Characterization of CHD Protein Subfamily-1 and Regulation of Gene Expression

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Abstract- Chromatin Remodelers are required to facilitate complete packaging of the genome, specialization of the chromatin and ensuring DNA accessibility in the packaged regions, performing various ATP dependent alterations like dissociation of histone-DNA interactions, vigorous movement of Histone octamers etc. Also, the approachability of nucleosomal DNA to transcription factors increases greatly. CHD proteins, a group of ATP dependent chromatin remodeling enzymes, help in maturation of histone octamers into native nucleosomes. CHD proteins consist of three different subfamilies widely distributed among various organisms like *C.elegans*, yeast, drosophila, humans and mice. This review highlights the progress made in understanding the regulation of gene expression by CHD1 and CHD2 (members of CHD subfamily1) proteins and how these two proteins influence various diseases like Estrogen Receptor Positive Breast Cancer, prostate cancer and neurodevelopmental disorders.

Keywords – CHD1, CHD2, chromatin remodeler, cancer, H3.3 and H2A.Z histone variants, miR26a, NKX2-1

I. INTRODUCTION

In the nucleus of eukaryotic cells, DNA is condensed to form chromatin. Nucleosome, a complex of 8 histone proteins and 146 bp (approx) of DNA, is the fundamental building block of chromatin. Nucleosomes are capable of getting wrapped into 30nm fibers resulting in the formation of tightly packed chromatin. DNA replication, transcription and repair are highly influenced by the degree of compaction of the chromatin. Maintenance of the compact chromatin structure is highly dependent on the ATP-dependent chromatin remodeling enzymes[1]. The chromatin remodeling enzymes are members of the DNA translocase family capable of changing the position of the histone octamer to a different site on DNA. They are capable of application of ATP- dependent torsional strain to DNA. These enzymes are capable of regulating the availability of nucleosomal DNA. They do so by exposing certain regions of DNA to promote cellular interaction; whereas it can also shield certain regions of DNA. There are four broad families of chromatin remodeling enzymes (CHD, SWI/SNF, ISWI, and INO80).

2. CHROMATIN REMODELING AND VARIOUS REMODELING ENZYMES

2.1 Chromatin remodeling:-

The complex organization of DNA within the nucleus is achieved by its folding into Chromatin, a highly compact structure. Chromatin shows a 'bead on a string appearance', where the beads are referred to as nucleosomes [2]. The organizational unit of chromatin is called histone. The major protein in chromatin, highly basic and rich in arginine and lysine residues is called Histone protein. They are capable of modifying the nucleosome structure thereby directly influencing the regulation of gene expression. The N-terminal tails of core histones(H2A,H2B,H3,H4) protrude outwards from the complex chromatin structure and are responsible for various post-translational modifications like acetylation, methylation, ubiquitination, sumoylation, glycosylation etc.[3]. Two histone molecules of H2A, H2B and one molecule each of H3 and H4 are assembled to form a histone octamer. . The nucleosomal core proteins are separated by H1/H5 linker proteins. Their length varies from 7 bp to 100 bp based on the organism[4]. The compaction in DNA packaging restricts the access of the transcription factors and other members of the transcription machinery to the genes targeted. Chromatin remodeling makes the compact DNA structure accessible to the transcription machinery. This phenomenon brings about notable changes in nucleosome positioning along the genomic DNA, directly influencing the positive and negative regulation of transcriptional activity [5][6]. Chromatin remodelers directly influence the packaging and unpackaging of DNA and guide the proper execution of DNA replication, repair, recombination alongside influencing gene transcription [7].

2.2 Chromatin remodeling enzymes:-

The ATP-dependent Chromatin complexes bring about alterations in the DNA-nucleosome interactions by utilizing the energy obtained from ATP hydrolysis. These interactions allow or restrict access of DNA to several types of regulation machinery. Four distinct chromatin remodeling enzymes along with their subunits are conserved currently from yeast to humans. The SNF/SWI enzymes consist of 8-14 subunits and were purified from *S.cerevisiae*[8] DNA- dependent ATPase activity is exhibited by SWI/SNF like ATPase subunits present in the complex. The C-terminal bromodomain is an essential motif and domains I and II having unknown functions which have been identified[9]. The ISWI (imitation switch) family of remodeler consist of 2-4 subunits. Among the domains of ISWI enzymes, are the ATPase domains together with the SLIDE domain which plays a significant role in Nucleosome recognition. Also, the HAND SANT domain shows proper interaction with the histone tails. The INO80 (inositol requiring 80) family of remodeler are inclusive of the SWR1 related complexes and consist of more than 10 subunits. They were obtained after purification from S.cerevisiae. INO80p/SWR1 ATPase alongside INO80 Remodeling complex and SWR remodeling complex are intrinsic components of this particular family. Positive and negative transcription regulation via ATP-dependent chromatin remodeling action is a primary function of INO80[10].

