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# The Expanding Mi-2/NuRD Complexes: A Schematic Glance

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**Abstract:** This mini-review will schematically update the progress of the expanding Mi-2/Nucleosome Remodeling Deacetylase (NuRD) complexes in cancer and in normal development such as stemness, with a focus on mammals and the increasingly popular and powerful model organism *Caenorhabditis elegans*. The Mi-2/NuRD complexes control gene activity during the development of complex organisms. Every Mi-2/NuRD complex contains many different core polypeptides, which form distinct multifunctional complexes with specific context-dependent regulators. The Mi-2/NuRD complexes have unique ATP-dependent chromatin remodeling, histone deacetylase, demethylase activities and higher order chromatin organization. They can regulate the accessibility of transcription factors or repair proteins to DNA. In this review, we summarize our current knowleges in the composition, interaction and function of the subunits within the Mi-2/NuRD complex, the methodology used for the identification of Mi-2/NuRD complexes, as well as the clinical and therapeutic implications targeting the Mi-2/NuRD subunits.

**Keywords:** Mi-2/NuRD complex, cancer, stemness, mammals, *Caenorhabditis elegans*

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## Overview

To gain an insight into the functions of the Mi-2/NuRD complex, we first need to understand the composition of this complexes. The Mi-2/NuRD complex is a expanding family of protein complexes, which control gene activity spatiotemorally during the development of complex organisms. In general, it has been regarded as one biochemical entity containing a number of core polypeptides but forming distinct multifunctional complexes with specific context-dependent regulators.[1,2] Some of these complexes are active during normal development such as stemness[3–7] and in cancer[8,9] (Table 1). These complexes are characterized by their unique ATP-dependent chromatin

**Table 1.** The neoplasms associated with core subunits of the Mi-2/NuRD compelx.

| Core subunit | Cancers associated with deregulation of core subunits of the Mi-2/NuRD complex | References |
|---|---|---|
| Mi-2 α | Breast and non-Hodgkin lymphomas. | Wang et al[8]<br>Pencil et al[11]<br>Toh et al[12,13] |
| Mi-2 β | Breast, ovarian, lung, pancreatic, stomach and colorectal cancers;<br>Non-Hodgkin lymphomas. | Hill et al[10]<br>Wang et al[8] |
| MTA1 | Breast, pancreatic and prostate cancers;<br>Lymphoma;<br>Thymoma;<br>Head and Neck Squamous Cell Carcinoma (HNSCC) and Non-Small Cell Lung Cancer (NSCLC);<br>Gastrointestinal cancer and adenocarcinoma. | Wang et al[8]<br>Toh et al[9]<br>Mazumdar et al[14] |
| MTA2 | Breast metastasis and ovarian cancer. | Wang et al[8]<br>Ji et al[16]<br>Cui et al[15]<br>Toh et al[9] |
| MTA3 | Breast cancer | Wang et al[8]<br>Toh et al[9] |
| RBBP4 | Breast, oropharyngeal and prostate cancers;<br>Primitive neuroectodermal tumor of the Central Neruous System(CNS); Retinoblastoma;<br>Skin tumor | Wang et al[8] |
| RBBP7 | Breast, lung and prostate cancers;<br>Primitive neuroectodermal tumor of the CNS;<br>Retinoblastoma;<br>Skin tumor | Wang et al[8] |
| HDAC1 | Breast, testicular, prostate, lung, colorectal, gastric, ovarian and pancreatic cancers;<br>Hematopoietic disorders | Wang et al[8]<br>Kim et al[17]<br>Minucci et al[18]<br>Choi et al[19] |
| HDAC2 | Breast, colon, pancreatic, cutaneous T-gastric, cervical, prostate and colorectal cancers;<br>Cell lymphoma | Wang et al[8]<br>Kim et al[17]<br>Minucci et al[18] |
| LSD1 | Breast, prostate, colon cancer and bladder cancers. | Wang et al[8]<br>Shi et al[20]<br>Huang et al[21] |
| MBD2 | Breast, prostate, colorectal, bladder and colon cancers;<br>Oral carcinogenesis | Wang et al[8]<br>Le Guezennec et al[22]<br>Zhu et al[23] |
| MBD3 | Breast, lung and colon cancers;<br>Mouse lymphosarcoma;<br>Oral carcinogenesis | Wang et al[8]<br>Le Guezennec et al[22]<br>Zhu et al[23] |

remodeling, histone deacetylase, demethylase activities and higher-order chromatin organization.[2,24] Nucleosomes form the building blocks of chromatin and, in general, these nucleosomes inhibit processes that require access to the DNA template, such as transcription and DNA repair. Mi-2/NuRDs use ATP hydrolysis to change the nucleosomes in DNA and they can regulate the accessibility of transcription factors or repair proteins to DNA.[25] The two highly homologous proteins Mi-2α/CHD3 and Mi-2β/CHD4 represent the catalytic ATP hydrolyzing subunits in the complex. The histone lysine-specific demethylase (LSD1/KDM1) was recently shown to be recruited to the NuRD complex via interaction between the Tower domain and metastasis-associated proteins MTA1-3 in breast cancer cells.[8] This newly identified subunit demethylates both di- and mono-methylated K4 (H3K4me2/1).[26] In addition, the core of the Mi-2/NuRD complex contains:

- at least two histone deacetylases, HDAC1 and HDAC2;
- the retinoblastoma-associated tumor suppressors RbAp48 (RBBP4) and/or RbAp46 (RBBP7);
- MTA1 and/or 2 and/or 3;
- GATAD2b (p66α) and/or GATAD2a (p66β);
- mCpG-binding domain proteins MBD2 and/or MBD3.[27]

Importantly, the stemmed Mi-2/NuRD complex emerges from these core components. The gene targeting mechanism of MBD2-recruitting Mi-2/NuRD (ie, the MeCP1 complex)[28] and the gene targeting mechanism of the transcriptional factor-interacting M-2/NuRD complex (eg, the lymphoid transcription factor Ikaros,[29] the transcriptional corepressor KAP-1,[30] the tumor suppressor p53[31] and Bcl-6)[32,33] have been determined as the interactions that are mediated by Mi-2α (Ikaros and KAP-1), MTA-2 (p53) or MTA-3 (Bcl-6), and therefore different transcriptional regulators use distinct NuRD subunits for recruitment. However, the details of the mechanisms of many other co-regulators (Supplementary Table 1) interacting with Mi-2/NuRD complexes remain largely unknown. The distinct tissue- and cell-specific Mi-2/NuRD complexes can execute their multifunctional, fine and spatiotemporal regulation in a variety of fundamental biological processes. In principle, this diversity could challenge the capture of all or most interactors from one purification experiment using core subunits of the Mi-2/NuRD complex tagged with a single condition; on the other hand, for cancer, cell-specific transcriptional factors and co-regulators of the Mi-2/NuRD complexes offer unexpected therapeutical benefits for designing both systemic and local site treatments (eg, RNAi therapeutics) in the future and may enable different therapeutic strategies tailored to patients with different profiles. These studies have benefitted from the comparative advantages of interspecies Mi-2/NuRD complexes.[34,35] Finally, increasing our knowledge of the Mi-2/NuRD complex could significantly advance cancer therapy.

## The Interacting Proteins and Functions of the Core Subunits within the Mi-2/NuRD Complex

### The Mi-2α, β/CHD3, 4 proteins

The evolutionarily conserved chromatin remodeling factor Mi-2 protein was first identified as an autoantigen in patients with dermatomyositis[36,37] and belongs to the chromodomian CHD3-CHD4 ATPase subfamiliy (CHDs) II. Mi-2 has been isolated from human cell lines, mice, plant, fly, worm and Xenopus egg extracts[27,36–43] (Fig. 1). The human genome contains two genes encoding isoforms of Mi-2: Mi-2α/CHD-3 and Mi-2β/CHD-4.[44]

Mi-2 proteins contain several domains: two PHD zinc fingers, two chromodomains (chromatin organization modifier), a SWI2/SNF-like helicase

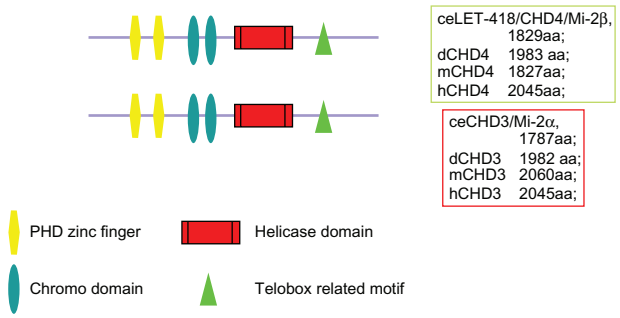


**Figure 1.** The structural domains of Mi-2α,β/CHD3,4 proteins. Schematic representation of protein domains found within *C. elegans*, drosophila, mouse and human Chd genes of sub family II. Members of this sub-family are characterized by paired PHD Zn-finger-like domains (yellow oval), tandem chromodomains (blue oval), and an SNF2-like ATPase domain (red rectangle) and a telobox (green triangle).

domain and a telobox-related motif[43] (Fig. 1a). PHD zinc finger domains are thought to be involved in protein-protein interactions. In fact, the Mi-2 PHD domains were shown to be required for Mi-2's interaction with HDAC1.[43] At least part of the nucleosomal recognition is contributed by Mi-2 itself, since the ATPase activity of the recombinant enzyme is strongly stimulated by nucleosomes, but does not react to the presence of free histones or DNA.[45] Mi-2 and its complex regulate gene expression by modifying chromatin accessibility during development. The Mi-2 subunits are implicated in a wide variety of cancers (Table 1). Mi-2β acts as a transcriptional repressor and as an activator, which is required for hematopoietic stem cell self-renewal and differentiation.[46,47] The Mi-2/NuRD complex has significant roles in DNA damage and repair.[25,35,48–50] TWIST1 interacts with several components of the Mi-2/NuRD complex, Mi-2β, RbAp46, MTA2 and HDAC2, and recruits them to the proximal regions of the E-cadherin promoter for transcriptional repression. Depletion of these TWIST1 complex components from cancer cell lines, which depend on TWIST1 for metastasis, efficiently suppresses cell migration and invasion in culture and lung metastasis in mice.[51]

Previous reports showed that Mi-2α and Mi-2β can coexist within the same complex.[39,42] So far, it remains unknown whether these Mi-2α and Mi-2β are forming heterodimers or whether they are assembled into distinct complexes.

But it is likely that all 3 types of complexes exist like ie, Mi-2α/NuRD,[30] Mi-2αβ/NuRD and Mi-2αβ/NuRD. Furthermore, though the increasing number of published interactors of Mi-2 proteins show up (Fig. 2; supplementary Fig. 1), we are in await for a systematic screening of interactors of Mi-2 proteins via current proteomic assay, which has evolved and matured to level where it is able to assess the complexity of the human proteome.[52]

Two *Caenorhabditis elegans* Mi-2 orthologs, LET-418 and CHD-3, have been cloned and characterized.[53] *let-418* has proven to be an essential gene expressed in most, if not all, nuclei of the worm. Mutations in *let-418* have pleiotropic phenotypes, including vulval defects and sterility and, without maternal contribution, result in L1 larval arrest.[53]

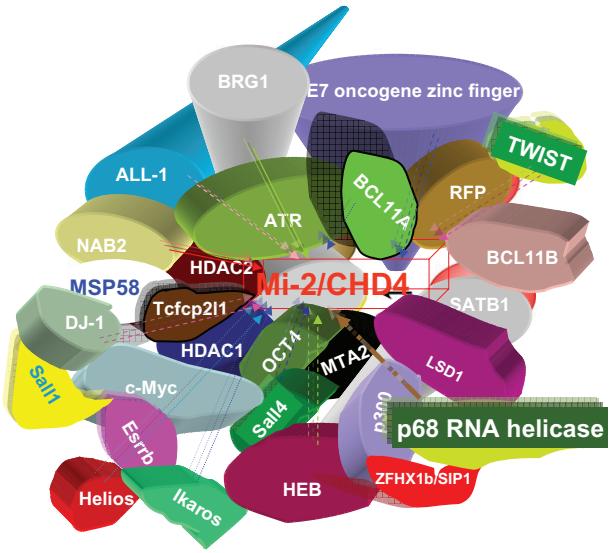


**Figure 2a.** The interactors of mammalian Mi-2β/CHD4.
The figure has been simplified to demonstrate the interactors of the core subunit of Mi-2/NuRD complexes (eg, Mi-2β/CHD4); but other mutual interactions of neighbor proteins have not beendetermined. The figures highlight the recently-published interactors; for a full list, refer to Supplementary Table 1.

