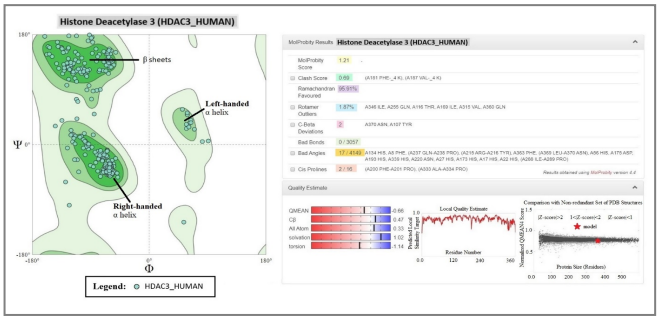

Fig. 11. Ramachandran plot of HDAC3 and regions of unusual arrangement. In the left half, the Ramachandran plot for HDAC3, the allowed secondary structures and the color coding (bottom) are represented. The y-axis represents psi (Ψ) angles, and the x-axis refers to phi (Φ) angles. In the right half, goals, relative percentages for Ramachandran plot, clash score, torsion angle, C-Beta deviations and bad values are shown. In the bottom part of the chart we find three graphs for the Quality Estimate (QMEAN, Local similarity Target and Z-score). Data collected and modified from: [19], [20].

2.4. Tertiary and Quaternary Structure

The tertiary structure refers to the three-dimensional arrangement of all atoms in the polypeptide chain. HDAC3 is a globular protein and its tertiary structure consists of a single polypeptide chain with the corresponding secondary structures (β-sheets, α-helixes, turns, and loops), (see Fig.12, left).

The quaternary structure of HDAC3 is composed of two HDAC3 subunits (chains A & B), present in specific complexes containing two monomers of NCOR2 (chains C & D), and an IP4 molecule per NCOR2 chain acting as an intermolecular glue between the two proteins (see Fig. 12, right) [20].

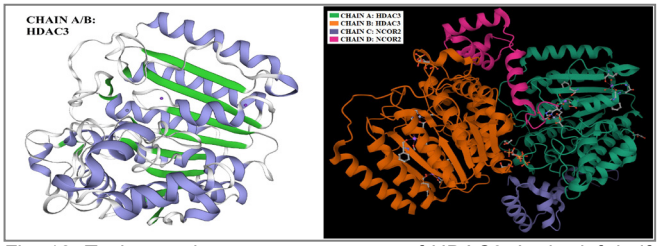

Fig. 12. Tertiary and quaternary structure of HDAC3. In the left half, the tertiary structure of one chain is shown (β-sheets in green, α-helixes in purple, and turns and loops in white). In the right half, the quaternary structure of HDAC3 (chains A & B) bound to NCOR2 (chains C & D) and to other Het groups, such as IP4. Each chain is color-coded (top). Data collected from: [18], [19].

2.5. Interactions

HDAC3 interacts with proteins of the HDAC family, such as HDAC1, 7, 9, and 10. It forms a heterologous complex with the zinc-finger transcription factor Yin-Yang-1 (YY1), interacts with NR2C1 (Nuclear Receptor Subfamily 2 Group C Member 1), and with XBP1 (X-Box Binding Protein 1) in endothelial cells under disturbed flow. Its weak interaction with CRY1 (Cryptochrome Circadian Regulator 1) is enhanced in the presence of FBXL3 (F-Box and Leucine-Rich Repeat Protein 3) and ARNTL/BMAL1 (Aryl hydrocarbon receptor nuclear translocator-like protein/ Brain and Muscle ARNT-Like 1). It interacts with Retinoic Acid Receptor Alpha (RARA), causing the inhibition of Retinoic Acid Response Element (RARE) DNA element-binding. Interactions with BCL6 (B-cell lymphoma 6 protein), DAXX (Death Domain Associated Protein), GPS2 (G Protein Pathway Suppressor 2), and SRY (Sex-determining Region Y) are also present [12], [14].

