Cloning and Functional Characterization of HDAC11, a Novel Member of the Human Histone Deacetylase Family*

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We have cloned and characterized a human cDNA that belongs to the histone deacetylase family, which we designate as HDAC11. The predicted HDAC11 amino acid sequence reveals an open reading frame of 347 residues with a corresponding molecular mass of 39 kDa. Sequence analyses of the putative HDAC11 protein indicate that it contains conserved residues in the catalytic core regions shared by both class I and II mammalian HDAC enzymes. Putative orthologues of HDAC11 exist in primate, mouse, Drosophila, and plant. Epitope-tagged HDAC11 protein expressed in mammalian cells displays histone deacetylase activity in vitro. Furthermore, HDAC11’s enzymatic activity is inhibited by trapoxin, a known histone deacetylase inhibitor. Multiple tissue Northern blot and real-time PCR experiments show that the high expression level of HDAC11 transcripts is limited to kidney, heart, brain, skeletal muscle, and testis. Epitope-tagged HDAC11 protein localizes predominantly to the cell nucleus. Co-immunoprecipitation experiments indicate that HDAC11 may be present in protein complexes that also contain HDAC6. These results indicate that HDAC11 is a novel and unique member of the histone deacetylase family and it may have distinct physiological roles from those of the known HDACs.

In eukaryotes, DNA is packaged into chromatin structures, whose basic unit is the nucleosome. Each nucleosome consists of ~148 bp DNA wrapping around a core histone octamer, which includes two copies each of H2A, H2B, H3, and H4 (1). The packaging of DNA generally creates a repressive environment for gene expression; therefore, transcriptional activation of many genes requires chromatin modifications, such as the reversible acetylation of core histones. Transfer of an acetyl group from acetyl-CoA onto the ε-amino group of various lysine residues in the NH₂-terminal tails of core histones is a ubiquitous process found in all eukaryotes examined. The steady state level of acetylation is controlled by the competing activities of histone acetyltransferases and histone deacetylases (HDACs). In general, hypoacetylated chromatin is associated with gene silencing, whereas hyperacetylation correlates with gene activation (2–5). However, recent studies have shown that histone deacetylation can also play a significant role in transcriptional activation. For example, inhibition of HDACs by trichostatin A or trapoxin both activates and silences a small fraction of cellular genes in mammalian cells (6, 7). Furthermore, mutation of the yeast histone deacetylases RPD3 and SIN3 shows that both genes are required to fully activate or to repress specific promoters (6, 8–10). Finally, there is increasing evidence to support the idea that acetylation/deacetylation of non-histone proteins may also function in activation as well as repression of transcription (11–14).

Since the discovery of histone deacetylase RPD3 in Saccharomyces cerevisiae, numerous HDACs have been identified in mammalian cells, and are grouped into three classes based on sequence homologies. Members of the class I include RPD3 and its related proteins HDAC1–3 and 8 (15–19). These enzymes contain NH₂-terminal catalytic domains of ~400–500 amino acids and are primarily located in the nucleus. The class II histone deacetylases consist of proteins that closely resemble the yeast HDA1 protein, including HDAC4–7 (20, 21). Members of this class are larger proteins (~1000 amino acids), whose catalytic domains are located in the COOH terminus of the peptide with the exception of HDAC6, which contains a duplicate of the catalytic region in its NH₂ terminus as well (20). Interestingly, the recently identified class II member, HDAC10, harbors an amino-terminal catalytic domain as well as a carboxyl pseudo-repeat that shares significant homology with its catalytic domain (22). However, the deacetylase function for each domain is yet to be determined. Finally, the yeast SIR2 protein and its homologues form the third class. These enzymes contain nicotinamide adenine dinucleotide (NAD⁺)-dependent activities that are insensitive to the histone deacetylase inhibitor trichostatin A (23–25).

