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Role of *CHD5* in Human Cancers: 10 Years Later

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Abstract

CHD5 was first identified because of its location on 1p36 in a region of frequent deletion in neuroblastomas. CHD5 is the fifth member of a family of chromatin remodeling proteins, and it probably functions by forming a nucleosome remodeling and deacetylation complex that regulates transcription of particular genes. *CHD5* is preferentially expressed in the nervous system and testis. Based on its position, pattern of expression and function in neuroblastoma cells and xenografts, *CHD5* was identified as a tumor suppressor gene (TSG). Evidence soon emerged that *CHD5* also functioned as a TSG in gliomas and a variety of other tumor types, including breast, colon, lung, ovary and prostate cancers. Although one copy of *CHD5* is deleted frequently, inactivating mutations of the remaining allele are rare. However, DNA methylation of the *CHD5* promoter is found frequently, and this epigenetic mechanism leads to biallelic inactivation. Furthermore, low *CHD5* expression is strongly associated with unfavorable clinical and biological features as well as outcome in neuroblastomas and many other tumor types. Thus, based in its likely involvement as a TSG in neuroblastomas, gliomas and many common adult tumors, *CHD5* may play an important developmental role in many other tissues besides the nervous system and testis.

Keywords

CHD5; epigenetic; neuroblastoma; promoter methylation; tumor suppressor gene

CHD5: Mechanisms of Action

CHD5 is a member of the chromodomain-helicase-DNA binding (CHD) protein family

CHD5 was first identified based on its location on 1p36 in a region of frequent deletion in neuroblastomas (1). CHD5 is the fifth member of a nine-member family of CHD chromatin remodeling proteins (CHD1-CHD9) (**Fig. 1A**). These proteins can be classified into three subfamilies or classes, based on structural features and sequence homology— 1) CHD1 and CHD2, 2) CHD3-CHD5, and 3) CHD6-CHD9 (2-4). One report initially described CHD6 as CHD5 (5), leading to some confusion as to whether CHD5 belonged to subfamily 2 or 3 (2, 3). However, based on sequence homology, paired plant homology domain (PHD) motifs at the N-terminal region that are unique to these three members, and similar protein associations, CHD5 more appropriately belongs in the second subfamily with CHD3 and CHD4 (4).

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CHD5 forms a nucleosome remodeling and deacetylation (NuRD)-type chromatin remodeling complex, similar to CHD3 and CHD4

The canonical NuRD complex consists of CHD4 together with other proteins, including MTA1/2, HDAC1/2, RBBP4/7, GATAD2A/B, and MDB2/3 (6-11). This megadalton complex is presumably recruited to specific genes or DNA domains by interaction with other DNA binding proteins. We and others have evidence that CHD5 interacts with most members of a canonical NuRD complex, further supporting a similar function [(12) and unpublished] (**Fig. 1B**). CHD proteins in the second subfamily lack direct DNA binding motifs, so they must interact with transcription factors, cofactors, or other DNA binding proteins to regulate transcription. NuRD complexes are generally thought to be transcriptionally repressive, but they are also associated with transcriptional activation, as well as transcript elongation, termination, RNA processing, and DNA damage response (4, 13). The exact functions may depend on the cellular context, developmental stage, the proteins that form the complex, and the DNA binding proteins with which they interact (12).

CHD5 requires PHD domains for histone 3 binding and tumor suppression

Structurally, CHD5 is characterized by paired PHD domains and two tandem chromodomains at the N-terminal region, an SNF2-like helicase/ATPase domain in the central region, and a conserved, coiled-coil motif in the C-terminal region. The tandem PHD domains are zinc finger-like motifs that recognize histone tails, which also facilitate chromatin-based transcriptional regulation (14). CHD5 binds the unmodified N-terminus of H3 through its PHD domains at loci lacking H3K4me3, a mark of actively transcribed genes (14, 15). However, CHD5 can also bind to H3K27me3, a mark of inactive genes (16). Inactivating mutations in the PHD domains of CHD5 abrogate histone 3 binding, which is critical for modulation of target genes and for tumor suppression (15).