3. CHROMODOMAIN HELICASE DNA BINDING PROTEIN AND IT'S SUBFAMILIES

The CHD proteins, are majorly large proteins with a size range varying between 223 Kda and 252.5Kda and are homologous structurally to a large extent. The discovery of the first member of CHD occurred while searching for regulators of immunoglobulin promoters. The CHD family consists of 9 members as of today varying in organisms like *C.elegans*, yeast, drosophila, humans and mice[11].CHD1and CHD2 belong to subfamily I whereas subfamily II contains CHD3 and CHD4.CHD5 to CHD9 proteins belong to subfamily III .They help in assembling the nucleosome(ATP dependent) with the help of their tandem Chromodomains and SNF2-like ATPase domain[12].

3.1 CHD subfamily I(CHD-1 and CHD-2):-

The first CHD protein to be characterized was mouse CHD-1 (Delmas et al. in 1993). The human CHD1

gene was introduced by PCR screening [13]; which is 95% homologous to mouse CHD-1 and encodes for a 1079 amino acid long protein. Two chromodomains near the N- terminus, a SNF2 related helicase domain and a DNA binding domain at the C-terminal are the structural domains of this subfamily [14]. Human CHD-2 protein has 58.6% similarity with mouse CHD-1 and is 95.9% homologous with mouse CHD-2, and has similar structural domains when compared with CHD-1[14].

3.2 CHD subfamily II (CHD-3 and CHD-4):-

Identification of the subfamily II proteins; also known as MI-2alpha and MI-2beta was done in the form of autoantigen in the connective tissue related disease Dermatomyositis [15][16]. PHD(plant homeo domain), also known as LAP(leukemia associated domain) similar to Zinc finger domain is the primary domain of these proteins(60 amino acid protein approx.) which function in transcription regulation[17].

3.3 CHD subfamily III (CHD5 to CHD9):-

Thompson et al first identified CHD-5 while performing the mapping of deleted genes in human neuroblastomas. Two PHD Zinc-finger domains and a DEAH box type helicase domain are primarily found in this protein [18]. CHD-6 comprises of a 50 amino acid motif containing domain called SANT domain observed in many chromatin remodeling complexes [19]. CHD-7 was first observed as the mutated gene of CHARGE syndrome. It primarily contains two chromodomains, two helicase domains, two BRK domains and a SANT domain [20]. Like other members of CHD subfamily III, CHD-8 contains a SANT domain and two BRK domains. CTFC, which is a DNA binding transcription factor shows efficient interaction with CHD-8 to induce enhancer blocking insulation throughout the genome [21]. The last member of subfamily III is CHD-9;is also known as CReMM(chromatin related mesenchymal modulator). It shows maximum expression in mesenchymal progenitor and shows binding to skeleton-tissue specific CBFA-1, biglycan, osteocalcin exhibited in different way [19].

4. CHD PROTEIN SUBFAMILY 1(CHD-1 AND CHD-2)

4.1Position of CHD-1 and CHD-2 in the genome:-

CHD-1 protein is present on the 5th chromosome in human beings. Cytogenetic band is given by 5q21.1.
CHD-2 is located on the15thchromosome in human beings. Cytogenetic Band is given by 15q26.1.

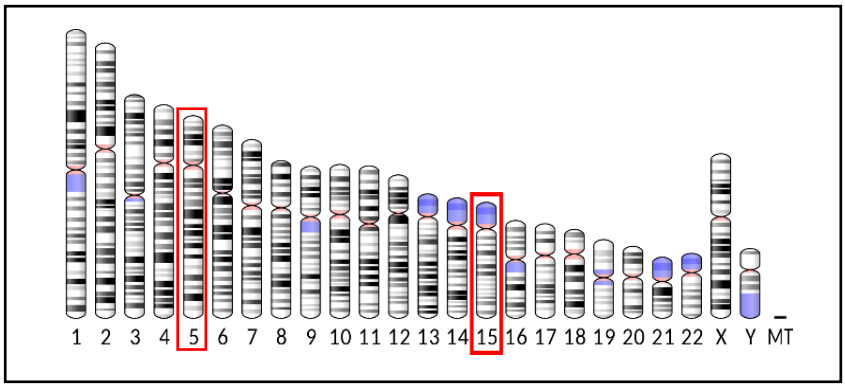

Fig1:- Position of CHD-1 and CHD-2 in the genome (https://m.ensembl.org/index.html)