Furthermore, *let-418* is required for the maintenance of somatic differentiation in *C. elegans*.[54] By contrast, mutations in *chd-3* do not display any obvious phenotype under standard culture. However, a requirement for *chd-3* becomes apparent in *let-418(lf); chd-3(lf)* double mutants, which display strong vulval defects and L4 arrest or, without any *let-418* maternal contribution, embryonic arrest. These results suggest that *let-418* and *chd-3* have essential and partially


**Figure 2b.** The interactors of mammalian Mi-2α/CHD-3.

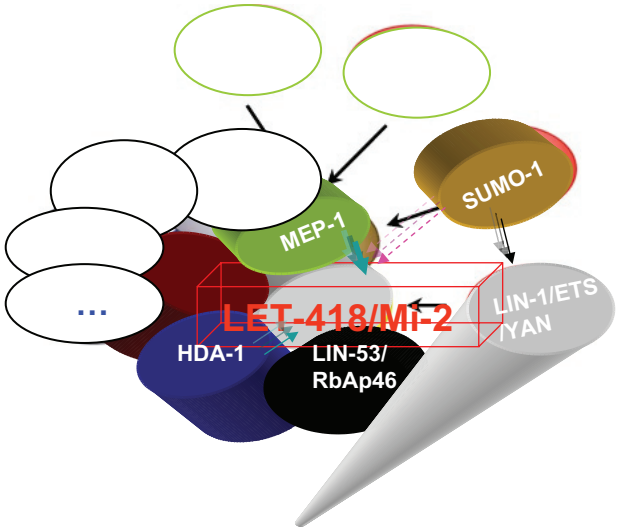


**Figure 2c.** The interactors of *C. elegans* Mi-2β/LET-418.

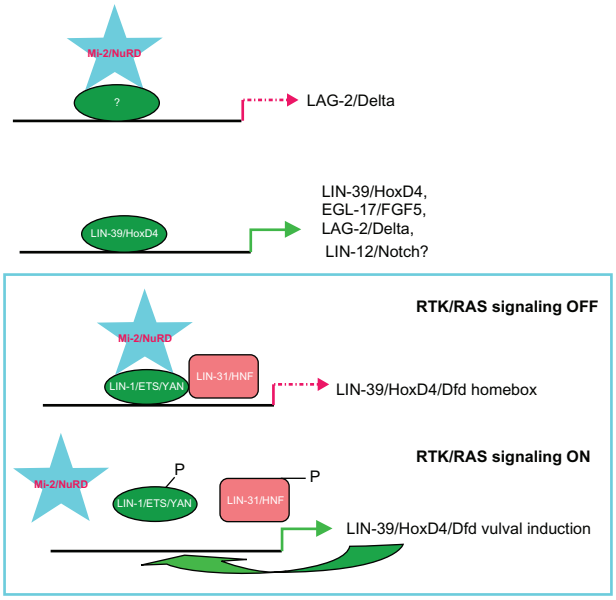


**Figure 3.** The downstream target genes of the Mi-2/NuRD complex in *C. elegans*. **Upper:** The LET-418/Mi-2/NuRD complex (blue star) represses *lag-2* transcription (red dashed line). **Middle:** LIN-39/HoxD4 binds to the promoters of *lag-2/Delta, egl-17/FGF5, lin-39/HoxD4* and possibly *lin-12/Notch*, and then promote the activities of respective genes (green line). **Bottom:** A model for how NuRD antagonises vulval cell fate. 1) In the absence of EGF/RTK/Ras signaling, LIN-1/ETS (red rectangle) binds to the promoter of *lin-39,* and it recruits a LET-418/Mi-2/NuRD complex and represses *lin-39* transcription by affecting histones and condensing the chromatin. The repression, however, is not complete, since basal levels of LIN-39 are required for normal vulval development. 2) EGF/RTK/Ras signaling results in LIN-1/ETS phosphorylation, which disrupts LIN-1/ETS interactions with the LET-48/Mi-2/NuRD complex and upregulates the target gene *lin-39* transcription required for vulval cell fate induction.

redundant functions during development.[53] Previously, we clarified the functions of LET-418/Mi-2β using genetic and biochemical techniques including Chromatin Immuno-Precipitation (ChIP), quantitative real time reverse transcriptase polymerase chain reaction (RT-PCR), co-immunopreciptation, reporter assays and suppression subtractive hybridization (SSH); we demonstrated that LET-418/Mi-2β along with LIN-1/ETS directly binds the promoter of the *lin-39/Hox* gene to repress its expression[55] and LET-418/Mi-2β directly regulates LAG-2/Delta in the LIN-12/Notch pathway.[56] LIN-39/Hox itself performs an auto-regulatory function as well as directly controlling EGL-17/FGF5 and LAG-2/Delta[55,57,58] (Zhang and Mueller, unpublished; Fig. 3). The role of regulation of Mi-2/NuRD in the LIN-12/Notch pathway has been demonstrated in different labs.[53,59,60] Furthermore, *spr-5* encodes the *C. elegans* LSD1 homolog and is implicate in Notch signaling.[61–64] Mittal et al recently observed high-level expression of Notch receptors and ligands, and its increased activation in several human breast cancers and early precursors. This places Notch signaling as a key player in breast cancer pathogenesis. Notch signaling cooperates with the Ras/MAPK pathway in transformation and it offers combined inhibition of the two pathways as a new modality for breast cancer treatment.[65] The cross-talk and precise molecular cooperation between EGF/RAS and LIN-12/Notch cell signalling pathways have been clearly shown in *C. elegans* vulva development.[66]

## The mCpG-binding domain (MBD) proteins

### MBD2/Mi-2/NuRD and MBD3/Mi-2/NuRD

As Figure 4 shows, MBD proteins have the mCpG-binding domain. MBD3's MBD domain has two point mutations that abolish the binding. MBD1, MBD2 and the methyl CpG binding protein 2 (MeCP2) have the Transcriptional Repressive Domain (TRD). MBD2 and MBD3 can form both homo- and heterodimers because both have the Coil-Coil (CC) domain. MBD4 is involved in DNA repair. Nearly all cases of Rett Syndrome are caused by a mutation in the MeCP2. The MBDs bind sites of hypermethylation in human cancer cell lines. MBDs function in transcriptional repression and long-range interactions in chromatin, and also appear to play a role in genomic stability, neural signaling[80] and transcriptional activation.[81] MBD3 was shown to be an integral component of the Mi-2/NuRD complex,[82] whereas murine MBD2, which

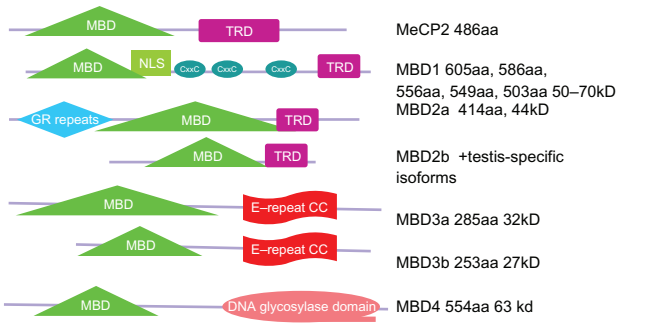


**Figure 4.** The structural domains of the mCpG-binding domain (MBD) proteins.
**Note:** All members of the MBD family contain the methyl-CpG binding domain that putatively binds to methylated DNA. MBD3 is the only member that does not bind to methylated DNA because of its two-point MBD domain mutations. MBD1 MBD2 and MeCP2 have the transcriptional represive domain (TRD). MBD2 and MBD3 can form both homo- and heterodimers because both have the Coil-Coil (CC) domain. MBD4 is involved in excision-based DNA repair through the glysecosyla domain. In addition to its MBD domain, MBD1 binds to unmethylated DNA via its third CxxC zinc-finger motif. MBD2 features a characteristic stretch of glycine and arginine residues (GR) and juxtaposed MBD and TRD domains.

can bind methylated DNA,[83] targets the Mi-2/NuRD to methylated CpG dinucleotides.[82,84] In general, MBD2 and MBD3 have been reported in a common Mi-2/NuRD complex, but different sets of inertactive proteins of MBD2 or MBD3 have been reported (Fig. 5). MBD2/Mi-2/NuRD complex (ie, the MeCP1 complex) was shown to be present at very early stages of development and to inactivate methylated promoters in the mouse embryo prior to implantation.[85] However, in MBD2/MBD3 tagged complex purification, in oral carcinogenesis and colon cancer, the

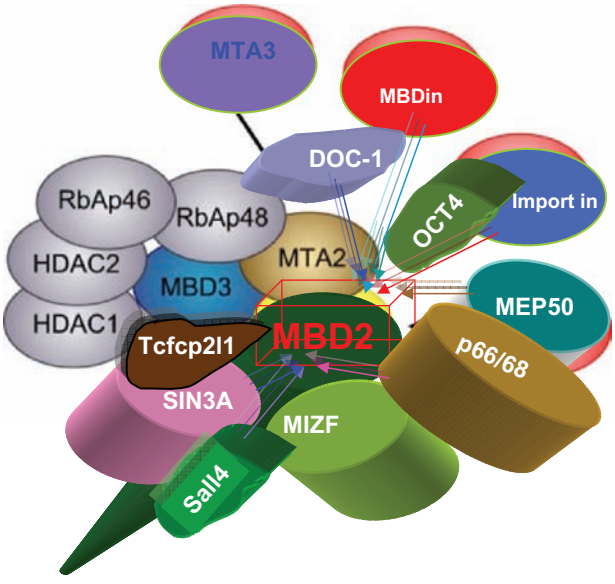


**Figure 5a.** The interactors of mammalian MBD2.

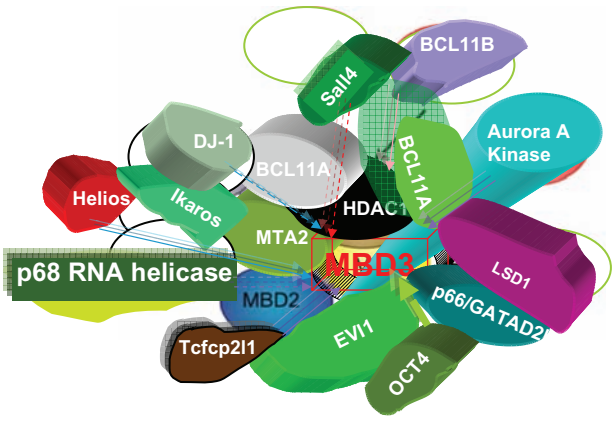


**Figure 5b.** The interactors of mammalian MBD3.

MBD2 eluate but not that of MBD3 contained the arginine methyltransferase PRMT5, MEP50 and importin α nuclear transport proteins. The association between MBD2 and importins may indicate that MBD2 shuttles between the cytoplasm and the nucleus. The over-expression of DOC-1 identified in purification results in a G1 arrest and significant growth retardation compared to wild-type cells, which is consistent with loss of the protein in tumors. A proposed model is shown in Figure 6c,d: the MBD2/NuRD complex deacetylates the nucleosomes surrounding

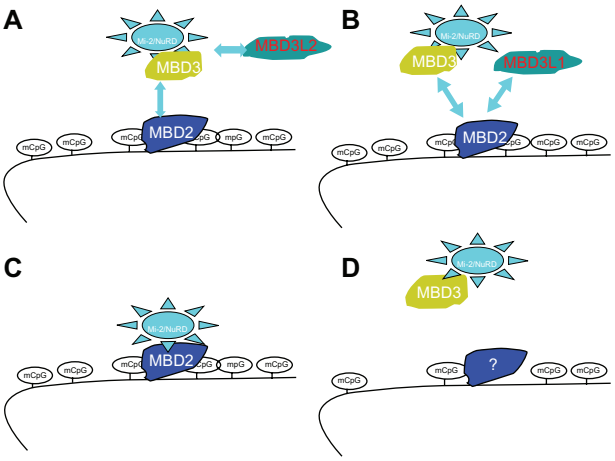


**Figure 6.** Summary of MBD/Mi-2/NuRD complex models. **A**) The inhibition model of mammalian MBD3L2 on MBD2 binding of CpG. The direct binding of MBD2 and MBD3 recruits the MBD2/MeCP1/NuRD complex to CpG-methylated DNA MBD3L2 can interact with MBD3, then compete with the MBD2, potentially leading to a loss of mCpG binding in specific forms of the MeCP1 complex and gene reactivation. The interactions of MBD2 and MBD3L2 with MBD3 may be mutually exclusive. **B**) The mutual antagonistic inhibition model of mammalian MBD3L1 and MBD2 interacting with MBD2 binding on CpG. Because MBD3L1 interacts with MBD2 but not MBD3 *in vivo,* and MBD3L1 and MBD3 bind to overlapping regions on the MBD2 protein, MBD3L1 replaces MBD3 and may form a special "isoform" of the MeCP1 complex. **C, D**) Independent model of the mutual exclusion of mammalian MBD3 and MBD2 to bind to CpG.

the targeting site;the addition of transcriptional repressive arginine methyl marks the H4 tail by its associated PRMT5. The hypoacetylated and arginine methylated nucleosomes surrounding the MBD2/PRMT5 targeting site in turn may provide a binding scaffold for the MBD3/NuRD complex. The MBD2/NuRD and MBD3/NuRD complexes co-occur on some CpG islands. The MBD3/NuRD complex can further deacetylate the nucleosomes and then facilitate the spreading of deacetylation and maintenance of transcriptional repression.[22] Unlike the other MBD members, MBD3 is unable to bind methylated DNA Human MBD3 and HDAC1 are localized at Aurora-A-positive centrosomes in the M phase.[86] MBD3 is transiently phosphorylated during the late G2 and early M phase, and Aurora-A is the candidate kinase for its phosphorylation.

In mice and humans, two proteins with high homology to MBD3 were identified: MBD3L1 and MBD3L2 (methyl-CpG-binding protein 3-like 1 and 2), which lack the MBD domain.[87] MBD3L1 can enhance MBD2-mediated repression in CpG-methylated promoters. MBD3L1 and MBD3 were shown to bind to overlapping regions of MBD2. MBD2 may interact with MBD3 or with MBD3L1 to form two different complexes with redundant function.[87] The Mi-2/NuRD complex may interact either with MBD2 or MBD3L2, via MBD3 (Fig. 3). MBD3L2 was shown to oppose MBD2/NuRD-mediated methylation silencing[88] by recruiting the complex away from methylated DNA and reactivating transcription. The hetero-oligomeric complexes can be formed between the four related proteins MBD2, MBD3, MBD3L1 and MBD3L2 and therefore different subforms of the Mi-2/NuRD complex (Fig. 6a,b).

Interestingly, the *C. elegans* genomic DNA is unmethylated, so *C. elegans mbd-2*[89] may exist in vestigial mammalian MBD3. The general role of MBD proteins in epigenetic regulation may be independent of methyl-DNA binding. Interestingly, while searching for the *C. elegans* homologs of components of the Mi-2/NuRD complex, we identified that CID-1, FLT-1 (now the *C. elegans* ACF1 homolog) adn R05D3.11 (now Met-2) have about 25% or better amino acid indentityl to the mammalian MBD domain, and knock-down (KD) animals with RNA interference against these genes had a low percentage of weak multivulvae, a phenotype of LET-418/Mi-2 deficiency. For the checkpoint protein CID-1, it remains tempting to know if it could transiently recruit Mi-2/NuRD complex in order to execute its function in longevity[90] (Zhang, unpublished).