It has been demonstrated that many HDACs exist in large protein complexes in vivo (reviewed in Ref. 26). For example, both HDAC1 and 2 have been found to associate with corepressor complexes Sin3 and Mi-2/ NuRD (27–29). In addition, HDAC3 has been shown to complex with N-CoR and SMRT (30–32). Factors that interact with class II HDACs include transcriptional corepressor CtBP, proteins of the 14-3-3 family as well as MEF2 transcription factors, which are involved in muscle differentiation (for review, see Ref. 33). The presence of different HDACs in distinctive complexes with different transcription regulators presumably contribute to their diverse biological activities.

In the present study, we report the cloning and functional characterization of a novel human histone deacetylase, HDAC11. Our data suggest that HDAC11 is a unique member

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§ The abbreviations used are: HDAC, histone deacetylase; AcuC, acetyl utilization protein; APAH, acetylpolyamine amidohydrolase; NAD, nicotinamide adenine dinucleotide; N-CoR, nuclear receptor co-repressor; NuRD, nucleosome remodeling and histone deacetylase complex; RT, real time.

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Novel Histone Deacetylase HDAC11

Experimental Procedures

Materials—[α-32P]dCTP (3000 Ci/mmol) and the Western blot Chemiluminescence Reagent Plus were purchased from PerkinElmer Life Science (Boston, MA). Rediprim DNA labeling kit, Hybond-P membrane, and Hyperfilm MP were purchased from Amersham Biosciences (Piscataway, NJ). Kodak BioMax MR film was from EASTMAN Kodak Co. (Rochester, NY). Human multiple tissue Northern blot sequences are in bold. The underlined, the STOP signal is in italic, and the FLAG-encoding sequences are in italic. The partial human HDAC11 amino acid sequence reveals an open reading frame of 347 residues with a corresponding molecular mass of 120 kDa. The predicted molecular mass as calculated by Trichloroacetic Acid (TCA) precipitation and silver staining was found to be 120 kDa.

Cloning of the HDAC11 cDNA—Reverse transcription-PCR was employed to generate the HDAC11 coding region, using cDNAs synthesized from normal dermal human fibroblast. PCR primers were designed based on a human renal epithelial cells cDNA EST sequence (GenBankTM accession number AK025890). Restriction enzyme sites and sequences encoding the FLAG epitope were added. The primers are: forward: 5'-CTTGTAATCCACACTGACTCATCAACACAC-3'; reverse: 5'-CCGTGTTAGACATCC-3'. (The XbaI site is underlined, and the translational start site is bolded). The 1.1-kb PCR product was cloned into the pcDNA3.1/Hygro (+) vector (Invitrogen). The resulting construct pcDNA3.1/HDAC11-Flag was verified by DNA sequencing.

Northern blot Analysis—The multiple human tissue Northern blot was purchased from CLONTECH. A 32P-labeled probe corresponding to the HDAC11 coding region (nucleotide numbers 181 to 1122) was prepared using the Redi-prime DNA labeling system (Amersham Biosciences) according to the manufacturer’s instructions. The DNA was further cloned into the pGEM-T easy vector (Promega) and sequenced. The Northern blot was pre-hybridized and hybridized in the presence of a 32P-labeled probe according to the manufacturer’s protocol. A control for the relative amount of mRNA loaded in each lane.

Real-time PCR (RT-PCR)—Total RNA from cultured cell lines were isolated with the Qiagen RNeasy™ kit according to the manufacturer’s instructions. The Northern blot was prepared from transfected 293 cells as described above. Nuclear and cytoplasmic extracts were prepared using NE-PER™ nuclear and cytoplasmic extraction reagents (Pierce) according to the manufacturer’s instructions. Ten µg of total RNA from each preparation were separated by SDS-PAGE, followed by Western blotting using an anti-FLAG antibody for detection of HDAC11-FLAG fusion proteins.