One of the most relevant interactions (mentioned and briefly described in the Quaternary structure section above) is the one established with NCOR2 in the N-CoR/SMRT repressor complex and with NCOR1. It is important to understand the correlation between the interactions and the coexpression of HDAC3 and other proteins (such as those mentioned above and others like TBL1X (Transducing Beta Like 1 X-Linked), KAT2A (Lysine acetyltransferase 2A), and EP300 (E1A Binding Protein P300)). Since they work as functional partners, they usually depend on each other to function or perform a certain activity (see Fig. 13).

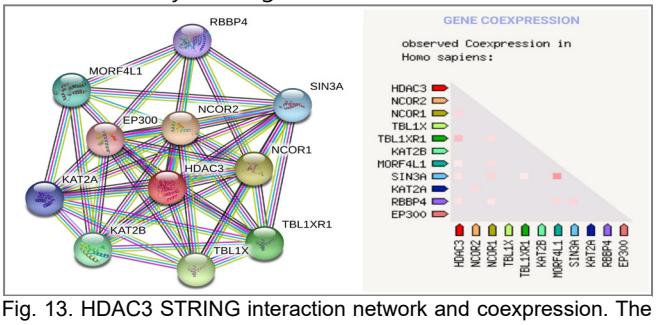

Fig. 13. HDAC3 STRING interaction network and coexpression. The network diagram in the left represents the most relevant interactions between HDAC3 (center, in red) and other proteins. In the right, coexpression scores in Homo sapiens based on RNA expression patterns and protein co-regulation are shown, plotting all the interacting proteins against each other. Data collected from: [21].

2.6. Ligands

HDAC3 presents four different types of ligands bound directly to it in its quaternary structure. These four ligands are glycerol (two per chain, four in total), zinc ions (one per chain, two in total), acetate ions (one per chain, two in total), and potassium ions (two per chain, four in total). Besides those four main ligands, when the two chains of HDAC3 are bound to two chains of NCOR2, another ligand comes into the picture and acts as an intermolecular glue between both proteins. This ligand, called D-Myo Inositol 1,4,5,6 tetrakisphosphate (commonly known as inositol phosphate or IP4) plays a crucial role in binding these two proteins for their correct function (there is one inositol phosphate per chain, two in total) (see Fig. 14). Each one of these ligands has specific binding sites throughout the HDAC3 sequence.

ID	Chains	Name / Formula / InChI Key	2D Diagram
I0P x1	C, D	**D-MYO INOSITOL 1,4,5,6 TETRAKISPHOSPHATE** $C_6H_{16}O_{18}P_4$ MRVYFOANPDTYBY-YORTWTKJSA-N	
GOL x2	A, B	**GLYCEROL** $C_3H_8O_3$ PEDCQBHIVMGVHV-UHFFFAOYSA-N	
ZN x1	A, B	**ZINC ION** Zn PTFCDOFLOPIGGS-UHFFFAOYSA-N	Zn^{2+}
ACT x1	A, B	**ACETATE ION** $C_2H_3O_2$ QTBSBXVTEAMEQO-UHFFFAOYSA-M	
K x2	A, B	**POTASSIUM ION** K NPYPAHLBTDXSSS-UHFFFAOYSA-N	K^+

Fig. 14. HDAC3 Ligands. HDAC3 ligands are shown, along with their 2D structure (right), their names and formulas (middle), the chains they are bound to (A,B for HDAC3 and C,D for NCOR2), and how many molecules are there of each ligand per chain (left). Data collected and modified from: [19], [20], [22], [23], [24].

2.7. Homologies

HDAC3 is very tightly conserved from plants to humans, so a very wide range of homologs of human HDAC3 is found in other species. These homologs are classified according to the percentage of identity with human HDAC3, going from 100% identity to only 50% identity among the different HDAC3s in other organisms (see Fig. 15).

Furthermore, the histone deacetylase domain of HDAC3 (amino acids 3 to 316) is partly homologous to the other class I HDACs (HDAC1, 2, and 8) although the carboxy-terminus part of the protein is highly differing. Thus, the HDAC3 protein is roughly 50% identical compared with other class I HDACs [2]. This is an important feature to take into consideration to differentiate the functions, activity, and localization of each kind of HDAC.