## The metastasis-associated (MTA) proteins

A decade ago, the function of the MTA (metastasis-associated gene) subunits of Mi-2/NuRD complex were largely unknown. At that time, MTA protein was a newly discovered family of cancer progression-related genes and their encoded products. The MTA gene family is clearly involved in oncogenesis and it is also used as an important marker of prognosis in cancer. The vertebrate MTA family of proteins is encoded by three genes that code for five isoforms: MTA1 and a splice variant known as MTA1s, MTA2, MTA3 and a longer isoform MTA3L, and the ZG29p (Fig. 7), an N-terminal truncated form of MTA1 that is present in the zymogen granules of the pancreas (Fig. 4a). MTA1 is the original member of a small family of metastasis-associated genes which also includes MTA2 and MTA3. The longer MTA1 isoform contains a bromo-adjacent homology (BAH) domain, an EGL-27 and MTA1 homology (ELM2) domain, a GATA-type zinc finger and a MYB domain. It contains four phosphorylation sites two serine and two threonine and two SRC homology-3 (SH3)-binding motifs.[93] MTA1s (short isoform) is missing the 285 AA C-terminal sequence and has a substituted 33 AA C-terminal compared to the long form. MTA1s contains an estrogen receptor binding motif (LRILL) not found in the long form. The distinct Mi-2/NuRD complexes are defined by different MTA variants with different composition and distinct functions that

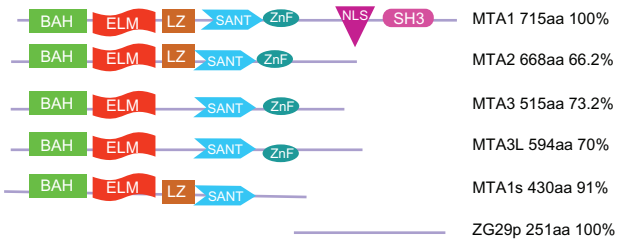


**Figure 7.** The structural domains of the metastasis-associated (MTA) family members. All major members can be a subunit of the Mi-2/NuRD complexes. The different role of each member of the family is still not clear but there is high similarity among them. The BAH domain is involved in protein-protein interaction, whereas the SANT domain binds to histone tails.

**Table 2.** A short list of target genes of the core subunits of the Mi-2/NuRD complex.

| Highlighted subunit | Validated/hightlighted targets | Method | Complex | References |
|---|---|---|---|---|
| HSF1 | *Hsp70, c-Myc, pS2* | ChIP, reporter assay | NuRD (MTA1, HDAC1/2, CHD3) | Khaleque et al[67] |
| Mi-2β | *Tek, Mpl, Kit receptor, Lxn, Ltbp3, Dmxl2, Il17re, Tgm2, Ndn, Dach1, Ddx4, Pkd2, sash1, Alox5ap, Il-27, Mamdc2, Tgm2, kit, Ccnd2, Csf1r, Il6Ra, IL6st, Egr1, Ccr1, Ccr2, Hba-a1, hbb-b1, Dntt, Thy1, Rag1, Dppa5, Hba-x, Tcfl5, etc.* | microarray analysis, RT-PCR, | NuRD | Yoshida et al[47] |
| *let-418/Mi-2β* | lin-39, lag-2, sqt-2, sqt-3 etc. | SSH, RT-PCR, ChIP | NuRD | Guerry et al[55]<br>Zhang et al[56] |
| MBD3 | *Pramel6, Pramel7, Dppa3, Gata6, Brachyury, Tbx2, Tpbpa etc.* | RT-PCR | NuRD | Kaji et al[68] |
| | *Cdx2, Eomesodermin, Hand, Cadherin 3 etc.* | RT-PCR, ChIP | NuRD | Zhu et al[69] |
| KAP1 | NANOG, OCT4 (POU5F1) | | HP1, NuRD, SETB1 | Rowe et al[70] |
| SALL4 | *pten, sall1* | RT-PCR, ChIP | NuRD | Lu et al[71] |
| LSD1 | *brachyury, hoxb7, hoxd8, RARgamma* and the aberrant transcription of 588 genes, | ChIP, microarray analysis, RT-PCR, | CoREST | Foster et al[7] |
| | *E cad, GLDN7, KRT8* | RT-PCR, ChIP, reporter assay | LSD1-Snai1 | Lin et al[72] |
| | *Mi-2β, MTA3, TGFB1, EGFR, RHOA, ANGPTL4, LAMININ ALPHA4, COLLAGEN VI, ENDOTHELIN-1, LMNB2, IGF1R, CCND1, ADK, PSEN1, RHOA, FGF21, APAF1, etc.* | ChIP-DSL, RT-PCR, re-ChIP | LSD1/MTA1/NuRD<br>LSD1/MTA2/NuRD<br>LSD1/MTA3/NuRD | Wang et al[8] |
| MTA3 | *E cad, Snail, CCL3 etc.* | RT-PCR, ChIP | BCL-6/NuRD | Fujita et al[32,33] |
| | *MTA, etc.* | ChIP-DSL, RT-PCR, re-ChIP | MTA3/LSD1/NuRD | Wang et al[8] |
| MTA1 | *Six3, Rhodopsin* | RT-PCR, ChIP | HDAC-MTA1 complex | Manavathi et al[73] |
| | *Mi-2, etc.* | ChIP-DSL, RT-PCR, re-ChIP | MTA1/LSD1/NuRD | Wang et al[8] |
| | *c-Myc, pS2* | RT-PCR, ChIP | NuRD (HDAC1/2, CHD4) | Mazumdar et al[14] |
| | *Ac-p53, BAX* | RT-PCR, ChIP | p53/NuRD | Kai et al[74,75] |
| | *Pax5, XBP-1, mAqp1, mStat1, mMaoa, mCdh1, mFabp1, mEps8, mEla1, mCdr2, mUbd, mSlamf8, mKLF4, mEmp1, mPik3r, mSerpinb1, mCcL-9, mLck, mPou2af, mSpint2, mMefc, etc.* | Double ChIP, microarray analysis, RT-PCR, Northern blot | HDAC2-MTA1 | Balasenthil et al[76] |
| | *AHF* | Reporter assay | FOG-2/NuRD | Roche et al[77] |
| | *Gαi2* | ChIP | MTA1-HDAC complex | Oshiro et al[78] |
| | *BCAS3* (breast cancer amplified sequence 3), *cyclin D* | ChIP cloning, RT-PCR, reporter assay | Not available | Gururaj et al[79] |
| HDA-1/ HDAC1/2 | *lag-2, lon-1, cli-2*/Cystain, and tissue-specific and extracellular matrix (ECM)-related genes | ChIP, microarray, RT-PCR | Not determined | Whetstine et al[34]<br>Zhang et al[56]<br>Dufourcq et al[59] |

presumably target different sets of gene promoters.[27,93] Presumably, MTA2 is expressed constitutively and may be involved in housekeeping functions of the Mi-2/NuRD complex, whereas MTA1 and MTA3 could be involved in cell type-specific transcription, since they are expressed in a tissue-specific manner.[27]

## MTA1/Mi-2/NuRD

Through differential cDNA screening, MTA1 was initially identified as being abundantly overexpressed in highly metastatic rat mammary adenocarcinomas.[11–13] Elevated expression of MTA proteins has been associated with many types of metastatic cancers such as gastrointestinal and esophageal carcinomas, and mammary adenocarcinomas, along with increased tumor aggressiveness, invasiveness, metastasis and poor prognosis[27,91–94] (Tables 1 and 2; Fig. 7).

Several studies have shown that these MTAs splicing variants form distinct protein complexes with Mi-2/NuRD components.[42,43,82,93] As mentioned above, the MTA1 gene yields two isoforms (MTA1 and MTA1s (Fig. 9a, b)) via alternative mRNA splicing and one novel zymogen granule protein (ZG29p) via alternative transcription initiation.[95,96] MTA1 is associated with the Mi-2/NuRD complex.[42] The splice variant MTA1s is cytoplasmic because it lacks a nuclear localization sequence.[27] ZG29p mediates an interaction with amylase and is involved in condensation-sorting in the exocrine rat pancreas, and it could possibly be linked to the deadly pancreatic cancer[9,97] (Fig. 4a). MTA1s plays an important role in breast cancer malignancy by sequestering estrogen receptors in the cytoplasm. MTA1 could affect the transcription of disease-related genes via chromatin remodeling. However, Yao and Yang reported that MTA1 associates with specific NuRD complexes that contain HDAC1/2, RbAp46/48, and MBD3, but neither Sin3 nor Mi2.[93]

The NuRD-dependent mechanisms of tumorigenicity induced by MTA1 have been intensively studied by researchers such as Toh and Kumar.[9] MTA1 is involved in regulation of genes such as the estrogen receptor (ER-α), c-Myc, BRCA1, p53 and many more (Fig. 8, Table 2 and Supplementary Table 1). Indeed, MTA1 converts breast cancer cells to a more aggressive phenotype by repression of the estrogen receptor (ER-α) trans-activation function through deacetylation of the chromatin in the ER-responsive

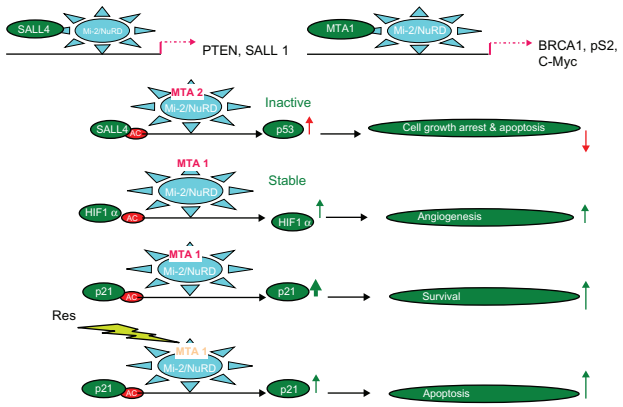


**Figure 8.** The downstream paths of the Mi-2/NuRD complex in carcinogenesis and cancer progression. **Upper left:** The Sall4/Mi-2/NuRD complex represses the promoter activities of PTEN and SALL1. **Upper right:** The MTA1/Mi-2/NuRD complex represses the promoter activities of BRAC1, pS2 and c-Myc. **Bottom:** 1) x4 Tumor suppressor p53 protein is deacetylated and inactivated by both MTA1 and MTA2 proteins in NuRD complexes, prompting inhibition of growth arrest and apoptosis. 2) x4 A hypoxia inducible factor-1a (HIF-1a) is deacetylated and stabilized by MTA1 protein, leading to angiogenesis. 3) x4 Normally, MTA1/Mi-2/NuRD complexes repress transcriptional activation of the genes implicated in cell cycle and apoptosis (p21) through deacetylation, which results in cell cycle progression and survival. 4) x4 With downregulation by resveratrol, MTA1 has less stable interactions in Mi-2/NuRD complexes, decreasing Mi-2/NuRD complex activity, increasing acetylated p53. In parallel, resveratrol switch one the acetylation mechanisms by HATs increasing global protein acetylation. This favors the expression of genes implicated in cell cycle arrest and apoptosis.

element of ER-responsive genes. Furthermore, MTA1 plays an essential role in c-MYC-mediated cell transformation. Overexpressed MTA1 (the long isoform) in specific NuRD complexes may modulate tumor aggressiveness via interference with estrogen signaling. Activation of the heregulin-beta1/HER2 pathway[14] of breast cancer cells induces MTA1-dependent repression of estrogen receptor transactivation, leading to enhanced anchorage-independent growth and hormone independence.[96–101] Since MICoA and NRIF3 are MTA1-binding partners, their coactivators and corepressors may coexist in a single super complex. c-Myc upregulates MTA1 expression (Fig. 8) and activation of NuRD complexes containing MTA1.[102] Importantly, c-Myc is one of four induced pluripotent stem (iPS) cell factors and has been linked to the dedifferentiation of somatic cells to the iPS cell phenotype.[103,104] MTA1/Mi-2/NuRD complexes have been shown to repress the BRCA1 tumor suppressor gene, and this resulted in an abnormal centrosome number, and chromosomal instability and inhibition of p53-induced apoptosis.[105,106]

MTA1s is upregulated by FGF-2 in breast cancer cells, and it sequesters ER-α in the cytoplasm and