4.2 Structural domains of CHD-1 protein:-

Chromatin remodeling enzymes bring about alterations in the nucleosome structure by catalyzing non-covalent changes in histone-DNA interactions. The action of different groups of remodeling enzymes is different. The method of coupling ATP hydrolysis to chromatin remodeling by the chromatin remodeling enzymes is yet to be understood. A conserved ATPase domain is common to all remodeling enzymes, but they also possess certain accessory domains which help to regulate the ATPase activity [23]. CHD1 contains a regulatory double chromodomain at the N-terminal, an Snf-2 ATPase domain and a DNA

binding domain at the C terminal [22]. CHD1 contains a regulatory double chromodomain at the N-terminal, an Snf-2 ATPase domain and a DNA binding domain at the C terminal [23].The chromodomain was identified originally as a homology region consisting of 37 amino acid residues shared by *D.melanogaster* epigenetic repressors, heterochromatin protein 1 and polycomb [24]. Currently, the chromodomain is considered as a 50 amino acid residue long region of shared homology by the aforementioned polypeptides[24][25]. At the transcriptional start sites of human, mouse and drosophila genes presence of localization of histone H3 methylated lysine 4 has been observed[26].Chromodomain hence is said to act as a mediator in influencing chromatin interactions between DNA , RNA and methylated histone H3[27]. Also, pieces of evidence have been observed regarding thebinding of yeast CHD1 and human CHD1 with H3K4me through the chromodomain[28][29].The ATP-dependent chromatin remodeling proteins are defined by Snf-2 like ATPase domain. Its involvement has been observed in DNA repair, DNA replication, transcription regulation and chromatin assembly [30][31]. This 400 amino acid long domain(approx.) has two subdomains:-

(i) A conserved N-terminal subdomain I assisting in ATP binding,

(ii) A C-terminal subdomain helping in energy transduction [11].

This domain is bi-lobal in nature and is capable of hydrolyzing ATP. Nucleosome sliding or repositioning of chromosomes is a common function of chromatin remodeling enzymes. This domain functions by moving along the DNA template and assisting in destabilizing protein-DNA interactions[13]. Sliding occurs by the translocation of the ATPase to an internal site on the nucleosomal DNA called the superhelical position 2. This is the primary function of the ATPase domain. There are two such SHL 2 sites hence two CHD 1 ATPases can bind to these two sites. The chromodomains also bind to DNA at superhelical location 1[32].The DNA binding C-terminal domain is capable of accelerating the action of Snf2 like ATPase domain to induce nucleosome spacing. The presence of SANT (*S*WI3, *A*DA2, *N-Co*R, and TFIIIB) and SLIDE (SANT like ISWI domain) domains previously observed in ISWI remodeling enzymes were also noted. The crystal structure of the DNA binding domain helped in identifying the aforementioned domains. This discovery helped to formulate the idea that the SANT and SLIDE domains might play an important role in nucleosomal spacing. Slight mutations in these two domains may lead to the exhibition of wild-type levels of binding to the nucleosome and ATPase activity, but defects were observed during the occurrence of nucleosomal sliding. The two domains thus can act as a cooperative unit to bind DNA[33].

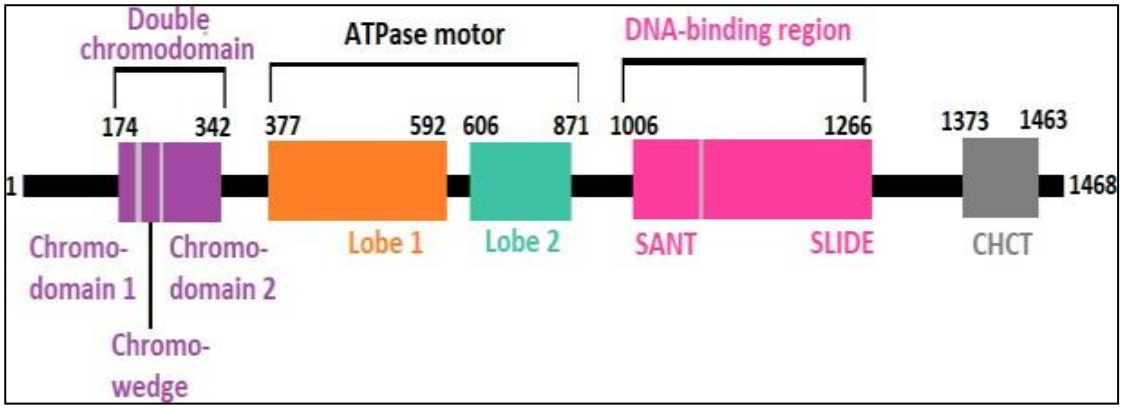

Fig2:The domain architecture of CHD-1 along with indication of residues at domain boundaries[23]