100 % Identity

Entry name	Protein names	Organisms	Length
HDAC3_HUMAN	**Histone deacetylase 3**	**Homo sapiens (Human)**	**428**
G1SW87_RABIT	Histone deacetylase 3	Oryctolagus cuniculus (Rabbit)	493
D2GUM1_AILME	Hist_deacetyl domain-containing protein (Fragment)	Ailuropoda melanoleuca (Giant panda)	406

90% Identity

Entry name	Protein names	Organisms	Length
Isoform 2 of HDAC3_HUMAN	**2, RPD3-2A**	**Homo sapiens (Human)**	**429**
HDAC3_XENLA	Histone deacetylase 3	Xenopus laevis (African clawed frog)	428
HDAC3_PONAB	Histone deacetylase 3	Pongo abelii (Sumatran orangutan) (Pongo pygmaeus abelii)	428

50% Identity

Entry name	Protein names	Organisms	Length
HDAC3_RAT	Histone deacetylase 3	Rattus norvegicus (Rat)	428
HDAC3_XENTR	Histone deacetylase 3	Xenopus tropicalis (Western clawed frog) (Silurana tropicalis)	428
HDAC3_TETNG	Histone deacetylase 3	Tetraodon nigroviridis (Spotted green pufferfish) (Chelonodon nigroviridis)	428

Fig. 15. HDAC3 homologs classified according to their percentage of identity with HDAC3. This figure shows a few examples of homologs of human HDAC3 at different identity thresholds (100%, 90% and 50%). In each chart, the names, lengths, and organisms they appear in are shown. Data collected and modified from: [25], [26], [27].

2.8. Functions

HDAC3 is an enzyme that removes acetyl groups from N(6)-acetyl-lysine residues on a histone (see Fig. 16), resulting in chromatin condensation. Histone acetylation plays an important role in the regulation of gene expression, therefore, in eukaryotes [28], HDACs play a key role in the regulation of transcription and cell proliferation. Histone deacetylation gives a tag for epigenetic repression [2].

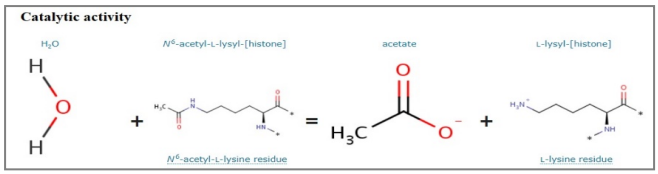

Fig. 16. Catalytic activity of HDAC3. HDAC3 removes the acetyl group of the lysine residues. Data collected from: [10].

HDACs modulate chromatin accessibility during transcription, replication, recombination, cell maturation, and repair. Thus, for re-establishing chromatin structure after transcription of a gene or the repair of a DNA double-strand break, the deacetylation of histones is required [29]. HDAC3 is in charge of the deacetylation of lysine residues on the amino-terminus of core histones and some other non-histone proteins (by associating with the acetyltransferases EP300 and CREB-binding protein-associated factor, it inhibits the myogenesis to reverse autoacetylation) [2]. By deacetylating the H3 'Lys-27' on enhancer elements, HDAC3 participates in the BCL6 transcriptional repressor activity, antagonizing EP300 acetyltransferase activity and repressing expression of proximal genes. Moreover, through its binding to YY1 and POU1F1 (POU Class 1 Homeobox 1), HDAC3 regulates their transcription. It acts as a molecular chaperone for phosphorylated NR2C1 to transport it to PML (Promyelocytic Leukemia protein) bodies for sumoylation. HDAC3 works with XBP1 isoform 1 in the activation of the gene expression of the NFE2L2 (Nuclear Factor, Erythroid 2 Like 2)-mediated HMOX1 (Heme Oxygenase 1) transcription factor. The NCOR1-HDAC3 complex regulates the circadian expression of the core clock gene ARTNL/BMAL1 in a deacetylase activity-independent manner, and the genes involved in lipid metabolism in the liver. HDAC3 plays a role in the repression of microRNA-10a and thereby in the inflammatory response when it is associated with RARA [10].