CHD5 is preferentially expressed in the nervous system and testis

Interestingly, CHD4 and CHD3 are expressed ubiquitously, whereas *CHD5* is preferentially expressed in the nervous system (brain, spinal cord, adrenal medulla, sympathetic ganglia) and testis (1, 12, 16-18). Within the nervous system, *CHD5* expression is restricted to neurons, and glial cells are consistently negative. Furthermore, expression is confined to the nucleus of mature neurons, consistent with its presumed role as a chromatin remodeling protein (12, 16, 17, 19). CHD5 is also required for proper spermiogenesis and chromatin condensation in sperm, and male mice with constitutional homozygous deletion of *CHD5* are infertile (18). Thus, the selective pattern of *CHD5* expression in the nervous system and testis suggests that CHD5 may have unique functions in these tissues. Nevertheless, *CHD5* is expressed at lower levels in many other tissues, and it has been implicated as a tumor suppressor gene (TSG) in a variety of non-neural cancers, suggesting it may also play an important developmental role in other tissues.

CHD5 expression is regulated by different mechanisms

CHD5 may be transcriptionally silenced by DNA methylation of the promoter, silencing the remaining allele in some cancers with 1p deletion (see below). One study examined the methylation status of the promoter in all the CHD family genes in various cancers, and the *CHD5* promoter was the most heavily methylated, (20). In addition, at least two other mechanisms of regulation have been identified. The lysine demethylase JMJD2A was identified because it cooperated with RAS activation to transform primary cells in culture, blocking cellular senescence (21). JMJD2A apparently causes transcriptional repression of *CHD5*, which leads to reduced p53 expression. MiRNAs can also regulate gene expression by binding to the 3' untranslated region of mRNAs, causing translational repression or mRNA instability (22). One group analyzed the CHD5 mRNA and predicted that miR-211

bound to the *CHD5* 3'-UTR (23). Transfection of colon cancer cells with an miR-211 expression vector substantially decreased *CHD5* expression and produced a more aggressive phenotype in vitro and in vivo, suggesting that *CHD5* is a direct target of miR-211. However, transcriptional regulation by other transcription factors, miRNAs or other mechanisms is still unknown.

CHD5 functions as a TSG in part by regulating p53

We first identified CHD5 as a candidate TSG in neuroblastomas (1). Bagchi and colleagues took an independent approach using chromosome engineering to identify *Chd5* as a TSG on mouse chromosome 4, a region orthologous to human 1p36 (24). They determined that *Chd5* functions as a TSG that controls proliferation, apoptosis, and senescence at least in part by upregulation of $p19^{Arf}$, which in turn upregulates p53 (24). CHD5 may have similar effects on $p14^{Arf}$ in human cells, although it is likely that its TSG function is not limited to this mechanism. Indeed, little is known about the other target genes that are regulated by CHD5 or CHD5-NuRD complexes.

CHD5 functions as a TSG in neuroblastomas and a variety of other cancers

Here we review the evidence supporting *CHD5* as a bona fide TSG in neuroblastoma, a common pediatric tumor, as well as gliomas and many other adult cancers, including cancers of the breast, colon, lung, ovary, prostate, stomach, larynx, and gallbladder. In many cases, one copy of *CHD5* is deleted, but the remaining allele is seldom unaltered (**Fig. 1C, Table 1)**. Nevertheless, expression of the remaining allele (or both alleles if one is not deleted) are frequently silenced by promoter methylation. Thus, both genetic and epigenetic mechanisms are involved in silencing this novel TSG.

Role of *CHD5* in Neuroblastomas

Neuroblastoma is a tumor of the sympathetic nervous system that is the most common extracranial solid tumor of childhood (25, 26). We first identified 1p deletion as a characteristic change in advanced stage neuroblastomas (27). Deletion of the short arm of chromosome 1 (1p) has been observed in 35% of primary neuroblastomas and 70-80% of neuroblastoma-derived cell lines (28-31). Deletion of 1p presumably reflects loss of one or more TSGs from this region. We refined the region of consistent deletion of 1p36 using DNA-based polymorphisms and narrowed the smallest region of consistent deletion to a ~2 Mb region on 1p36.31 (30, 32, 33). Although 23 genes mapped to this 2 Mb region, we identified *CHD5* as the most likely TSG contributing to neuroblastoma pathogenesis from this region (1, 34, 35).