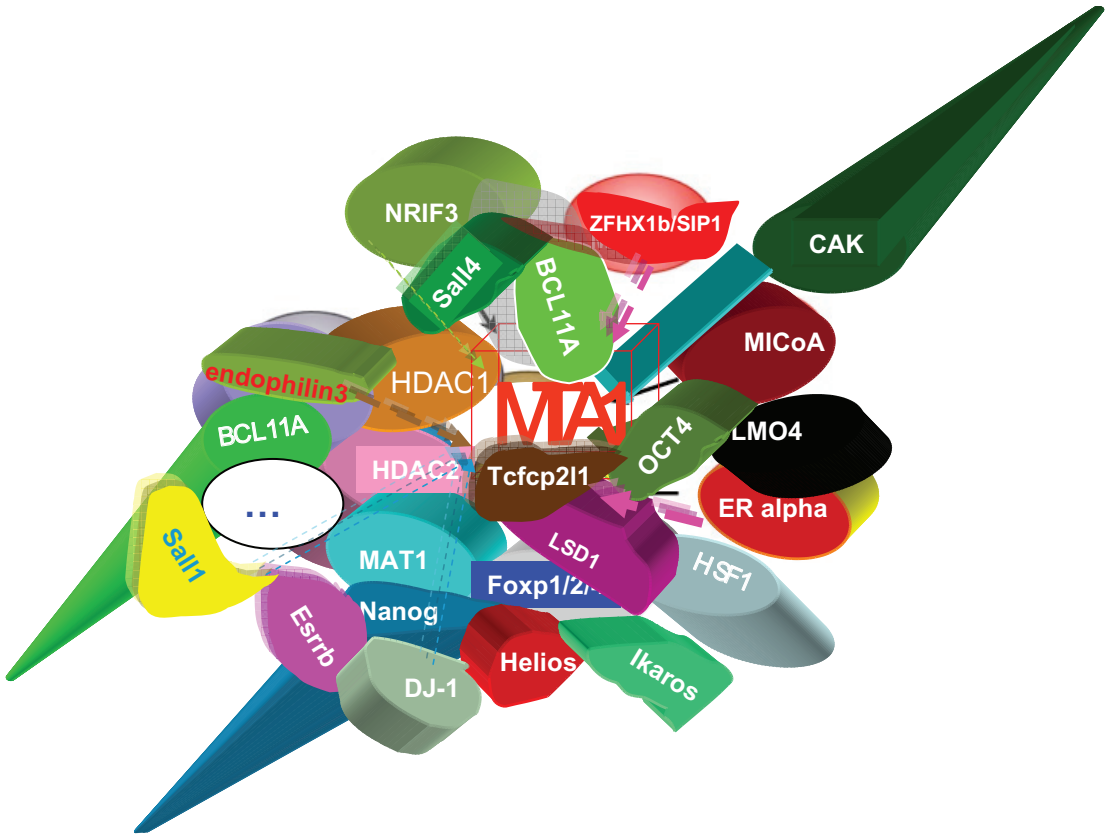


**Figure 9a.** The interactors of mammalian MTA1.

represses ER-α transcriptional activity, estrogen-induced proliferation and anchorage-independent growth of the human breast cancer cell line MCF-7.[107] In an animal model, MTA1s peptides block the tumor progression of MCF7 overexpressing ER-α, which promotes the malignant phenotypes.[108] MTA1s associates with casein kinase I-γ2, an estrogen-responsive kinase that phosphorylates and modulates the functions of MTA1s. The acetylated MTA1-histone deacetylase (HDAC) interaction facilitates the recruitment of the MTA1-HDAC complex to the Gαi2 regulatory element, and consequently for the repression of Gαi2 transcription and expression, which leads to activation of the Ras-Raf pathway.[78] MTA1 and MTA1s stimulate Wnt1 transcription through promoting its derepression from the Six3 corepressor.[109] Moreover, a hypoxia-inducible factor-1α (HIF-1α) is deacetylated and stabilized by MTA1, resulting in angiogenesis (Fig. 8). The HSF1, one interactor of MTA1 in the MCF7 cell line, has interactions with HIF-1α after stress (Li et al, unpublished). Thus, MTA1, and probably other MTA proteins, belong to the master co-regulatory molecules involved in carcinogenesis and the progression of various malignant tumors.

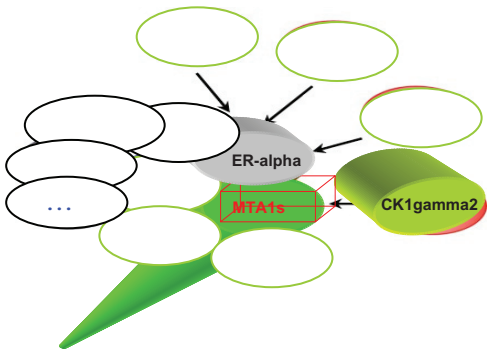


**Figure 9b.** The interactors of mammalian MTA1s.

The ELM2 domain in vertebrate and invertebrate MTA1, -2 and -3[27,110] (Fig. 7) was first described in a *C. elegans* protein, EGL-27, which is a nuclear protein involved in embryonic patterning, cell polarity, cell migration and vulval development.[111] Based on sequence, EGL-27 is likely to be an ortholog of atrophin in the nematode *C. elegans*. Therefore, EGL-27 may be involved in nuclear receptor signaling in *C. elegans*. *C. elegans* has MTA1 homologs,


**Figure 9c.** The interactors of mammalian MTA2.

*egl-27* and *egr-1*, which are related to embryonic patterning. The Mi-2/NuRD complex including *egr-1* antagonizes vulval development induced by the Ras signal transduction pathway, at least partly by promoting cell fusion between the vulval precursor cells and the hypodermal syncytium at an early larval stage. This inhibitory function of *lin-40* might be carried out by downregulating *lin-39* Hox

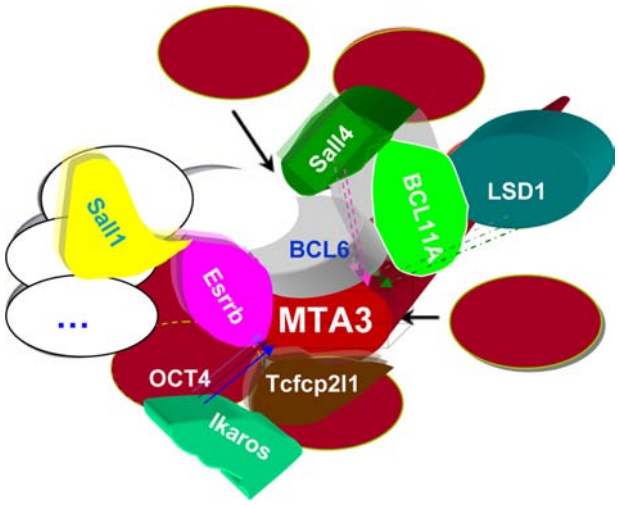


**Figure 9d.** The interactors of mammalian MTA3.

expression[112,113] (Fig. 3). Thus, understanding their physiological functions will be absolutely necessary to understand the pathological functions of MTA proteins in human cancers. However, one RNAi/*egr-1* study[60] reported a failure to detect ectopic expression of *lag-2::gfp,* but this could be caused by a lower efficiency of RNAi, especially with the genomic RNAi construct; it would be interesting to examine the ectopic expression of the *lag-2::gfp phenotype* in *egr-1* mutant and *egl-27* mutant backgrounds (Fig. 3).

## MTA2/Mi-2/NuRD

MTA2, along with MTA1 and MTA3 and their isoforms, is a member of the MTA family of novel nuclear receptor coregulators. MTA2 (Fig. 9c) appears in Mi-2/NuRD complexes composed of HDAC1/2, RbAp46/48, MBD3, Sin3 and Mi-2, and is associated with maintaining homeostasis of the cell. MTA2 is involved in the regulation of NuRD complexes, where it modulates the activity of the core histone deacetylase (HDAC) complex.[82]

MTA2 is in many cancers including breast cancer and epithelial ovarian cancer[15] but it is not correlated

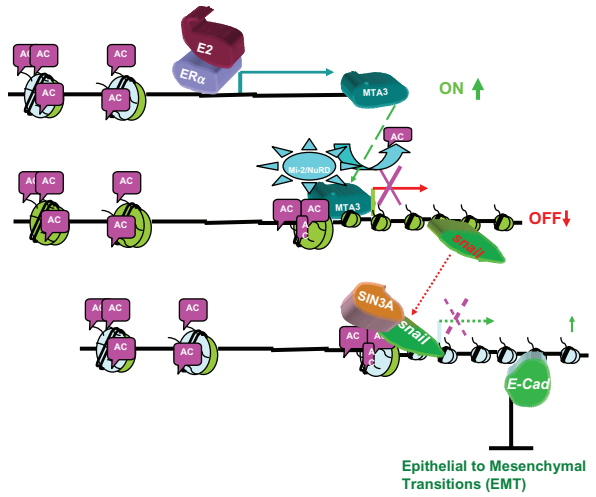


**Figure 10.** MTA3, a potent suppressor of "epithelial to mesenchymal transitions" (EMT). Through the promoter deacetylation of the Mi-2/NuRD complex, MTA3, an estrogen responsive gene, represses the expression of the transcriptional repressor Snail, thus enhancing the expression of the cell adhesion molecule E-cadherin and maintaining a differentiated normal epithelial status in breast cells.

as strongly with metastasis as MTA1. MTA1 and MTA2 both exert histone deacetylase and gene repressor activity. MTA2 (but not MTA1) forms complexes with the transcription factors YY1 and FKBP25.[93] YY1 is required for development. The MBD domain of MBD3 is necessary and sufficient for binding to HDAC1 and MTA2.[114]

MTA2 has normal physiological roles. In experiments with MTA2 null mice, MTA2 was shown to be important for embryonic survival and to be involved in modulating IL-4 and IFN-gamma expression in T-cell immune responses. These null mice develop lupus-like autoimmune symptoms.[115] MTA1 and MTA2/PID expressiones repress p53-dependent transcriptional activity such as p53-mediated cell growth arrest and apoptosis via deacetylation of p53[31] (Fig. 8). TWIST1 interacts with MTA2/NuRD to repress the E-cadherin promoter for transcription of E-cadherin and has essential roles as a component of the Mi2/NuRD complex in cancer metastasis; TWIST1's role is independent of another master regulator in "epithelial to mesenchymal transitions" (EMT), Snail, which is regulated by MTA3/NuRD, as discussed below.[51] In addition, BMI1/PRC1and EZH2/PRC2 also interact with TWIST1, and they bind to promoters of E-cadherin and p16/INK4a. It would be interesting to know how these activities are coordinated. MTA2/NuRD complex has a role in mouse genomic imprinting, and is involved in proper imprinted expression of H19 and Peg3 during mouse preimplantation development.[116]

## MTA3/Mi-2/NuRD

Both isoforms of MTA3 (Fig. 9d) are associated with the vertebrate Mi-2/NuRD complex.[117] MTA3 has been shown to be part of an estrogen-dependent pathway that regulates growth and differentiation of mammary epithelial cells.[117] MTA3 is induced by estrogen and represses the expression of the transcriptional repressor Snail, a master regulator of EMT, resulting in the expression of the cell adhesion molecule E-cadherin and maintenance of a differentiated normal epithelial phenotype in breast cells (Fig. 10).

Depletion of MTA3 leads to aberrant upregulation of the transcriptional repressor protein Snail. An increased expression of Snail is correlated with loss of differentiation and metastasis in breast tumors. MTA3 is also required to regulate B lymphocyte differentiation[32,33] by acting as a co-repressor for the transcriptional repressor BCL-6.

The systematic screening of the target genes of the MTA/Mi-2/NuRD complex is being developed. First, LSD1, MTA1/2/3 ChIP-DSL gives a list of genes, including components of the TGFβ signaling pathway, which is a key player in metastatic tumor invasion. Many of these targets are in the main cell signaling pathways, which are involved in apoptosis, Wnt pathways, MAPK, cell communication, cell cycle, focal adhesion, the actin cytoskeleton, etc.

## The histone binding proteins/ retinoblastoma A tumor suppressor RbAp46/P48

The Mi-2/NuRD complex contains RbAp46 and RbAp48 (Fig. 11), two proteins that bind to the retinoblastoma A tumor suppressor.[39–42,118] They contain a number of WD repeats, a sequence motif that forms the basis for the beta-propeller structure of the beta-subunit of the G protein transducin.[119] These two proteins are presumed to be structural subunits of the NuRD complex, with the potential for different blades of the propeller structure to act as protein interaction surfaces. Interestingly, RbAp46 and RbAp48 have been shown to be components of several


**Figure 11a.** The interactors of mammalian RBBP7/RbAp46/LIN-53.

multiprotein chromatin modification complexes,[120] in which they interact directly with core histones H3 and H4[121] as an adaptor that attaches to tetramers.[122,123]

The Rb complex component LIN-53 (mammalian RbAp48/46)[124,125] antagonizes a *C. elegans* Ras pathway and a synthetic multivulva B (synMuv B) pathway, which includes the homologs of the core subunits of Mi-2/NuRD complex, such as LET-418/Mi-2β, HDA-1/HDAC1, dcp66, etc. The mutations in the Rb pathway components enhance RNA interference (RNAi) and cause somatic cells to express genes limited to germline-specific P granules. Furthermore, gene inactivations that disrupt RNAi reverse the cell lineage transformations of Rb pathway mutants. These findings suggest that mutations in Rb pathway components cause cells to revert to patterns of gene expression normally restricted to germ cells. Rb may act via a similar mechanism to transform mammalian cells.[126] RBBP7 interacts with DOC1R, a MAP kinase substrate that controls microtubule organization of metaphase II mouse oocytes.[127,128]


**Figure 11b.** The interactors of mammalian RBBP4/RbAp48.

## The histone deacetylases

Human histone deacetylases (HDACs) are targets for cancer therapy, and they are classified into three types.[129] The Class I histone deacetylases HDAC1 and HDAC2 are ubiquitously expressed but restricted to the nucleus. Histone deacetylases sometimes combine with histone acetyltransferases (HAT) to form a super HAT HDAC complex.[130]

HDAC1 and HDAC2 have no preference for specific DNA sequences; therefore they associate with coactivators and co-repressors which bind to DNA in a specific manner. They both are part of the Mi-2/NuRD complex and bind to the histone binding proteins RbAp46/48 to form a HDAC core complex. HDAC1 and HDAC2 are also associated with other different co-regulators (Fig. 12; Fig. 13a, b Supplementary Table 1). Rb was shown to depend on HDAC1 for the transcriptional repression of E2F target genes.[131] They promote nonhomologous DNA end-joining in the DNA damage response.[50]

The microarray analysis using *C. elegans* embryos identified tissue-specific and extracellular matrix (ECM)-related genes as major HDA-1 targets. Ectopic expression of HDA-1 or *C. elegans* cystatin, an HDA-1 target identified from the profiling, significantly affected mammalian cell invasion. Similarly, RNAi depletion or overexpression of human HDAC1 also affected cell migration. Therefore HDA-1/HDAC1 may play a critical evolutionarily conserved role in regulating the extracellular microenvironment.[34,59] The sumoylation of Smo-1 on HDA-1 inhibits LIN-12/Notch signalling in the vulva.[60] HDA1 contributes to huntingin polyglutamine toxicity.[132] HDA1 is required for cell migration and axon pathfinding.[133]

## GATAD2a/b (p66/p68)

Some versions of the NuRD complex are also thought to incorporate a second structural and/or regulatory subunit, p66a or p66b,[45,134] also known as Gatad2a (p66)