Furthermore, HDAC3 plays unique roles in cell physiology and has substrates in the different cell compartments, in accordance with the restricted homology of HDAC3 with the other HDACs and its subcellular localization. Unlike HDAC1 and 2, HDAC3 is necessary for cell growth and affects most cell types by regulating pro-apoptotic genes. Moreover, HDAC3 plays a role in signal transduction, development, inflammation, and metabolism. HDAC3 is found in diverse complexes consisting of members of the NCOR/SMRT family involved in gene repression (Fig. 17).

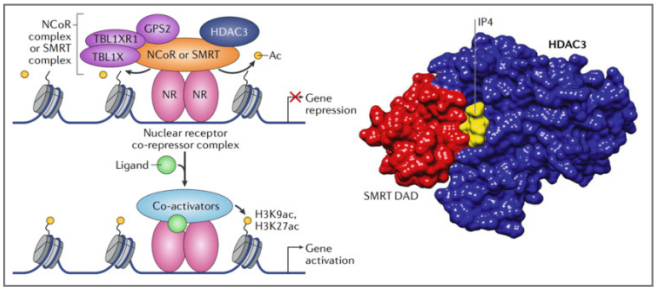

Fig. 17. Regulation of physiology of HDAC3 in the N-CoR/SMRT complex. The regulation process performed by the N-CoR/SMRT complex and HDAC3 is shown in the left half of the figure. In the right half, the structure containing NCOR2 (SMRT) and HDAC3 linked by IP4 is shown. Data collected from: [30].

Many transcription factors interact with HDAC3 and target it to certain promoters. HDAC3 is capable of controlling osteoblast differentiation and bone formation causing the inhibition of the trans-activity of the osteoblast master protein Runx2. Similarly, in hematopoietic stem cells, HDAC3, but not other class I HDACs, binds with GATA binding factor 2 suppressing its transcription [2].

HDAC3 and other class I HDACs are deregulated in a variety of cancers such as colon, ovarian, lung, stomach, muscle, bone, or skin cancers. Therefore, the downregulation of HDAC3 in cancer cells, in which it is overexpressed, results in cell growth inhibition, differentiation, and increased apoptosis. HDAC3 is recruited by MAGE-A (Melanoma-associated antigen) to block the activation of the tumor antigen p53 which keeps cells from dividing uncontrollably. In leukemia, the generation of oncogenic fusion proteins causes aberrant recruitment of N-CoR/SMRT-HDAC3 repressor complexes on promoters. Additionally, HDAC3 found in the nucleus plays an anti-apoptotic role that is determinant for the uncontrolled growth of cancer cells [2].

3. Discussion and Conclusions

In addition to the structural and functional characteristics of HDAC3, it is important to remark the roles that the histone deacetylase inhibitors (HDI) play in the regulation of the overexpression of HDAC3 to maintain the acetylation/deacetylation balance in genes. Therefore, HDIs are being used to treat a variety of cancers and other therapeutic uses, so it is essential to understand their action and potential side effects. Not only overexpression of HDAC3 must be determinant in genomic instability (that might lead to different diseases), but also the full inhibition of HDAC3 because the acetylation/deacetylation balance may be disrupted [29].

The other fundamental issue regards the possible applications of HDACs as emerging cancer drug targets. Several HDAC inhibitors are at various stages in clinical trials and two drugs, romidepsin and vorinostat, have been approved for the treatment of cutaneous T-cell lymphomas [20]. Several studies are performed with particular class I HDACs inhibitors for the treatment of pathological conditions like Spinal Muscular Atrophy, the Hodgkin lymphoma, myeloid leukemia, and myelodysplastic syndrome (associated with DNA methylation inhibitors) or of pancreatic cancers (associated with antimetabolites).

HDIs are currently being tested to enhance neuronal stability and survival in both *in vitro* and *in vivo* models of neurodegenerative diseases such as polyglutamine-related illnesses and amyotrophic lateral sclerosis. When joined with other antigens, HDAC3 may become a useful molecular biomarker as a diagnostical tool for a subset of colon cancer patients [2].