We first identified *CHD5* as a new member of the CHD family and a candidate TSG in neuroblastomas 10 years ago (1). Indeed, subsequent studies by ourselves and others strongly support *CHD5* as an important TSG in neuroblastomas, based on: 1) its function and expression profile, 2) its ability to suppress neuroblastoma growth in vitro and in vivo, and 3) the correlation of *CHD5* expression with prognostic variables and outcome (17, 34-37). However, other TSGs have been identified on 1p36 that may also play important roles in neuroblastoma pathogenesis, including *CAMTA1, miR-34a, KIF1Bβ, CASZ1* and *ARID1A* [reviewed in (38, 39)]. Most 1p deletions in neuroblastomas are quite large, so several of these other genes may be involved. However, at least for *CHD5*, simply deleting one copy is probably not sufficient to promote tumorigenesis, as expression the remaining allele in cases with 1p36 deletion is extremely low or undetectable (34, 37). Indeed, the remaining allele in cases with 1p deletion is seldom mutated (**Table 1, Fig. 1C**), suggesting that it is silenced transcriptionally by epigenetic mechanisms.

Functional analysis of *CHD5* after transfection into neuroblastoma lines demonstrates that it suppresses both clonigenicity and tumorigenicity (34). High *CHD5* is strongly correlated with favorable clinical and biological features as well as outcome, whereas low/absent expression is associated with unfavorable features including *MYCN* amplification, as well as a poor outcome (17, 37). Although one copy of *CHD5* is frequently deleted, the remaining copy is rarely mutated or inactivated by DNA rearrangement. However, the *CHD5* promoter is heavily methylated, especially between –780 and –450 in neuroblastoma cell lines with 1p36 deletions (34). Furthermore, *CHD5* can be re-expressed by exposure to the demethylating agent 5-aza-2'-deoxycytidine (5-Aza). Thus, *CHD5* is a bona fide TSG in neuroblastomas, but the remaining *CHD5* allele is usually silenced by epigenetic mechanisms rather than by inactivating mutations (35).

High *CHD5* expression by microarray analysis was strongly associated with favorable clinical and biological risk features as well as outcome in a panel of 101 neuroblastomas (34). Similarly, high *CHD5* mRNA expression (by quantitative real-time RT-PCR) was associated with favorable risk features and outcome in a study of 814 representative primary neuroblastoma (37). CHD5 protein expression was also shown to be a strong prognostic marker in an immunohistochemical study of 90 primary neuroblastomas (17). Finally, a study of differentially expressed genes from chromosomes 1 and 17 led to an expression signature that was predictive of neuroblastoma outcome. *CHD5* was identified as one of only three genes (*CHD5*, *PAFAH1B1*, *NME1*) that robustly classified patients into clinically relevant risk groups (36). Thus, there is strong structural and functional evidence that *CHD5* is a bona fide TSG in neuroblastomas, and high *CHD5* expression is strongly associated with both favorable features and outcome (and vice versa).

Role of *CHD5* in Other Cancers

Gliomas (Table 1)

Law and colleagues screened 17 glioma lines for 1p deletions using fluorescence in-situ hybridization, comparative genomic hybridization and DNA polymorphisms (40). They defined a 700 kb region of consistent deletion that contained five genes and four uncharacterized transcripts; *CHD5* was the most plausible TSG in this region. Bagchi and coworkers analyzed the expression of genes mapping to a 5.4 Mb region corresponding to 1p36 in 54 gliomas, and loss of *CHD5* expression was significantly associated with 1p36 deletion in these tumors (24). Finally, Wang measured *CHD5* expression in 128 gliomas, and down-regulation of *CHD5* expression was associated with unfavorable risk features and outcome in these patients (41). These data are consistent with a role for *CHD5* as a TSG in gliomas.