Results

Isolation of Human HDAC11 cDNA—To identify additional members of the HDAC family, we performed BLAST™ searches against GenBank™ and dbEST data bases using the yeast HOS3 protein (34) as the query sequence. The HOS3 protein is a unique member of the HDAC family. It contains functional deacetylase motifs that are shared by both class I and class II eukaryotic HDACs, as well as the prokaryotic HDAC-like proteins, which include the acetoin utilization proteins (AcuCs) (36) and the acetylpolyamine amidohydrolases (APAhs) (36), yet it is not grouped into any of the three HDAC classes. Our homology screens reveal a cDNA sequence in primary human renal epithelial cells (GenBank™ accession number AK025890) that potentially encodes a novel histone deacetylase, which we designate as HDAC11. In addition, we have identified putative HDAC11 orthologues in Mus musculus (accession number BC016208), Drosophila melanogaster (accession number CG10899, 52% identical to human), and Arabidopsis thaliana (accession number AF149413, 51% identical to human) based on sequence homologies at the amino acid level (data not shown). The predicted human HDAC11 amino acid sequence reveals an open reading frame of 347 residues with a corresponding molecular mass of 39 kDa (Fig. 1A). Searches of genomic data bases indicate that...
FIG. 1. A, nucleotide and predicted amino acid sequence of the human HDAC11 cDNA clone. The GenBank™ accession number for the cDNA clone is AK025890. The initiation codon is in bold face. The stop codon is indicated by a black dot (●).

The polyadenylation signal (AAATAAA) is in bold face and italic. B, multiple sequence alignment of HDAC11 with other HDACs and HDAC-like proteins. HDAC11 contains all nine blocks of sequences conserved in other eukaryotic histone deacetylases and the prokaryotic AcuC hydrolases and acetylpolyamine amidohydrolases (36). Active site residues involved in catalysis, metal chelation, and ligand binding, as shown in the crystal structure of the bacterial HDAC-like protein, are marked with a black dot (●). The human proteins are preceded by h, the yeast proteins are preceded by y, the prokaryotic proteins are preceded by pro, and the Drosophila protein is preceded by d.

The human HDAC11 and its putative fly orthologue are labeled in red. The following GenBank™ entries are included in the alignment: hHDAC1 (U50079), hHDAC2 (U31814), yRPD3 (S66438), hHDAC3 (U75697), yHOS2 (Z72716), hHDAC5 (AF290097), yHO51 (Q12214), A. aeolicus acetyl utilization protein proAcuC (AE000719), hHDAC11 (AK025890), hHDAC2 (U31814), hHDAC3 (U75697), yHOS2 (Z72716), hHDAC5 (AF290097), and M. ramosa acetylpolyamine amidohydrolase proAPAH (D10463).

C, phylogenetic relationships of known HDACs. A phylogenetic analysis suggests that the HDAC enzymes diverged from a common prokaryotic ancestor. The yeast proteins are preceded by y, the prokaryotic proteins are preceded by pro, and the Drosophila protein is preceded by d. Catalytic domains of human HDAC6 are marked separately: N, indicates the NH₂ terminus and C, represents the COOH terminus. The eukaryotic class I HDACs are shown in blue, the class II HDACs are shown in green, the human and Drosophila HDAC11 are shown in red, and the prokaryotic HDAC-like proteins as well as the yeast HOS3 proteins are shown in black.

The HDAC11 gene is located on human chromosome 3p25.2, spanning ~25-kb in length. It contains 9 exons and 8 introns, as predicted by spliced junction sites (data not shown). Sequence comparisons at the amino acid level show that HDAC11, over the entire length of the protein, is only slightly homologous to the existing HDAC family members, including
the yeast HOS3 protein. However, HDAC11 contains all nine blocks of conserved sequences that are potentially important for the deacetylase function (Fig. 1B) (20, 35, 36). Furthermore, alignment of HDAC11 with other members of the HDAC family reveal a putative catalytic core region that includes almost all of the invariant active site residues shared by the eukaryotic HDACs as well as the prokaryotic HDAC-like proteins (Fig. 1B) (37). These findings suggest that the newly identified cDNA might encode a protein that possesses histone deacetylase enzymatic activity.