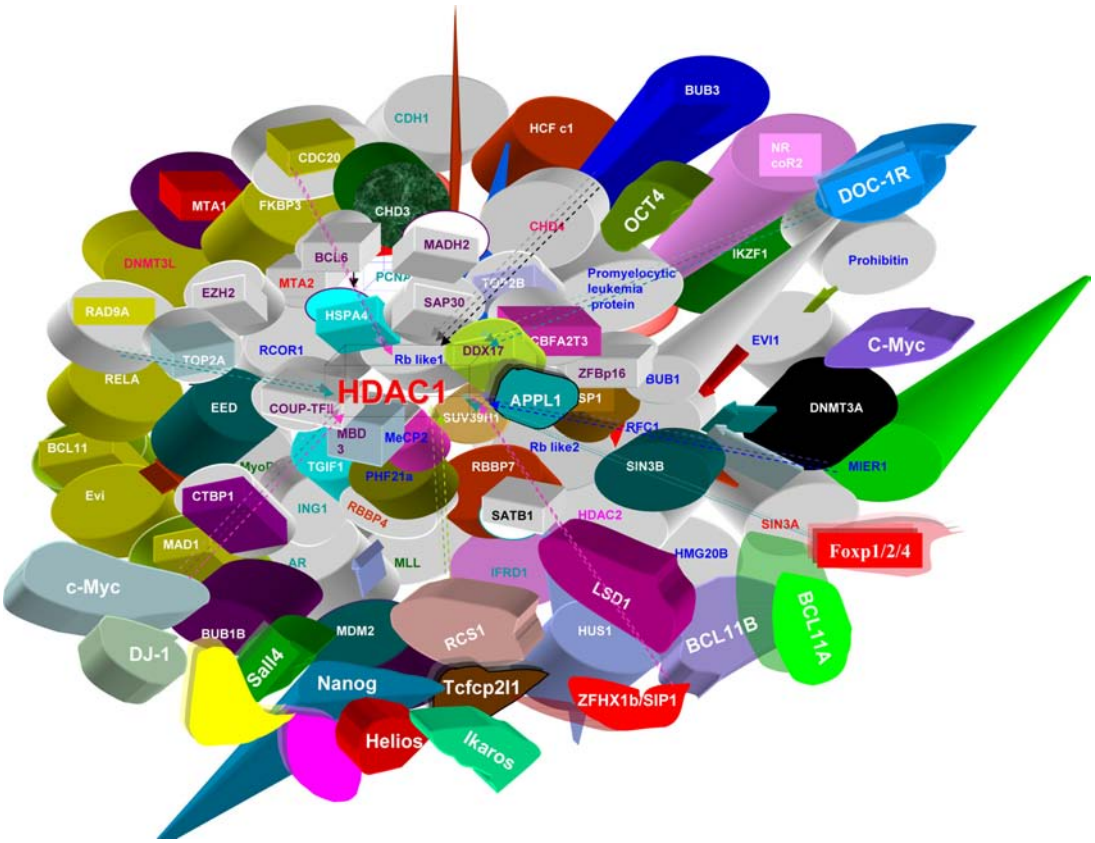


**Figure 12a.** The interactors of mammalian HDAC1.


**Figure 12b.** The interactors of mammalian HDAC2.

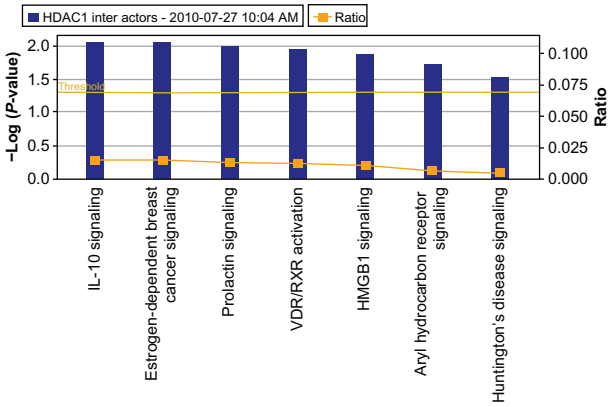


**Figure 13a.** The pathways of interactors of mammalian HDAC1. Reprinted with permission from ingenuity® systems, www.ingenuity.com

and Gatad2b (p68) (Fig. 14). As previously mentioned, the p66 subunits interact with the MBD2 subunit and may be involved in interactions between the Mi-2/NuRD complex and methylated DNA.[28] Like the RbAp46/48 subunits, both p66 isoforms have the capacity to interact directly with core histones.[135] Small ubiquitin-like modifier (SUMO)-modified forms of p66 efficiently interact with HDAC1, whereas RbAp46 binds to SUMO-p66.[136] The *C. elegans* p66 negatively regulates the expression of the Delta homolog *lag-2* and RNAi knock-down has low penetrance of multiple Muv phenotypes[60] (Zhang and Mueller, unpublished).

The Histone 3 Lysien-Specific Demethylase LSD1 independent, font big as the equivalents/other components of Mi-2/NuRD. Since its identification in 2004, LSD1 has been shown to be essential for many cellular processes. Significantly, Wang et al identified LSD1 as an integral component of the human Mi-2/MTA1-3/NuRD complexes that had profound implications for oncogenesis.[8] In breast cancer cells, LSD1 interacts directly with all MTA1-3 proteins and these different MTA/Mi-2/NuRD complexes have different genomic DNA landing signatures. MTA2 is required for nucleosome LSD1 demethylation. Furthermore, BRCA2-LSD1/Mi-2/NuRD complexes are associated with cancer susceptibility. LSD1/Mi-2/NuRD complexes suppressed breast cancer metastasis. Overexpression of LSD1 has been correlated with prostate cancer and tumor recurrence during therapy. The association of LSD1 with the androgen receptor switches its substrate specificity from H3K4me/me2 to H3K9me/me2. Polyamine analogues inhibit LSD1 and result in re-expression of aberrantly silenced genes in colon carcinoma.[20,137–139] A 3′ domain of the first identified long intergenic noncoding RNAs (LincRNA), HOTAIR, binds the LSD1/CoREST/REST complex, and, consequently, CoREST prevents LSD1 degradation. This coordinated tethering of PRC2 and LSD1 to chromatin couples histone H3 lysine 27 methylation and lysine 4 demethylation of target genes.[140]

LSD1 function is implicated in the DNA damage response by demethylating p53. LSD1 maintains DNA methyltransferase, Dnmt1. Loss of LSD1 demethylase activity results in reduced levels of Dnmt1 and global DNA methylation. Genetic ablation of LSD1 causes early embryonic lethality. Loss of LSD1 in embryonic stem (ES) cells reveals a reduction

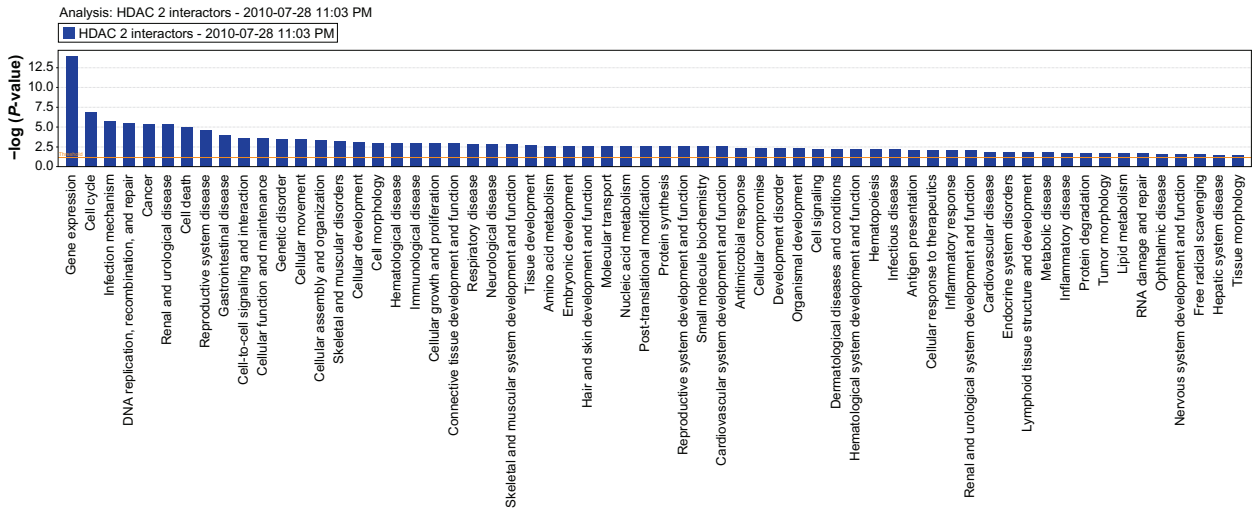


**Figure 13b.** The pathways of interactors of mammalian HDAC2. Reprinted with permission from ingenuity® systems, www.ingenuity.com.


**Figure 14.** The interactors of mammalian p66/68 (GATAD2a/b).

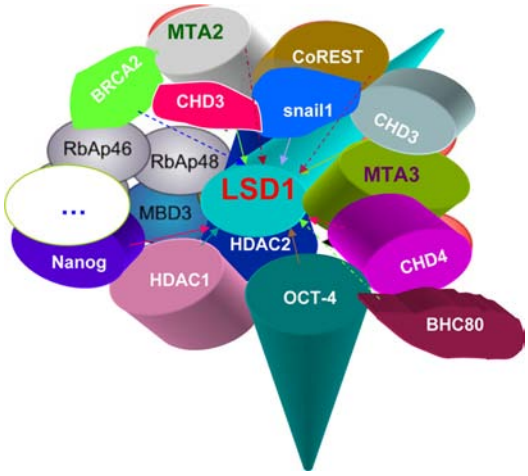


**Figure 15.** The interactors of mammalian LSD1.

in CoREST levels and the aberrant transcription of 588 genes[7] (Table 2). Like MTAs, LSD1 has SANT and ELM domains. It is unclear whether LSD1 and MTA proteins could have any redundant functions with such domains in a subset of target genes.

As mentioned earlier, *spr-5* encodes the *C. elegans* ortholog of the human histone demethylase LSD1; loss of *spr-5* activity can phenotypically suppress mutations in *sel-12*/presenilin and derepresses expression of *hop-1*, the second *C. elegans* presenilin in the LIN-12/Notch pathway. SPR-5 interacts with SPR-1, the *C. elegans* ortholog of CoREST.[61–63]

## Lamin A and the Mi-2/NuRD complex

Hutchinson-Gilford Progeria Syndrome (HGPS) is caused by mutations in the LMNA gene, which encodes nuclear lamins A and C. The *in vivo* physical interaction of lamin A with RBBP4, RBBP7 and HDAC1 points to lamin A being a co-regulator for the nuclear lamina and the Mi-2/NuRD complex in normal cells. Multiple Mi-2/NuRD components are lost in HGPS. Similar to RBBP4/7KD, silencing of any subunit increased the percentage of cells lacking H3K9me3 and HP1γ heterochromatin foci. Furthermore, the KD of HDAC1, MTA3, CHD3 or CHD4 in primary human fibroblasts increased the percentage of cells containing phosphor-H2AX positive foci. Progerin causes the loss of RBB4/7, which is an early event in ageing-associated chromatin defects. Loss of any Mi-2/NuRD component and reduction of HDAC1 activity is sufficient to recapitulate several ageing-associated chromatin defects. Induction of progerin or the KD of RBBP4 and RBBP7 results in changes within the heterochromatin structure, followed by accumulation of DNA damage. Loss of RBBP4/7 compromises the histone modifications and higher order chromatin structure, possibly making chromatin more susceptible to DNA damage. Thus the Mi-2/NuRD complex is a mediator of ageing-associated chromatin defects.[190]

## Post-transcriptional modifications and upstream cell signaling pathways of Mi-2/NuRD

A large number of different posttranslational modifications were identified in all major Mi-2/NuRD subunits. These include phosphorylation sites in Mi-2α, Mi-2β, p66α, p66β, HDAC1 and HDAC2. The role of p66 in the NuRD complex arises from its sumoylation.

SUMO-modified forms of p66 efficiently interact with HDAC1, whereas RbAp46 binds to SUMO-p66.[136] MTA proteins display tissue-specific differential expression giving rise to distinct Mi-2/NuRD complexes. Sumolyation of HDA1 in *C. elegans* is evident. The Mi-2/NuRD complex has many phosphorylation sites, especially on sites in MBD2, MTA1, MTA2 and MTA3. Several post-translational modifications have been conserved, and these might therefore have a role in regulating protein -protein or protein DNA interactions, or in fine-tuning of enzymatic activities. More potential amino acid modifications in chromatin remodeling are determined by DNA methylation and by multiple histone modifications including methylation, ubiquitination, sumoylation, phosphorylation and acetylation.

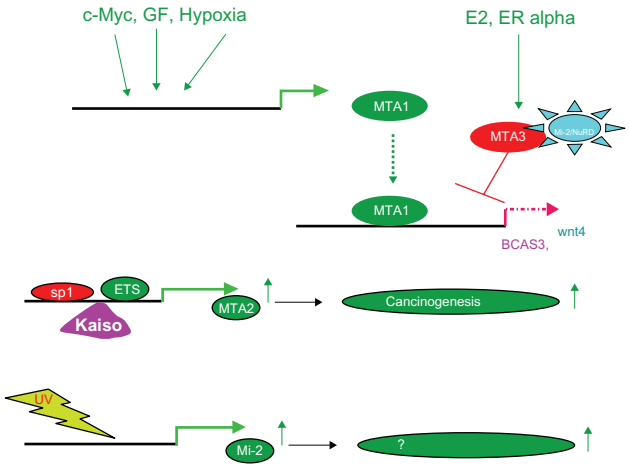


**Figure 16.** Model of upstream effects on the Mi-2/NuRD complexes. **Upper left and right:** The upstream processes of the MTA1, such as hypoxia, oncogenic growth factor heregulin and oncogene c-Myc, induce MTA1 expression. MTA1 protein is included in NuRD complex that represses the transactivation function of ER-α, rendering breast cancer cells more phenotypically aggressive. Moreover, the MTA1 protein in NuRD complexes enhances the stability and functions of HIF1, and also increases metastasis. MTA1 itself could promote the activities of BCAS3 and Wnt4, which is antagonized by the repression of the estrogen-activated MTA3/Mi-2/NuRD. **Middle:** sp1, ETS and Kaiso can upregulate the activity of MTA2, and promote the cancinopgenesis. **Bottom:** UV treatment increases the protein level of Mi-2.