Colorectal cancer (Table 1)

Ragnarsson analyzed several solid tumor types for 1p allelic loss using microsatellite markers, and they identified 1p deletion in 20-30% of cases (42). Mokarram studied the methylation status of a set of cancer-related genes in 102 colon cancers from Iranian and African-American populations (51 each) (43). The methylation status of the *CHD5* promoter was significantly higher in cancers from the African-American population (78%) compared to the Iranian patients (47%; $p<0.002$). In addition, *CHD5* showed a lower level of methylation in the distal colon (43), suggesting that *CHD5* may play a role in the incidence or aggressiveness of colorectal cancer in this population. Fatemi found that the *CHD5* gene was repressed, either epigenetically or by chromosomal deletion, in colon adenomas compared to surrounding normal tissue (44). Cai also investigated the effect of epigenetic silencing of *CHD5* on colorectal tumorigenesis (23). A colorectal cancer cell line (HCT-116) was stably transfected to overexpress miR-211, which was predicted to bind to

the 3' untranslated region of *CHD5*. The transfected cell line showed a 50% decrease in CHD5 protein level. In vitro and in vivo studies showed that cell proliferation, cell migration and tumor growth were significantly higher than control cells. These results suggest *CHD5* is a direct target of miR-211, and epigenetic silencing of *CHD5* may play a role in colorectal tumorigenesis.

Breast Cancer (Table 1)

Wu analyzed the role of *CHD5* in breast cancer by screening 55 tumors for mutations, 39 tumors for promoter methylation, 90 tumors for *CHD5* RNA expression, and 289 tumors for CHD5 protein expression (45). They correlated *CHD5* expression changes with clinico-pathological characteristics of breast cancer. They also assessed functional effects of *CHD5* on cell proliferation, invasion and tumorigenesis. Two *CHD5* mutations were found out of 55 cases examined (**Table 1**). One was a base pair deletion leading to a premature stop codon and a truncated protein, and the other was a conserved codon at the N-terminus in a region of unknown function. *CHD5* mRNA and protein expression was significantly reduced in breast cancer tissue, which was accompanied by genomic deletion and promoter methylation, and treatment with 5-Aza restored *CHD5* expression. Low *CHD5* mRNA expression was correlated with lymph node metastasis ($p=0.026$), and lack of CHD5 protein expression was correlated with higher tumor stage, ER/PR-negativity, HER2 positivity, distant metastasis and worse patient outcome ($p\leq0.01$). Transfection of breast cancer cells with *CHD5* inhibited cell proliferation and invasion in vitro, and tumorigenesis in nude mice (45). These studies suggest that decreased *CHD5* expression, mediated in part by promoter methylation, contributes to the development and progression of human breast cancer.

Lung Cancer (Table 1)

Zhao studied the epigenetic modification and tumor-suppressive ability of *CHD5* by measuring *CHD5* mRNA and protein expression in lung cancer cell lines and tissues (46). *CHD5* expression ranged from low to absent in the lung cancer lines and tissues examined, and this correlated with *CHD5* promoter hypermethylation. Clonigenicity and tumor growth were abrogated in lung cancer cell lines A549 and H1299 upon restoration of *CHD5* expression (46). These observations suggest that *CHD5* served as a potential TSG in lung cancer.

Ovarian Cancer

Gorringe analyzed 123 primary ovarian cancers for *CHD5* mutations (47). Missense mutations were identified in 3 of 123 tumors, but no inactivating mutations were found (**Table 1, Fig 1C**). They referred to an earlier study that identified one potentially pathogenic mutation in CHD5 out of 10 tumors examined (48) (**Table 1, Fig 1C**). Although none of these mutations were inactivating, all were in codons encoding conserved amino acids (**Supplemental Fig. 1**). Promoter methylation was seen in another 3 of 45 samples tested, and copy number loss was seen in 6 of 85 tumors. Overall, deletion, mutation or promoter methylation was seen in about 16% of cases (47). Wong compared *CHD5* expression between 72 primary ovarian tumors and 12 normal ovarian tissues, and found that *CHD5* expression was downregulated at least 2-fold in 32 out of 72 (41%) tumors compared to normal tissues (49). *CHD5* down-regulation was associated with shorter disease-free and overall survival ($p<0.05$). These studies suggest a possible role for *CHD5* in the pathogenesis of at least a subset of ovarian cancers.