In conclusion, the single structural-functional characterization of HDAC3 and the understanding of the role that its inhibitors play, are key for present and future advances in the comprehension of genomic mechanisms with critical repercussions in clinical research, medical biotechnology, and biochemistry (see Fig. 18).

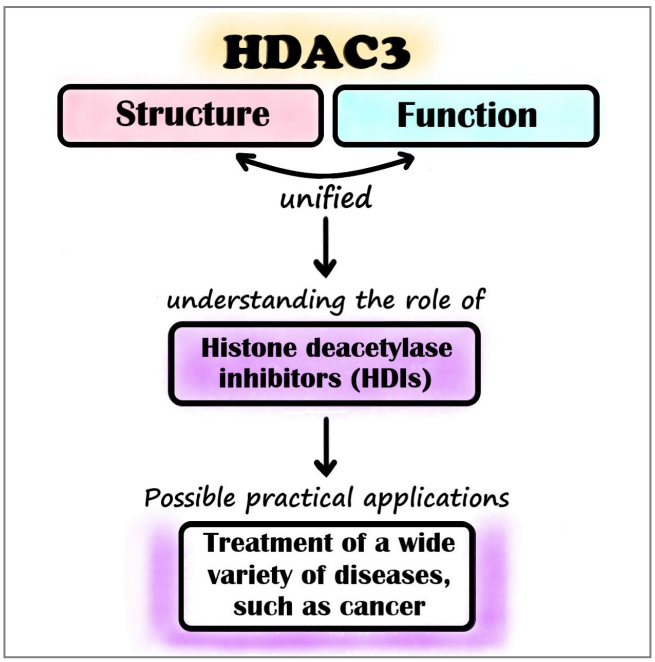

Fig. 18. Summary diagram proposed for the unified structural-functional approach.

ACKNOWLEDGMENTS

The author thanks Professor Dr. Juan R. Tejedo for encouraging the realization of this review.

REFERENCES

[1] M. Haberland, R.L. Montgomery and E.N. Olson, "The many roles of histone deacetylases in development and physiology: Implications for disease and therapy," *Nat. Rev. Genet.*, vol. 10, pp. 32–42, Jan 2009, doi: 10.1038/nrg2485.

[2] F. Escaffit, "HDAC3 (histone deacetylase 3)," *Atlas Genet Cytogenet Oncol Haematol.*, vol. 12, no. 2, pp. 104-107, 2008, doi: 10.4267/2042/38492.

[3] "Histone deacetylase 3 (HDAC3) (cd10005)," *InterPro*, 2020, https://www.ebi.ac.uk/interpro/entry/cdd/CD10005/.

[4] S. Emiliani, W. Fischle, C. Van Lint, Y. Al-Abed and E. Verdin, "Characterization of a human RPD3 ortholog, HDAC3," *PNAS*, vol. 95, no. 6, pp. 2795-2800, Mar 1998, doi: 10.1073/pnas.95.6.2795.

[5] "Transcript: HDAC3-201 (ENST00000305264.8) – Summary," *Ensembl genome browser,* 2020, https://bit.ly/2As6rIz.

[6] "Homo sapiens histone deacetylase 3 (HDAC3) mRNA, complete cds," *NCBI*, 1999, https://www.ncbi.nlm.nih.gov/nuccore/AF039703.

[7] R. Rahhal and E. Seto, "Emerging roles of histone modifications and HDACs in RNA splicing," *Nucleic Acids Res.*, vol. 47, no. 10, pp. 4911-4926, Apr 2019, doi: 10.1093/nar/gkz292.

[8] "Domains within Homo sapiens protein HDAC3_HUMAN (O15379)," *SMART*, 2020, https://bit.ly/31KpKb9.

[9] "Histone deacetylase 3 [Homo sapiens]," *NCBI,* 1998 https://bit.ly/2VHyD1u.