Expression of HDAC11 in Human Tissues and Cell Lines—To determine the tissue distribution pattern for HDAC11 in normal human tissues, we performed Northern blotting experiments using a probe that hybridized to the coding region (see “Experimental Procedures”). These analyses reveal two HDAC11 transcripts in human tissues. One is ~1.7 kb, which agrees with the estimated size of the human cDNA clone; the other is ~3.2 kb and appears to be expressed more abundantly (Fig. 2A). Interestingly, additional GenBank search identified a 3160-bp Macac fascicularis (primate, aka crab-eating macaque) brain cDNA (accession number AB052134) that shared extensive homology with the human cDNA. Therefore, the longer transcript we observed on the Northern blot might represent the human homologue of the macaque cDNA. Sequence alignment of the two cDNAs show that although the macaque cDNA contains additional nucleotides in both the 5′- and 3′-untranslated regions, the predicted macaque polypeptide is almost identical to that of the human (only 3 conservative amino acid substitutions), missing just the first 28 amino acids (Fig. 2B). This finding suggests that the
two transcripts seen on the Northern blot are probably the result of alternative gene splicing. Furthermore, Northern analyses indicate that the overall expression level of HDAC11 mRNA is low in many human tissues and that the high expression level is limited to brain, heart, skeletal muscle, and kidney (Fig. 2A). On the contrary, previous reports have shown that many members of the histone deacetylase family are ubiquitously expressed (20, 35, 38, 39). Thus, we envision that HDAC11’s function may be tissue-specific.

Real-time PCR technique was also employed to examine HDAC11 expression in normal human tissues and eight human cancer cell lines. These experiments confirm findings of the Northern analyses; in addition, they reveal high expression levels of HDAC11 mRNA in testis (Fig. 2B). Furthermore, our

![Figure 2A](image.png)

**Fig. 2. Tissue distribution of HDAC11 mRNA in human.** A, Northern blot analysis of HDAC11 expression. The blot containing mRNA from the indicated tissues were probed with $^32$P-HDAC11 cDNA (top panel), stripped, and re-probed with $^32$P-actin cDNA (bottom panel). B, the predicted open reading frames of the human and macaque cDNA were aligned using the ClustalW program. Identical amino acids are marked by an asterisk (*) and similar amino acids are marked by a colon (:). C, RNAs from human tissues and cultured cells (right 8 bars) were assayed by RT-PCR (see “Experimental Procedures”). Three tissues were derived from the same donor (black bars). Indicated are tissue type, pool size, sex, and age of the donor(s).
data show that large amounts of HDAC11 transcripts are found in four cancer cell lines, most notably, a rhabdomyosarcoma muscle tumor line (Fig. 2B). Taken together, these results suggest that HDAC11 may function not only in normal human tissues, but also in the development and/or maintenance of certain human cancers.

**HDAC11 Contains Histone Deacetylase Activity**—To determine whether HDAC11 possesses histone deacetylase activity, we expressed HDAC11 as a COOH-terminal FLAG epitope tag (Fig. 3A) in 293 cells which is detected on a Western blot using the α-FLAG antibody (upper panel) and total protein loaded in each lane is revealed by Ponceau S staining (lower panel); C, in vitro histone deacetylase enzymatic activity of HDAC11. D, in vitro HDAC11 enzymatic activity following 200 nM trapoxin (TPX) treatment, the inset shows the enzymatic activity of HDAC3.