Gastric Cancer (Table 1)

Wang examined the expression and promoter methylation status of 7 gastric cancer cell lines and 15 primary gastric tumors (50). *CHD5* expression was downregulated in all gastric

cancer lines. *CHD5* promoter methylation was detected in all seven lines, and exposure to 5-Aza substantially restored expression levels towards that seen in normal gastric mucosa. Methylation status of the *CHD5* promoter was examined in 15 primary gastric cancers, and methylation was found in 11 of 15 primary tumors. Furthermore, ectopic expression of *CHD5* resulted in significant growth inhibition in colony formation assays (50). In another study, Qu conducted a study of the methylation status of cancer-related genes (including *CHD5*) in gastric cancer (51). The percent methylation in gastric cancers was twice that found in normal gastric mucosa. These results suggest that *CHD5* play a role as TSG in gastric cancer, and expression may be downregulated by promoter hypermethylation.

Laryngeal squamous cell carcinoma (Table 1)

Wang examined *CHD5* mRNA and protein expression in 65 patients with laryngeal squamous cell carcinomas (SCC) (52). Both *CHD5* RNA and protein expression were significantly lower compared to normal laryngeal tissue, and the level of *CHD5* expression correlated with the extent of promoter hypermethylation. Furthermore, ectopic expression of *CHD5* in laryngeal SCC cells led to significant inhibition of growth and invasiveness (52). These data suggest that *CHD5* acts as a TSG that is epigenetically downregulated in laryngeal SCC.

Gallbladder carcinoma (Table 1)

Du and colleagues studied *CHD5* mRNA and protein expression in 120 primary gallbladder carcinomas and 20 normal gallbladder specimens (53). The expression levels of *CHD5* mRNA and protein were both significantly lower in the gallbladder carcinomas than those in the normal gallbladder epithelium (mRNA: $p<0.006$; protein: $p<0.01$). Survival analysis showed that low *CHD5* expression in gallbladder carcinomas was associated with shorter disease-free ($p=0.01$) and overall survival ($p=0.008$) compared to patients with high *CHD5* expression in their tumors (53). Decreased expression of *CHD5* is an unfavorable prognostic marker in patients with primary gallbladder carcinoma.

Prostate Cancer

Robbins conducted a comprehensive genomic survey for somatic events in metastatic prostate tumors using both high-resolution copy number analysis and targeted mutational survey of 3,508 exons from 577 cancer-related genes in eight tumors using next generation sequencing (54). Two novel missense mutations were identified in *CHD5* **(Table 1)**, but no copy number change of *CHD5* was found in these tumors. However, two different missense mutations were found in two different samples (54). These studies indicate that deep genomic analysis of advanced metastatic prostate tumors can identify somatic alterations of genes that may contribute to aggressive prostate cancers, although no functional evidence was presented that *CHD5* was a TSG in prostate cancer.

Conclusions

CHD5 was first identified as a new member of the CHD family and a candidate TSG deleted from 1p36 in neuroblastomas (1). Subsequent evidence showing suppression of clonigenicity and tumorigenicity, as well as correlation with risk factors and outcome, support its function as a TSG in this tumor (17, 34, 36, 37). Indeed, there is increasing evidence that *CHD5* functions as a TSG in many other types of cancer, including cancers of the colon, breast, lung, and ovary, gliomas and others (**Table 1**). However, the pattern established in neuroblastoma is consistently seen in essentially all other tumor types, to the extent it has been studied: 1) one *CHD5* allele is frequently lost by deletion; 2) the remaining *CHD5* allele is rarely inactivated by mutation or structural rearrangement; 3) the *CHD5* promoter is transcriptionally silenced by methylation or other epigenetic

mechanisms; 4) *CHD5* expression can be upregulated by exposure to demethylating agents, like 5-Aza; 5) transfection of tumor cells with a CHD5 expression inhibits tumor growth in vitro and in vivo; 6) low *CHD5* expression is seen tumor tissue compared to adjacent normal tissue, and lower levels in high-risk versus low-risk tumors; 7) low *CHD5* expression is associated with adverse risk factors and poor survival. Thus, *CHD5* may be a master regulator, controlling cellular development, differentiation, proliferation, senescence and cell death for neuroblastomas and a variety of other tumors.