[10] "O15379 (HDAC3_HUMAN) - Histone deacetylase 3 gene, protein & align results," *UniProt*, 2020, https://www.uniprot.org/uniprot/O15379.

[11] G.H. Eom and H. Kook, "Posttranslational modifications of histone deacetylases: Implications for cardiovascular diseases," *Pharmacol. Ther.,* vol. 143, pp. 168–180, Aug 2014, doi: 10.1016/j.pharmthera.2014.02.012.

[12] "HDAC3 Gene (Protein Coding)," *GeneCards*, 2020, https://bit.ly/38qUtvh.

[13] "HDAC3 - Histone deacetylase 3 – Proteomics," *NeXtProt*, 2020, https://bit.ly/3ipSlsj.

[14] "Histone deacetylase 3 (O15379, human)," *PhosphoSitePlus*, 2020, https://bit.ly/38mhiQM.

[15] "4a69 - Structural details," *PDBj*, 2020, https://pdbj.org/mine/structural_details/4a69.

[16] "Browse CATH-Gene3D Hierarchy," *CATH*, 2020, http://www.cathdb.info/browse/sunburst?from_cath_id=3.

[17] "Transcript: HDAC3-201 (ENST00000305264.8) - Domains & features," *Ensembl genome browser,* 2020, https://bit.ly/3eRKCkx.

[18] "Histone deacetylase 3 in PDB entry 4a69," *PDBe*, 2012, https://www.ebi.ac.uk/pdbe/entry/pdb/4a69/protein/1.

[19] "O15379 (HDAC3_HUMAN) – Structure Assessment and Interactive Modelling," *SWISS-MODEL*, 2020, https://swissmodel.expasy.org/repository/uniprot/O15379.

[20] P.J. Watson, L. Fairall, G.M. Santos and J.W.R. Schwabe, "Structure of HDAC3 bound to co-repressor and inositol tetraphosphate," *Nature*, vol. 481, pp. 335-340, Jan 2012, doi: 10.1038/nature10728.

[21] "HDAC3 protein (human) - STRING interaction network & coexpression view," *STRING*, 2020, https://string-db.org/network/9606.ENSP00000302967.

[22] "4A69: Structure of HDAC3 bound to corepressor and inositol tetraphosphate," *RCSB PDB*, 2012, http://www.rcsb.org/structure/4A69.

[23] "4a69 structure summary," *PDBe*, 2012, https://www.ebi.ac.uk/pdbe/entry/pdb/4a69.

[24] "Histone deacetylase 3," *PDBe-KB*, 2020, https://www.ebi.ac.uk/pdbe/pdbe-kb/proteins/O15379.

[25] "UniRef - Cluster: Histone deacetylase 3 (100%)," *UniProt*, 2020, https://www.uniprot.org/uniref/UniRef100_O15379.

[26] "UniRef - Cluster: Histone deacetylase 3 (90%)," *UniProt*, 2020, https://www.uniprot.org/uniref/UniRef90_O15379.

[27] "UniRef - Cluster: Histone deacetylase 3 (50%)," *UniProt*, 2020, https://www.uniprot.org/uniref/UniRef50_O15379.

[28] "CATH Domain 4a69A00," *CATH*, 2020, http://www.cathdb.info/version/latest/domain/4a69A00.

[29] S. Bhaskara *et al.*, "Hdac3 is essential for the maintenance of chromatin structure and genome stability," *Cancer Cell*, vol. 18, no. 5, pp. 436-447, Nov 2010, doi: 10.1016/j.ccr.2010.10.022.

[30] Q. Zhao *et al.*, "HDAC3 inhibition prevents blood-brain barrier permeability through Nrf2 activation in type 2 diabetes male mice," *J. Neuroinflammation*, vol. 16, no. 103, May 2019, doi: 10.1186/s12974-019-1495-3.

Leyla M. Sánchez-Palancar is currently a student of the Biotechnology Degree at the Faculty of Experimental Sciences (Universidad Pablo de Olavide). Her study motivations include different topics of biochemistry and genetics, and her research interests involve genetic engineering, health biotechnology, and specific aspects regarding genetic disorders and pathologies.