From Table 1, we analyze the interactors of HDAC1 and HDAC2 or pooled interactors of the subunits of the Mi-2/NuRD complex, except for HDAC2, by using the Ingenuity Pathways Analysis (IPA) program for functional pathway analysis (Fig. 13a, b; Fig. 17).

Finally, the expression of MTA2 is partly regulated by SP1 and ETS elements in its promoter[141] and by Kaiso, a component of the human N-CoR complex.[142] Kaiso recruits the NCoR complex to the MTA2 promoter in a DNA methylation-dependent manner, resulting in hypoacetylation and methylation at H3K9 at the promoter region.[142] Therefore, this establishes that Kaiso is a DNA methylation-dependent transcriptional repressor of the MTA2 gene (Fig. 16, middle).

## The Methodology for Identifying of Mi-2/NuRD Complex Composition

Classical biology has become "big biology", ie, high-tech, high throughput and high computation. Identifying protein protein interaction is becoming increasingly important in gaining a molecular understanding of protein function and regulation in cells, in organisms under normal development and in diseased states. However, conventional chromatography has led to the discovery of the Mi-2/NuRD complex.[28,82,83] Moreover, single tagging studies like GST contributed significant insights for interacting proteins of the cell or tissue-specific Mi-2/NuRD[67,143] (Table 3). The yeast two-hybrid system further identified fruitful co-regulators of the Mi-2/NuRD complex[9,67,73,101,144] (Table 3).

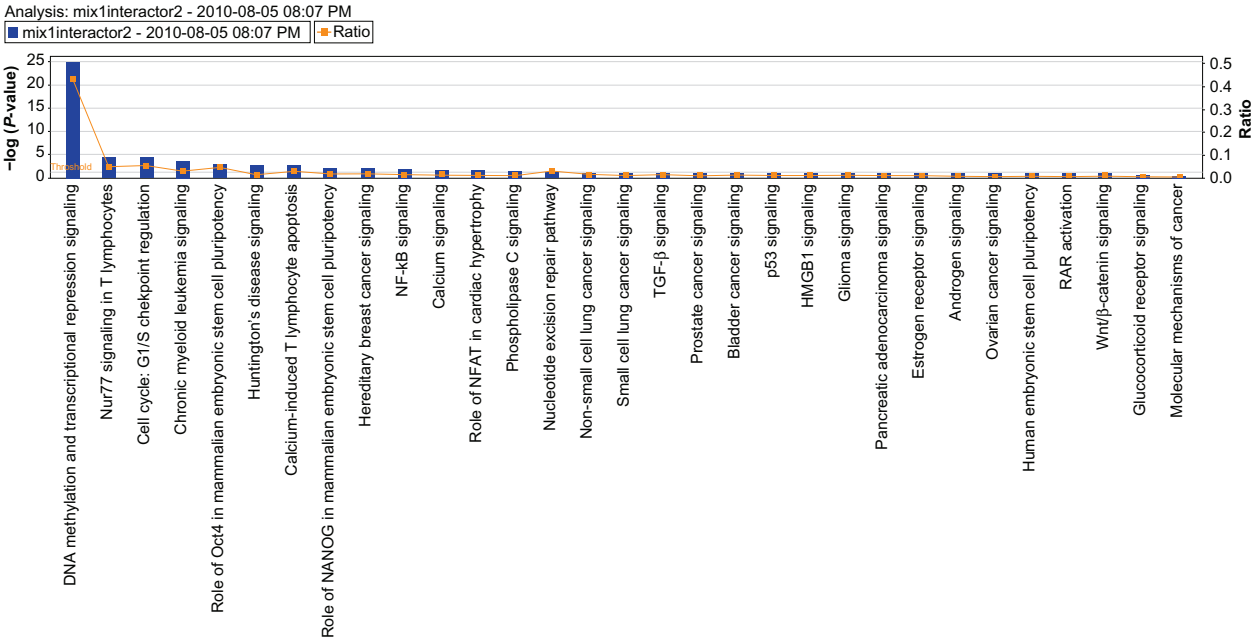


**Figure 17.** The pathways of pooled interactors of the core subunits of Mi-2/NuRD (excluding those of HDAC2). Reprinted with permission from ingenuity® systems, www.ingenuity.com.

**Table 3.** Advantages and disadvantages of the methods used for the identification of Mi-2/NuRD complexes.

| | Advantages | Disadvantages |
|---|---|---|
| Yeast two-hybrid | 1. Routinely performed in many labs.<br>2. Able to provide first hint for the identification of interaction partners.<br>3. Scalable to large scale screen for interactions among many proteins.<br>4. Sometimes generate a similar quality to data generated by the co-affinity purification and mass spectrometry (AP/MS).<br>5. Able to capture some transient interactions.<br>6. Having well-established bioinformatic platform, esp. for functional modules and networking. | 1. A high number of false positive (and false negative) identifications as high as 50%. The reason for this high error rate lies in<br>i. Over-expression can result in non-specific interactions;<br>ii. Fusion proteins;<br>iii. In the Yeast. A mammalian protein is sometimes not correctly post-transcriptionally modified in yeast eg, missing phosphorylation;<br>iv. In the nucleus. Some proteins might specifically interact when they are co-expressed in the yeast and nucleus, although in reality they are never present in the same cell at the same time.<br>2. All interactions should be confirmed by a high confidence assay, for example co-immunoprecipitation of the endogenous proteins. |
| GST tag | 1. Commonly used to create fusion proteins.<br>2. Simple and straightforward<br>3. Many commercial GST-tagged plasmids include a thrombin domain for cleavage of the GST tag during protein purification.<br>4. Increase the solubility of insoluble or semi-soluble proteins expressed in E. coli.<br>5. Others<br>i. Efficient translation;<br>ii. Initiation;<br>iii. Inexpensive affinity resin;<br>iv. Mild elution conditions. | 1. The tag is roughly 26 KDa, which, compared to other tags like the FLAG-tag, is quite big and possible to affect the native functions and structure of protein of interest<br>2. Generally fused to the N-terminus of a protein.<br>3. The over-expression of GST fusion proteins can result in non-specific interactions.<br>4. The conformation of the GST after expressing might be challenge, esp. for membrane proteins.<br>5. Others<br>i. High metabolic burden;<br>ii. Homodimeric protein;<br>iii. Does not enhance solubility. |
| HIS tag | 1. The most widely used fusion tags.<br>2. Small in size, less immunogenically active, and suitable for both purifying protein and downstream applications.<br>3. A large number of commercial vectors ready for expressing His-tagged proteins.<br>4. Be placed at either the amino-terminus (N) or carboxy-terminus (c), or in association with other tags.<br>5. The interaction of the His-tag does not rely on the tag structure, possible to purify insoluble proteins using denaturing conditions.<br>6. If solubility is not an issue, the constructs already exist in 6X or 8x HIS based vectors for the characterization of interactions.<br>7. It was used for FLAG–HIS (FH) TAP and have a high yield in both drosophila and mammalian systems. | 1. Specificity of IMAC is not as high as other affinity methods.<br>2. Does not enhance solubility. |

(*Continued*)

**Table 3.** (*Continued*)

| | Advantages | Disadvantages |
|---|---|---|
| | 8. Relatively high purity, and yield.<br>9. Others:<br>i. Low metabolic burden;<br>ii. Inexpensive affinity resin;<br>iii. Mild elution conditions;<br>iv. Tag works under both native and denaturing conditions. | |
| FLAG tag | 1. Very small size;<br>2. Many high quality antibody is commercially available;<br>3. Widely used;<br>4. Be placed at either the amino-terminus (N) or carboxy-terminus (c), or in association with other tags.<br>5. It will not usually interfere with the fusion protein expression, proteolytic maturation or activity.<br>6. Relatively high purity, and yield.<br>7. Others:<br>i. Low metabolic burden;<br>ii. High specificity. | 1. Expensive affinity resin.<br>2. Harsh elution conditions. |
| STREP tag | 1. A recombinant form of streptavidin with a near-neutral pI is commercially available. With no carbohydrate modification and a near-neutral pI, it has the advantage of much lower nonspecific binding than avidin.<br>2. It was used for FLAG–STREP (FS) TAP and have a high yield.<br>3. Relatively high purity, and yield<br>4. Others<br>i. Low metabolic burden;<br>ii. High specificity;<br>iii. Mild elution conditions. | 1. Expensive affinity resin;<br>2. Does not enhance solubility. |
| CBP | 1. Low metabolic burden.<br>2. High specificity.<br>3. Mild elution conditions.<br>4. Relatively high purity, and yield. | 1. Expensive affinity resin.<br>2. Does not enhance solubility. |
| TAP | 1. Low false positive.<br>2. The novel modified TAPs get much improvements.<br>3. High purity, moderate yield and specificity in comparison with single epitope tag affinity purification. | 1. Relatively low yield in mammalian classic TAP tagged purification.<br>2. A big size and possible to affect the functions of fusion proteins.<br>3. Stringent conditions to discriminate nonspecific interactor, loss of weak interaction, TEV remnant. |
| Chromatin immunoprecipitation and mass spectrometry | Gain insights for both real time protein-protein and protein-DNA interactions | The quality of fixation reversal is a critical challenge. |

Mass spectrometry-based proteomics, combined with tandem affinity (TAP)-tag-based protein purification,[145] is one of the most effective strategies for isolating and identifying protein complexes and has revolutionized proteomic experiments (Table 3). Generally speaking, mass spectrometry can identify medium or high abundance proteins. Therefore, previously infrequently expressed proteins could need a crosslink fixation[146] (Zhang, unpublished). Now it has become routine to allow protein identification with

high sensitivity and accuracy, and it recently produced several protein interaction network reports. The classic TAP using Protein A and CBP tags has proven successful in yeast to allow rapid purification of protein complexes and minimize the background. However, this classic TAP is not suitable for the purification and identification of proteins from tissues and cell lines. The large tags on either the N-terminus or the C-terminus destabilize the protein (Table 3). Some alternative other tags (eg, FLAG and His (FH-TAP), FLAG and CBP (FC-TAP), protein G and the streptavidin-binding peptide (GS-TAP), and FLAG and STREP (SF-TAP)) provide protein yields from tissues and mammals that are about an order of magnitude higher than the classic one[147–149] (Table 3; Zhang et al in preparation). Semi-quantitative specificity filters that are based on peptide spectral count measurements could derive an accurate complex composition.[150] Furthermore, to be extended to targeted therapy, such proteomics-based strategies could identify novel biomarkers that indicate a response of cancer cells to PI3K pathway inhibitors.[151] For Mi-2/NuRD, Le Guezennee et al. succeeded in identifying MBD2/NuRD and MBD3/NuRD complex compositions; to purify Ikaros/NuRD complexes under reduced stringency conditions, a two-step TAP procedure was successfully used.[152]

However, some studies in mammals and *C. elegans* rely on cDNA overexpression driven by exogenous promoters or transgenic random integration approaches. These transgenes can lack alternate promoters, enhancer elements, and 3 untranslated region (UTR) elements, which play critical roles in the control of gene expression *in vivo*. In *C. elegans,* for example, both the *daf-12* and *fah-1* genes have important enhancer elements that lie outside of the proximal promoter which were missed in promoter-only constructs. Furthermore, many transgene constructs use the *unc-54* 3′UTR, which prevents regulation by the appropriate microRNA genes. Consequently, generating transgenes with large segments of mammalian and worm genomic DNA would be ideal for capturing all of promoters, splice variants, and 3′UTR control elements. Along with mammalian Bacterial Artificial Chromosome (BAC) or fosmid library, a *C. elegans* fosmid library, which consists of ~40 kb regions of genomic DNA and covers almost all of the genome, has been recently constructed. Previously, we developed fosmid DNA recombineering technology to carry out profiling studies of the DAF-12 targets by powerful genome-wide screening techniques including ChIP-tiling arrays and assay of interactors by proteomic analysis on transgenic *C. elegans* lines[153,154] (Hochbaun et al in preparation). Importantly, we could capture most, if not all, previously identified targets, and thus their related mechanisms, as well as thousands of novel targets. For the proteins expressed with low abundance, like DAF-12, the integration of a few copies rather than a single copy in transgenic animals significantly increased the capture of *bona fide* target genes (unpublished).

Recently, an epitope-tagging strategy for the purification with recombination of a full-length BAC that contains Oct4 has pulled down the stemned Mi-2/NuRD complexes in mouse ESCs. As previously mentioned, fosmid recombinant cloning has the advantage of maintaining the endogenous promoter and therefore natural transcriptional regulation. The technology is amenable to high-throughput delivery, as demonstrated by random integration of tagged BAC transgenes,[155] and should considerably facilitate systematic tagging of genes and analysis of protein complexes with roles in development in different contexts, be it in stem cells, differentiated cell types or even mouse tissue.[156] Previous proteomic studies of Oct4 protein complexes needed lengthy single or tandem purifications from nuclear extracts with streptavidin capture[157] or anti-Oct4 antibodies,[3] and yielded small datasets; in contrast, this novel approach has produced by far the most extensive analysis of Oct4-associated proteins to date. The advantage of using whole extracts and nuclear extracts is that the analysis is not restricted to the nuclear environment, and the dataset encompasses diverse aspects of the life of Oct4, both nuclear and non-nuclear. With such a broad dataset, Oct4 obviously is involved in diverse cellular processes that can have an impact on many aspects of stem cell biology.

For the dissection and assay of different tissue/organ cancers, going small is the new big, ie, it is able to achieve comprehensive integrated molecular views of such defined cell populations. For the downstream sequences of target genes, low-quantity digital gene expression (LQ-DGE)[158] allows mRNA profiles of as few as 250 cells to be characterized without an amplification step. The new application of chromatin immunoprecipitation combined with high-throughput sequencing (ChIP-seq) from a limited number of cells

(10,000) hematopoietic mouse progenitors.[159,160] The mass spectrometry-based proteomics assays have become ready for human proteomes.[161] Such a strategy is certainly a hypothesis generator,[161] with some of the most interesting connections revealed being completely unanticipated.