CHD5 likely functions as a part of a NuRD-type chromatin remodeling complex, but it is unknown if it has functions independent of this complex. CHD5-NuRD presumably functions predominantly as a transcriptional repressor, and it may also contribute to transcriptional activation, as well as transcript elongation, termination, RNA processing, and DNA damage response (4, 13). Nevertheless, as a nuclear protein, CHD5 may be a difficult target. Fortunately, the remaining allele in cases with 1p36 deletion is almost always intact, and it is usually silenced by promoter methylation, which is amenable to modification. The region that is methylated varies somewhat from one study to another, but the region between –400 and –800 bp from the *CHD5* start site seemed most frequently involved (20, 34, 37, 46, 50, 52). Upregulation of *CHD5* expression can be accomplished by exposure to 5-Aza or other demethylating agents, although there is no way to selectively target the *CHD5* promoter. *CHD5* expression may be repressed by transcription factors or miRNAs that may be more tractable as targets. Also, the targets of CHD5 transcriptional control are largely unknown, but they may be more easily druggable. Nevertheless, *CHD5* has emerged as an important TSG that clearly plays a critical role in neuroblastomas, gliomas and a variety of other cancers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

5-Aza	5-aza-2’-deoxycytidine
CHD	Chromodomain-Helicase-DNA binding
NuRD	Nucleosome Remodeling and Deacetylation
PHD	Plant Homology Domain
SCC	squamous cell carcinoma
TSG	Tumor Suppressor Gene

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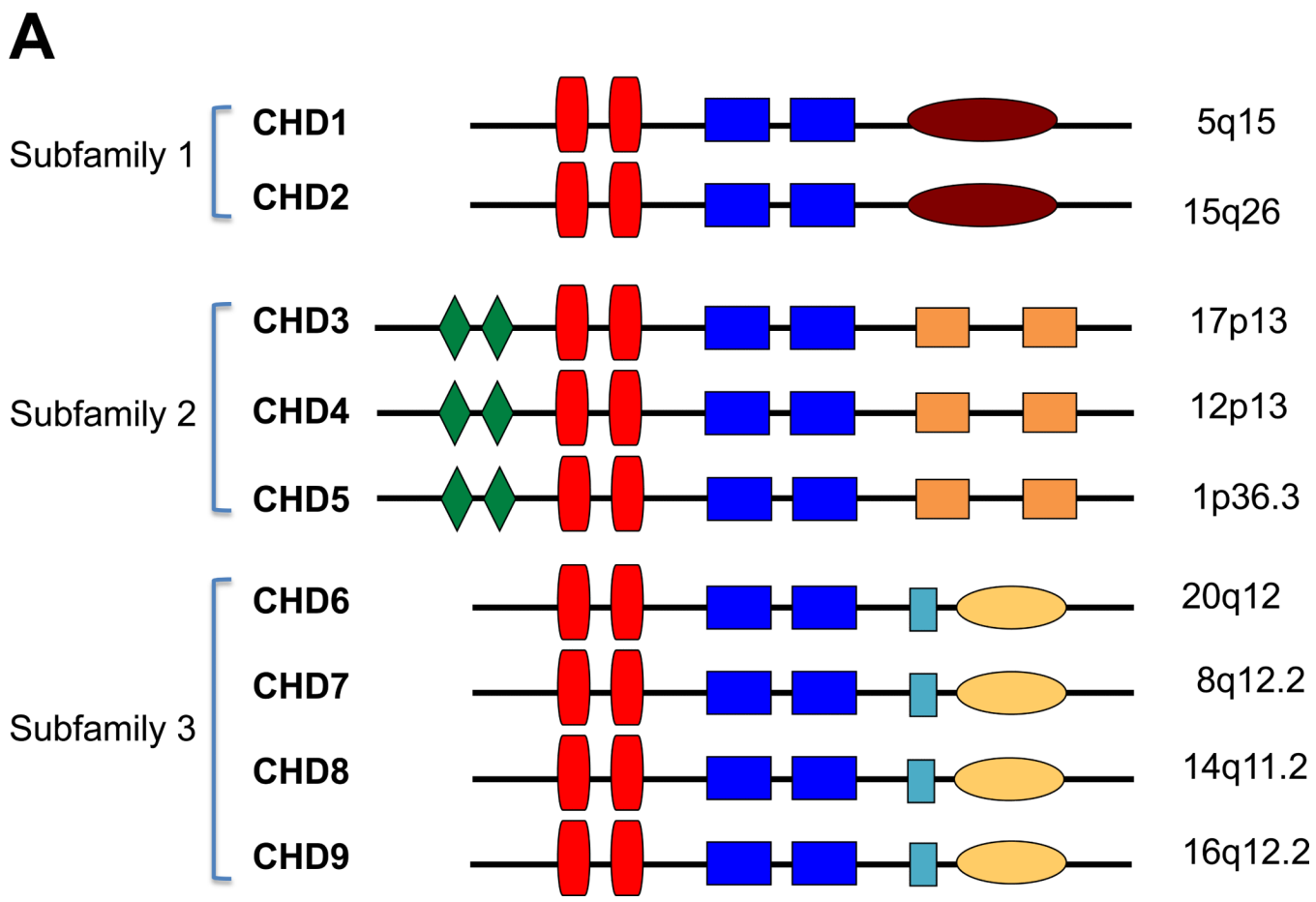