**HDAC11 and HDAC6 Are Present in Common Multiprotein Complexes**—Previous studies have shown that both class I and class II HDACs function in large protein complexes that include many transcription repressors/corepressors and other HDAC members (20, 21, 31, 40). To determine whether HDAC11 participates in similar complexes, we performed co-immunoprecipitation experiments. Our results indicate that unlike the class I and class II HDACs, HDAC11 does not associate with mSin3A (Fig. 4A), RbAp48, N-CoR, or SMART in vivo (data not shown), suggesting that HDAC11 is probably biochemically distinct from the existing HDACs. Interestingly, we find that HDAC6, which is also highly expressed in kidney and testis (20), coimmunoprecipitates with HDAC11 (Fig. 4B). Furthermore, the reciprocal experiments show that the anti-HDAC6 antibody is able to coimmunoprecipitate the HDAC11-FLAG fusion protein (Fig. 4C). Therefore, these two HDAC members probably associate with common proteins or each other in vivo. No previous protein-protein interactions have

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**Fig. 3.** HDAC11 encodes a histone deacetylase. A, a schematic drawing of HDAC11 polypeptide with a COOH-terminal FLAG epitope tag; B, transient expression of the HDAC11-FLAG fusion protein in 293 cells is detected on a Western blot using the α-FLAG antibody (upper panel) and total protein loaded in each lane is revealed by Ponceau S staining (lower panel); C, in vitro histone deacetylase enzymatic activity of HDAC11. D, in vitro HDAC11 enzymatic activity following 200 nM trapoxin (TPX) treatment, the inset shows the enzymatic activity of HDAC3.

**Fig. 4.** HDAC11 associates with HDAC6 in vivo. 293 cells were transfected with the indicated plasmids. Immunoprecipitation were performed using either the α-FLAG or the α-HDAC6 antibody as shown. The immune complexes were analyzed by Western blotting using the indicated antibodies. F represents the FLAG epitope tag. A: upper panel, α-mSin3A; lower panel, α-FLAG; B: upper panel, α-HDAC6; lower panel, α-FLAG; C: upper panel, α-HDAC6; lower panel, α-FLAG.

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2 F. Asselbergs, unpublished data.
been demonstrated for HDAC6, whose biological activity is still largely unknown.

**HDAC11 Is a Predominantly Nuclear Protein**—It has been shown that some members of the histone deacetylase family localize exclusively to the nucleus (16), whereas others shuttle between the cytoplasm and the nucleus (26, 29, 41). To examine the subcellular localization of HDAC11 protein, we transected 293 cells with the HDAC11-FLAG expression vector and performed cell fractionation studies. Our data shows that the HDAC11-FLAG fusion protein is found in the whole cell lysates and the nuclear extracts, but not in the cytoplasmic extracts (Fig. 5). As expected, we also detect the endogenous HDAC1 protein in the same nuclear extract preparation. Therefore, we conclude that HDAC11 proteins are located mostly in the nuclei.

**DISCUSSION**

In this report, we describe the identification and characterization of a new human histone deacetylase, HDAC11. We have shown that, when expressed in mammalian cells, the cloned cDNA lead to production of an active HDAC enzyme. Furthermore, the HDAC11 mRNA is abundantly expressed in specific normal human tissues as well as several cancer cell lines. Finally, we have demonstrated that the HDAC11 protein resides in the nucleus and is found in protein complexes with another HDAC family member, HDAC6.

The histone deacetylase family is a large and expanding family with the recent additions of human HDAC9 (42) and HDAC10 (22). Together with the prokaryotic HDAC-like proteins AcuC and APAHs, eukaryotic HDACs are members of an ancient protein superfamily (36). Previous studies have shown that the eubacterium protein *Bacillus subtilis* AcuC and the proteobacterium protein *Mycoplana ramosa* APAH may have diverged from a common ancestor and subsequently evolved to give rise to the eukaryotic class I and class II HDACs, respectively (20). HDAC11 is the smallest member of the HDAC family discovered so far and the entire protein contains mostly the catalytic domain. Similarly, the prokaryotic HDAC-like proteins AcuC and APAH are relatively small enzymes whose catalytic domains span their entire sequences (36). Analyses of the conserved active site residues as well as phylogenetic studies (Fig. 1C) indicate that HDAC11 is more closely related to the prokaryotic AcuC protein and the class I eukaryotic HDACs than to the APAH protein and class II HDACs. Furthermore, these findings suggest that together with AcuC, APAH, and the yeast HOS3 protein, HDAC11 might have diverged early in evolution and thus more closely related to the common ancestral gene(s) from which the eukaryotic HDACs evolved.