As high-sensitivity and high throughput mass spectrometry, microarray transcriptional profiling and DNA sequencing become more common, assays that scan an entire genome, proteome, ribonome or metabolome will generate a huge amount of data. Bioinformatics tools such as Cytoscape, DAVID, Metacore and Ingenuity Pathway Analysis (IPA) could certainly provide us with functional modules and/or genetic cause-and-effect regulatory networking.[162,163]

## The emerging stemnessed Mi-2/NuRD complexes

Oct-4 expression must be tightly regulated; too much or too little will cause differentiation of the cell. Oct4 can both activate and repress transcriptional targets in mouse and human ESCs.[164,165] To date, Oct4 has been shown to be associated mainly with members of repressor Mi-2/NuRD complexes.[3,5,6,157] MTA1/Mi-2/NuRD may have an essential role in normal development as a mediator of pluripotency. MTA1 appears to be the preferred core component of NuRD complexes for Nanog and OCT4 interaction[3] as a potential regulator of pluripotency. Recently, Sall4, a well-known Oct4 partner, and other members of the Spalt-like family of transcriptional cofactors have been shown to associate with NuRD.[166] One of the stemness factors for iPS, c-Myc, is also associated with the Mi-2/NuRD complex.

## Clinical and therapeutic implications

Human cancer is heterogeneous, and this is driven by progressive genetic and epigenetic abnormalities. The Mi-2/NuRD complex is the master regulator of epigenetic alterations in changed patterns of histone modification. In contrast to genetic changes, the epigenetic alterations in cancer cells are reversible by the epi-drugs, for example, the inhibitors of histone deacetylases, DNA methyltransferases and histone demethylases.

## MTAs: targets for drug development

MTA1 protein, as a master co-regulator, or its gene, could be an excellent molecular target for cancer therapy, as well as being useful in cancer diagnosis or prognosis. The antisense silencing against MTA1 mRNA had a growth-inhibitory effect on human metastatic breast cancer cell lines.[167,168] RNAi knock-down of MTA1 in a human esophageal squamous cell carcinoma cell line resulted in significant