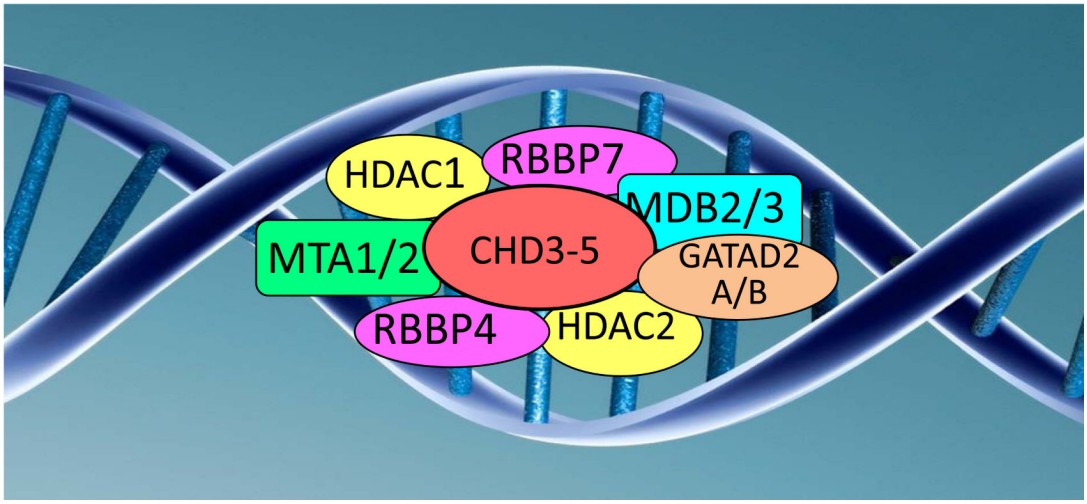

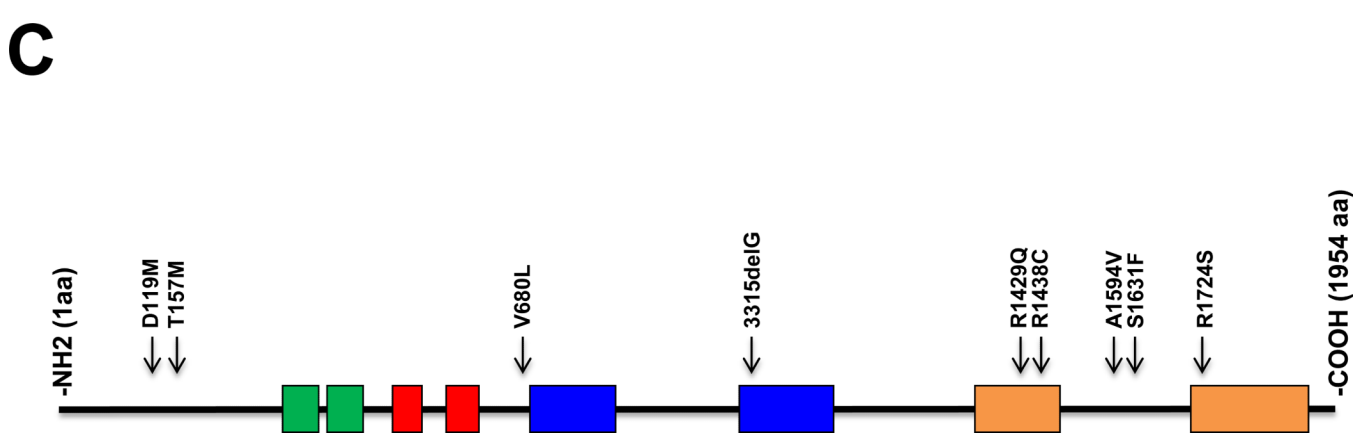

Figure 1. CHD5 Chromatin Remodeling Protein

A. CHD family. Shown are diagrammatic representations of the nine members of the CHD (Chromodomain-Helicase-DNA binding) family of proteins. They are divided into three subfamilies based on sequence and structural homology as well as function. The conserved motifs are shown: green diamonds = PHD finger domains; red vertical boxes = chromodomains; blue rectangular boxes = split SNF2-like helicase/ATPase domain; burgundy oval = DNA binding motif; orange boxes = domains of unknown function (DUF1, DUF2); light blue box = SANT domain; yellow oval = BRK domains. The chromosomal locations of the nine CHD family members are shown to the right.

B. CHD5 NuRD complex. Shown is a diagrammatic representation of the hypothetical CHD5-NuRD complex, which is identical to a CHD4-NuRD complex. The canonical components of this complex are HDAC1 and HDAC2; MTA1 and MTA2; RBBP4 and RBBP7; GATA2DA and GATA2DB; and MDB2 and MDB3.

C. CHD5 mutations. Shown is a diagram of the CHD5 protein with conserved motifs, as indicated in **1A**. The relative position of 9 CHD5 mutations in various cancers is listed in Table 1 and shown on this diagram. All of the mutations (8 of the 9) involved highly conserved amino acids (see **Supplemental Figure 1**). The G base pair deletion at nucleotide position 3315 changed the reading frame and led to a premature stop codon.