The COOH-terminal FLAG-tagged HDAC11 fusion protein, when expressed in 293 cells, possesses HDAC activity that is active on a synthetic peptide derived from the NH2-terminal domain of histone H4. Compared with activities of other HDAC members in the same in vitro enzyme assay, HDAC11’s activity is much lower (data not shown). However, we have found that the HDAC11-FLAG fusion protein is expressed at a lower amount compared with all other FLAG-tagged HDAC members we have examined; thus, the reduced in vitro activity does not necessarily imply that HDAC11 is a weaker HDAC. Furthermore, the activity is measured in an in vitro assay using a synthetic peptide that might not be the endogenous substrate(s) in vivo. These may include other histones as well as non-histone proteins. It is possible that HDAC6, due to its association with HDAC11 in vivo, is contributing to the observed in vitro enzymatic activities. However, HDAC6’s enzymatic activity is highly resistant to trapoxin (43), whereas the activity derived from immunoprecipitated HDAC11 is completely inhibited at 200 nM. Therefore, we conclude that HDAC11 is a bona fide histone deacetylase. Based on results of this and other similar studies, we hope that the availability of this new HDAC will permit development of more selective HDAC inhibitors with possible beneficial pharmaceutical properties.

Unlike the class I HDAC enzymes, which are ubiquitously expressed in normal human tissues, expression of HDAC11 mRNA is limited to brain, heart, skeletal muscle, kidney, and testis, suggesting that its function might be tissue-specific. Interestingly, we have found an extremely high level of HDAC11 mRNA in several human cancer cell lines. Preliminary studies suggest that the aberrant expression is not due to gene amplification, but rather a result of deregulation of HDAC11 gene expression. It is tempting to speculate that misregulation of HDAC11 gene expression may contribute to carcinogenesis and/or metastasis in certain human cancers. We have observed two HDAC11 transcripts on Northern blots, most likely a result of alternative gene splicing. The larger transcript, which might encode an NH2-terminal truncated protein, appears to be expressed more abundantly in normal human tissues. It will be interesting to examine whether the truncated HDAC11 protein contains histone deacetylase activity and whether it is the predominant form in vivo. An antibody against the endogenous HDAC11 protein will aid in addressing these questions.

It has been widely accepted that HDACs modulate transcriptional gene expression at specific promoters by associating with transcriptional corepressors, often in large protein complexes (26). Most of the evidence on the role of HDACs in vivo has been pieced together by studying the interacting partners. Immunoprecipitation experiments of epitope-tagged HDAC11 suggest that, in contrast to class I and class II HDACs, HDAC11 does not associate with the mSin3a or the SMRT-N-CoR complex. Interestingly, we find that virtually all of the HDAC11 in vivo is present in a protein complex that can be immunoprecipitated by the HDAC6 antibody (Fig. 4C). HDAC6 is a unique member of the class II enzymes that contains two catalytic domains. Unlike other members of the class II, such as HDAC4 and HDAC5, HDAC6 has not been found in protein complexes containing HDAC3 or RbAp48. It has been suggested that HDAC6 may not interact with histones in vivo, but rather may deacetylate other substrates (20). Its precise biological function has not been established. We speculate that both HDAC11 and HDAC6...
may utilize non-histone proteins as their substrates; they may exist in a distinctive protein complex from the known co-repressor complexes and play roles other than to directly modulate chromatin structure. Further studies of HDAC11 will not only provide information on its biological functions but also potentially shed some light on HDAC6’s mechanism of action.

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