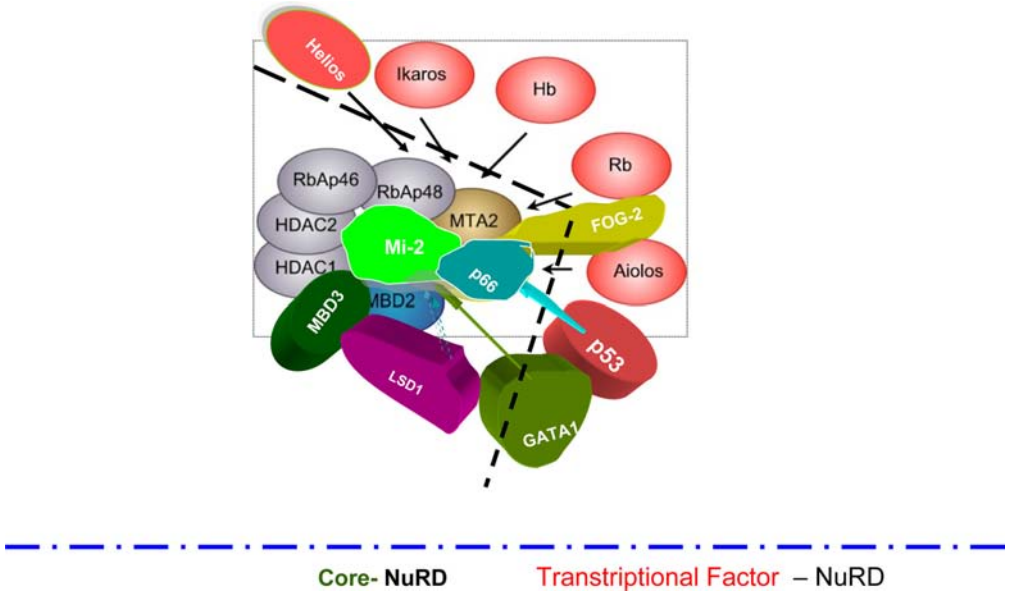


**Figure 18a.** The core-NuRD and transcriptional factor-NuRD.

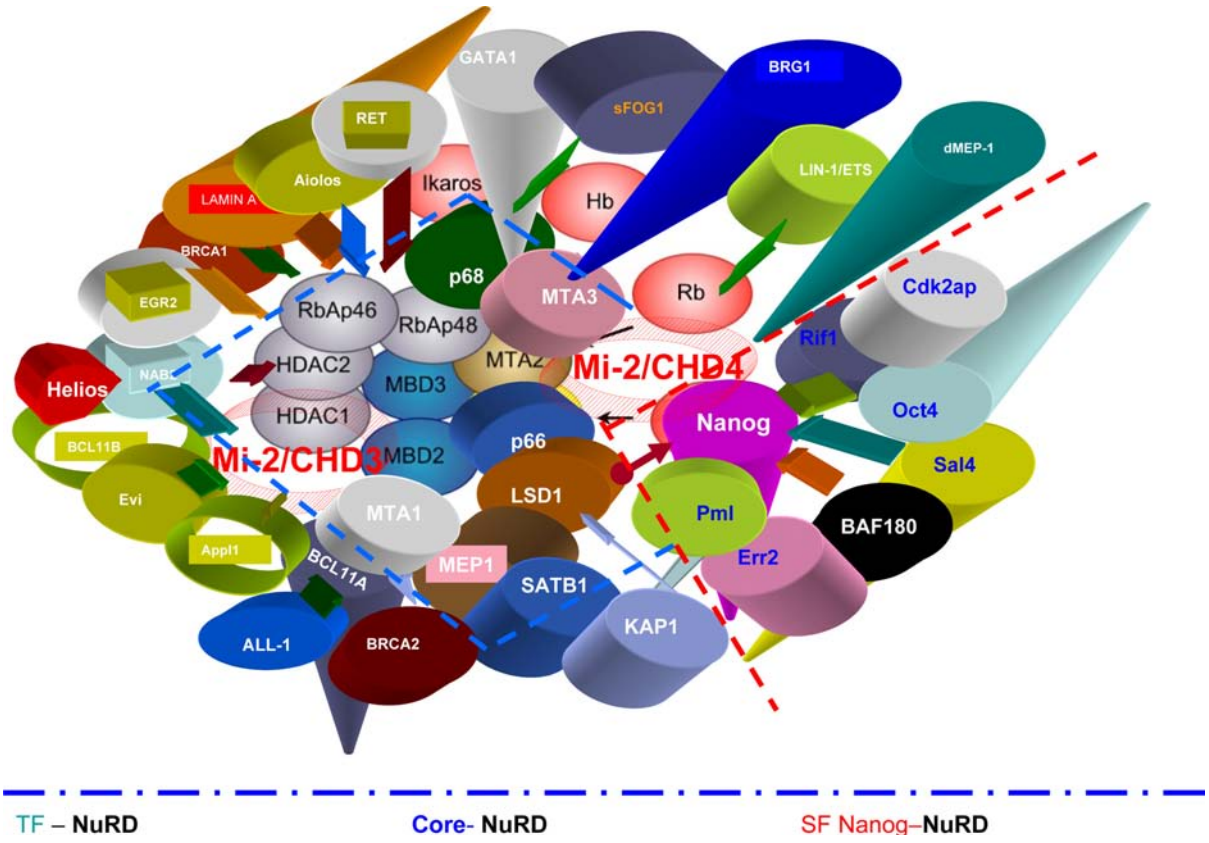


**Figure 18b.** The core-NuRD, transcriptional factor-NuRD and stemmed Nanog-NuRD.

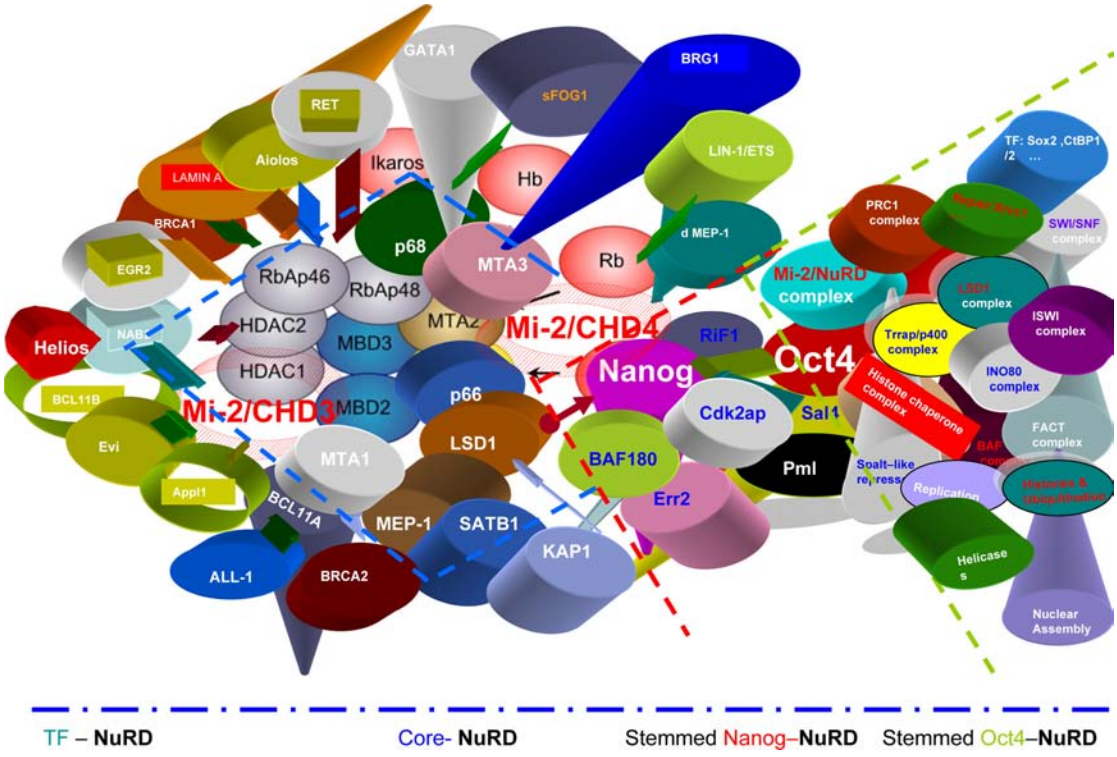


**Figure 18c.** The core-NuRD, transcriptional factor-NuRD, Stemmed Nanog-NuRD and stemmedOrchestra Oct4-NuRD.

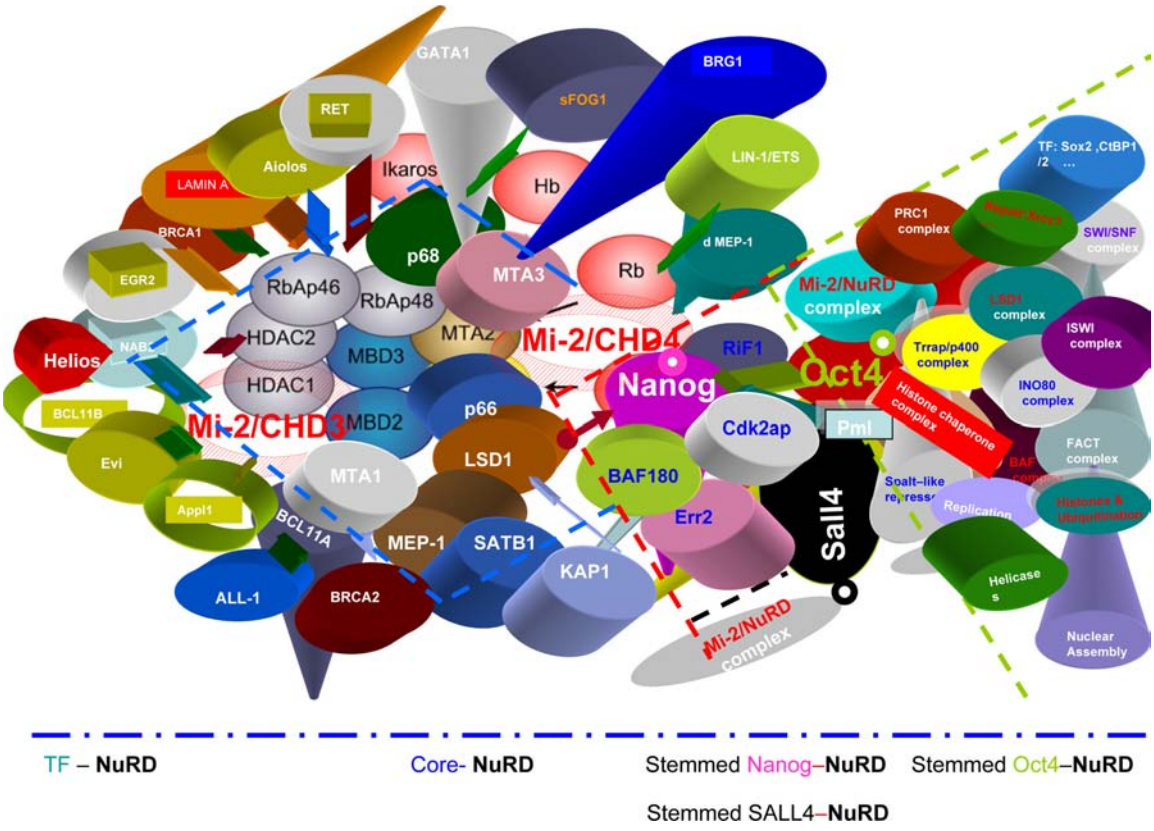


**Figure 18d.** The core-NuRD, transcriptional factor-NuRD, stemmed Nanog-NuRD stemmed Oct4-NuRD and stemmed SALL4-NuRD.

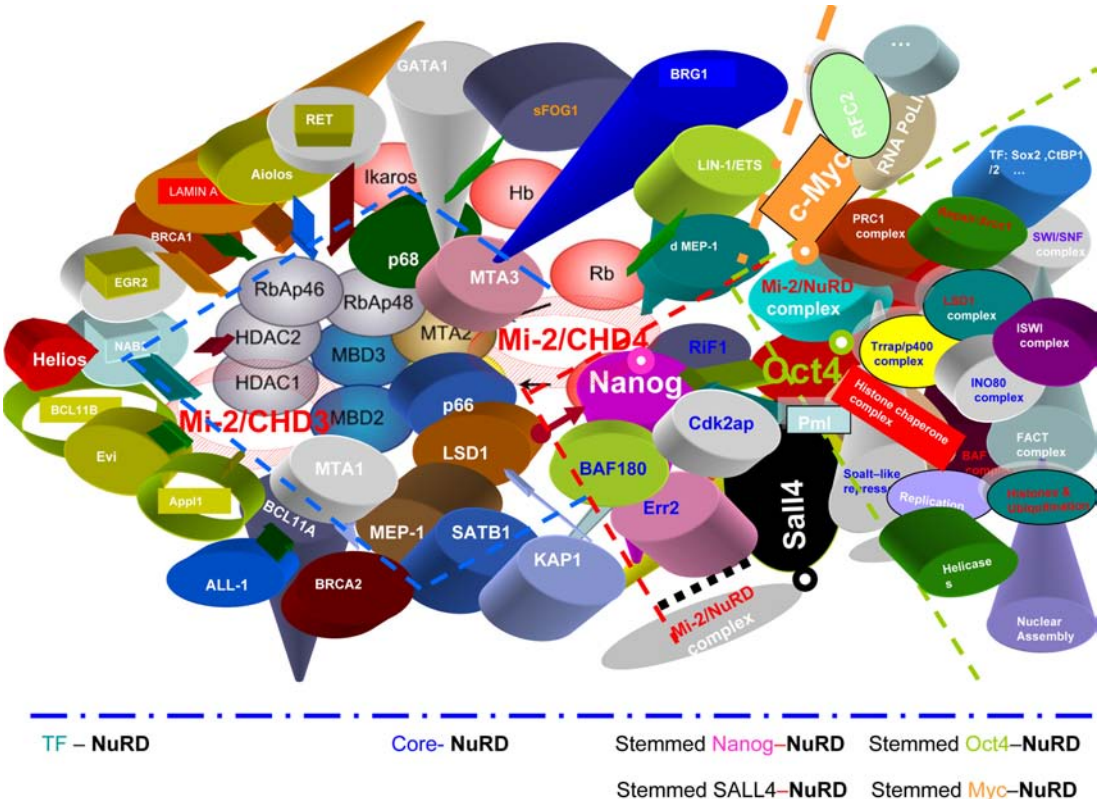


**Figure 18e.** The core-NuRD, transcriptional factor-NuRD, stemmed Nanog-NuRD, stemmed c-Myc-NuRD and stemmed Oct4-NuRD.

inhibition of *in vitro* invasion and migration properties of the cancer cells. In malignant melanoma cells, knock-down of MTA1 by RNAi successfully suppressed the growth *in vitro* and experimental metastasis of mouse melanoma cells *in vivo*. MTA1s may also be a useful target for fighting breast cancer. In an animal model, the effect of the MTA1s peptide blocks the tumor progression of MCF-7, which overexpresses ER-α. MTA1 is a promising antigen for tumor rejection in that it is overexpressed in many different tumors and is expressed at lower levels in normal tissues. An initial study demonstrated the presence of immunogenic MHC class I-restricted peptides of MTA1.[169]

## MBDs: targets for drug development

MBD proteins may be important modulators of tumorigenesis through the gene silencing mechanism of DNA hypermethylation and may be excellent novel therapeutic targets.[170] Some intensively-investigated DNA methylation inhibitors include the pyrimidine nucleoside analogs decitabine (Dacogen, SuperGen, Inc.) and azacitidine (Vidaza, Celgene), and the nonnucleoside inhibitor hydralazine. Azacitidine and decitabine are both US Food and Drug Administration (FDA) approved for the treatment anemia and chronic myelogenous leukemia (CML). Hydralazine was originally approved for use as an antihypertensive and recently used to reactivate the expression of tumor suppressor genes in cancer. Pharmacologic inhibition of DNA methylation blocks DNMTs and their targeted degradation, and desilences genes that have been aberrantly silenced by hypermethylation. Finally, it causes inhibition of clonal expansion and tumor cell growth, induction of cell differentiation, and cancer cell death. Only rapidly dividing cells such as tumor cells will be targeted as these agents function by being incorporated into newly replicated DNAs.

## HDACs: targets for drug development

Epigenetic therapy tries to reverse the aberrations with natural compounds and/or synthetic molecules that are active on specific epi-targets. Vorinostat (Zolinza, Merck) is the first HDAC inhibitor that has been licensed for clinical use. HDAC inhibitors have been tested as promising agents in treating blood-borne cancers, and also in treating polyglutamine diseases.[171–174] Two HDACs, hydralazine and magnesium valproate, can sensitize tumor cells to chemotherapy in patients with advanced and solid refractory tumors.[175] Inhibition of histone deacetylation enhances tumor radiosensitization.[176] So far, more than 11 HDAC inhibitors are in clinical development for epigenetic cancer therapy.

## G9a: a target for drug development

The MTA proteins are associated with HDAC1/2 and methyltransferase G9a, and specific G9a inhibitors have been recently reported.[177] This raises the possibility that G9a inhibitors such as BIX-01294 might also be used, alone or together with the current FDA-approved HDAC inhibitors, for treating MTA-involved cancers and neurological diseases.[178]

## LSD1: a target for drug development

The development of LSD1 inhibitors may represent a significant weapon against cancer. To date, only two drugs, pargyline[179] and tranylcypromine,[20,180] have been described as LSD1 inhibitors, but their action is not as specific as the well-known anti-Monoamine Oxidase (MAO) agents. Pargyline blocks demethylation by LSD1, and consequently it blocks androgen-receptor-dependent transcription. Thus, modulation of LSD1 activity offers a new strategy to regulate androgen receptor functions, which may turn out to be important in prostate cancer models.[179]

## RNAi and potential combined therapy

Hypoxia inducible factor (HIF)-1 accumulation favors tumor angiogenesis.[181] 5-AZA-CdR represses the hypoxia response pathway by downregulation of HIF-1 targets vascular endothelial growth factor (VEGF).[182] HDACi also plays an important role in inhibiting tumor angiogenesis by concomitantly upregulating antiangiogenetic factors such as VHL and by downregulating pro-angiogenic factors such as HIF-1 and VEGF in cancer cells.[183] MTA1/NuRD has a role in tissue maintenance, via HIF-1a/VEGF expression against hypoxic condition.

Recently, ALN-VSP02 (KSP/VEGF siRNAs), a lipid nanoparticle containing two small interfering RNAs (siRNAs) for kinesin spindle protein (KSP) and vascular endothelial growth factor (VEGF), has demonstrated its potential antitumor activity. Upon intravenous administration, the siRNAs in KSP/VEGF siRNAs ALN-VSP02ALN bind to both KSP and VEGF messenger RNAs (mRNAs), preventing

translation of KSP and VEGF proteins; this may result in growth inhibition of tumor cells that overexpress KSP and VEGF.[184]

Interestingly, as already mentioned, some components of the Mi-2/NuRD complex (eg, LIN-53/RbAP48) in *C. elegans* could enhance RNAi. Furthermore, the long-term RNAi of Transcriptional Gene Silencing (TGS) with a chromatin remodeling-related mechanism might potentially be used in the future for therapeutic applications of RNAi for prolonged, epigenetic gene silencing.[185] Thus, it may be useful to apply such RNAi against components of the Mi-2/NuRD complex combined with thermo- or radiation- therapy in preclinical cancer.

Furthermore, the Mi-2/NuRD complex plays a crucial role in DNA damage and repair.[25,35,48–50] Curcumin (diferuloylmethane) targets DNA damage and repair.[186] A combined strategy of the HDAC inhibitors (eg, Vorinostat, ie, suberoylanilide hydroxamic acid (SAHA) or the safe dietary compound resveratrol) and curcumin therapy provides new hope for cancer patients.

## Future Mi-2/NuRD-based approaches

It is highly likely that greater understanding if Mi2/NuRD subunit structure could contribute to a breakthough in anti-cancer drug development since our knowledge in this field and the interdependence among the subunits is in its infancy (Tables 4 and 5). In the future, further understanding of the relationships between the Mi-2/NuRD complex structure and activity, as well as their biological mechanism(s) of action, will hopefully provide novel epigenetic approaches for fighting human cancer.

## Future Mi-2/NuRD stemness-based treatment

A deeper understanding the origin of cancer stem cells (CSC)[190] and their epigenetic characteristics is important for the rational design of cancer treatment. Cancer derived from CSC may be more prone to drug resistance. However, epigenetic changes are mainly reversible through the administration of epi-drugs. Such drugs have been developed for targeting total cell populations and not CSC's specifically. Finally, a stem cell-based vaccine has been shown to successfully address colon cancer.[192] An iPS-based vaccine has also been developed too.[192,193] Advancing our knowledge of the Mi-2/NuRD complex could possibly contribute to an improved design to achieve better effects.

**Table 4.** Crystal structure of subunit of Mi-2/NuRD complex.

| Subunit | Structure | References |
|---|---|---|
| CHD4/Mi-2β | + | PDB IDs: 2EE1, 1MM2, 1MM3 |
| CHD3/Mi-2α | n.a | |
| MTA1 | + | PDB |
| MTA2 | n.a | |
| MTA3 | + | PDB IDs: 2CRG |
| MBD2 | + (for MECP2) | PDB IDs: 1QK9 |
| MBD3 | n.a | |
| GATAD2a/p68 | n.a | |
| GATAD2b/p66 | n.a | |
| RBBP4/RbAp48 | n.a | |
| RBBP7/RbAp46 | n.a | |
| LSD1 | + | Chen Y et al PNAS[187]<br>2COM<br>2DW4<br>2EJR<br>2HKO<br>2IW5<br>2UXN<br>2UXX<br>2V1D<br>2W9I<br>2X0L<br>2XAF<br>2XAG<br>2XAH<br>2XAJ<br>2XAQ<br>2XAS<br>2Z3Y<br>2Z5U<br>3ABT<br>3ABU |
| HDAC1/HDAC2 | + | Vannini et al[188]<br>PDB IDs:<br>3HGT<br>3HGQ |

**Abbreviations:** n.a., not available; +, available; PDB, protein data bank.

## The significance of cross-validation with interspecies (Mammals and *C. elegans*): a personal view

*C. elegans* has almost the same number of protein-coding genes as a human—about 20,000 is the latest estimate—and most of those genes encode similar functions.[34,35] So the basic parts set for animal development was established several hundred million

**Table 5.** Interdependence of subunits of Mi-2/NuRD complex.

| | Mi-2α | Mi-2β | MTA1 | MTA2 | MTA3 | MBD2 | MBD3 | HDAC1 | HDAC2 | RBBP4 | RBBP7 | LSD 1 | p66/68 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MTA2$^{-/-}$ | n.d. | = | n.d. | ↓ | n.d. | = | = | = | = | n.d. | n.d. | n.d. | n.d. | Ma et al[115] |
| MBD3$^{-/-}$ | n.d. | n.d. | n.d. | ↓ | n.d. | = | ↓ | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | Reese KJ et al[189] |
| Lamin A$^{-/-}$ | ↓ | ↓ | n.d. | n.d | ↓ | n.d. | n.d. | ↓ | n.d. | ↓ | ↓ | n.d. | n.d. | Pegoraro[190] |
| CHD4$^{-/-}$ | n.d. | ↓ | n.d. | = | n.d. | n.d. | n.d. | = | n.d. | n.d. | n.d. | n.d. | n.d | Polo SE et al[125] |

**Abbreviations:** n.d., not determined; = , no change; ↓, Down.

years ago. Furthermore, *C. elegans* has two small evolution-distance nematode sister species, *C. briggsae* and *C. remanei,* and similar studies could provide very strong mutual cross-species validation.[58,195] This study of the Mi-2/NuRD complex in *C. elegans* proved that such cross-research can be a fruitful and probably cost-effective way to study cancer.[196] Besides, we are now able to perform more high-tech, high throughput assays, such as ChIP-seq, quantitative proteomics[197] and TAP on Mi-2/NuRD complexes and their interactors to learn more about them and their clinic implications for beating cancer. Screening all the genes or proteins in an organism is not much more difficult than analyzing a small subset, and robotics and high-throughput screening techniques are now within the reach of most labs. Second, the cost of systems biology scales sub-linearly while the payoffs scale super-linearly.[162] A huge amount of information will need to be analalyzed with current powerful bioinformatic tools[163] so we can obtain information on the behavior of gene groups at a system level, as well as their functional modules and networks. This could be done quickly with genetically tractable organisms like *C. elegans*, and then projecting its mechanisms to human beings, as has already been demonstrated successfully for *C elegans* originals such as RNAi, programmed cell death and miRNAs. Put simply, we should be ready to worm our way into elucidating differential regulatory networks between healthy and cancerous tissues.

## Discussion

We are towel on the road from "big biology" to "bigger biology"[198] with both "data first"[199] and "hypothesis first"[200] approaches being used together rather than alone; from HGP and NextGen Sequencing to the NextGen mass-spectrometry and HUPO initiatives. Since the international Human Genome Project and Celera Genomics Corporation completing the initial draft of the human genome, the past decade has witnessed remarkable progress in our understanding of the expanding family of Mi-2/NuRD complexes and their powerful master roles in gene regulation during normal development and carcinogenesis across species. This rapid trajectory can continue with the emergence of the human proteome project. The Cancer Genome Atlas, a comprehensive platform to accelerate our understanding of the genetics of cancer

using innovative genome analysis technologies, is now available. Weekly, even daily developments are emerging from this interesting Mi-2/NuRD complex family. Finally, all these new findings could be promisingly pave the way for a highly effective genetic and epigenetic therapy in the near future to cure cancer.

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## Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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