Table 1

Cancers with 1p36 deletion and possible role of *CHD5*

Malignancy	1p Deletion (%)	*CHD5* Mutation	*CHD5* Mutation(s)[+]	*CHD5* Expression	Promoter Methylation[*]	*CHD5* Growth Suppression	*CHD5* Clinical Correlation	References
Neuroblastoma	35%	1/30	V680L	Lower in HR tumors	Yes[*]	In vitro and in vivo	Risk factors Outcome	(32-35, 37)
Gliomas	30-50%	ND	-----	Lower in tumors	ND	ND	Outcome	(24, 40, 41)
Colorectal	20-30%	ND	-----	Lower in tumors	Yes[*]	In vitro and in vivo	Risk factors	(23, 42-44)
Breast	61%	2/55	3315delG T157M	Lower in tumors	Yes[*]	In vitro and in vivo	Risk factors Outcome	(45)
Lung Cancer	60%	ND	-----	Lower in tumors	Yes	In vitro and in vivo	ND	(42) (46)
Ovarian	55%	4/123	D119M R1429Q R1438C S1631F	Lower in tumors	ND	ND	Outcome	(42, 47-49)
Gastric	53-73%	ND	-----	Lower in tumors	Yes[*]	In vitro	ND	(42) (50) (51)
Laryngeal SCC	ND	ND	-----	Lower in tumors	Yes	In vitro	Risk factors	(52)
Gallbladder Ca.	ND	ND	-----	Lower in tumors	ND	ND	Outcome	(53)
Prostate	ND	2/8	A1594V R1724S	ND	ND	ND	ND	(54)

NA = Not applicable; ND – Not done.

[+] Nucleotide changes were converted to amino acid changes, and coding sequence positions modified based on RefSeq NM_015557.

[*] Re-expression by 5-aza-2-deoxycytidine (5-Aza)