4.3 Structural domains of CHD-2 protein:-

The structural domains of Chromosomal helicase DNA binding protein 2 are similar to that of CHD-1. However, in mCHD-1(mouse chromodomain helicase DNA binding protein), two chromodomains are located at the N-terminus, along with a Snf2 like helicase/ ATPase domain at the centre ,within which it has a DEAD like helicase domain and a Helicase domain at the C-terminus finally ending in a HMG-1 domain at

the C terminus. Four bipartite nuclear localization signals are present in CHD-2 unlike CHD-1 proteins having only one nuclear localisation signal. The DEAD-box proteins found in all eukaryotes and majority of prokaryotes belong to the DEAD-box family of helicase[14].They have been observed to possess RNA-dependent ATPase and ATP-dependent helicase like activities [14][34].The HMG domain induces binding to AT-rich regions of DNA and chromatin, bringing about long and short alterations in the binding site structures[35].

Fig.3- The structural domains of a wild type humanCHD-2 protein[24].

4.4 Remodeling structure of ScCHD-1:-

The structural information of chromatin remodeling enzymes is largely limited to domains. Very little has been deciphered regarding the yet to be structurally analyzed regions connecting these domains [37]. The ATP-dependent chromatin remodeling enzymes, like ISw1 and CHD1 help in positioning nucleosomes over coding regions. They are extended members of Snf 2 related chromatin proteins inducing reconfigurations in DNA-protein interactions. Structural information is available for CHD-1 among all the other members of the Snf2 family.

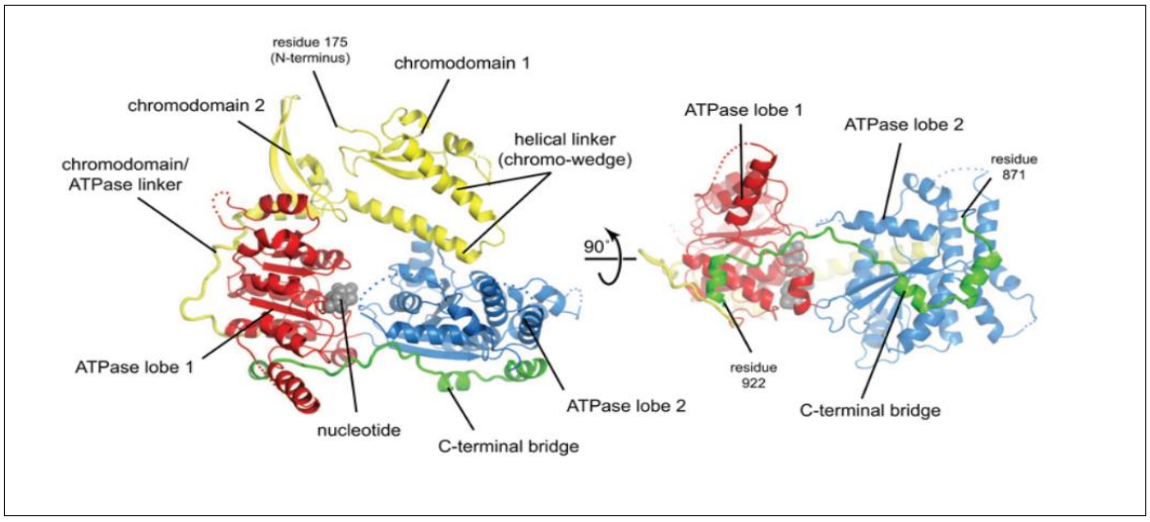

Fig4. Remodeling structure of ScCHD-1[36]

An overall flattened structure with a ring like appearance is observed. Across the central cleft of the ATPase motor, the double chromodomain unit is present which is in close association with two ATPase lobes. Two helices protruding from the chromodomains (wedge-shaped) connect the two chromodomains. These connecting helices are referred to as the 'Chromo-wedge'. Seated on the ATPase lobe 2, is the second helix of the chromo wedge. The second chromodomain is packed against the first ATPase lobe. A Chromodomain /ATPase linker (35 residues approximately) is seen to which the double chromodomain is tethered. The linker is packed against the ATPase lobe 1 and is organized with compaction in the crystal structure. A 50 residue (approx.) segment C-terminally extends from the second ATPase lobe on the other side of the double chromodomain. Initially, this segment referred to as the C-terminal bridge; crosses over a certain face of ATPase lobe 2 and then makes a turn to finally pack against the first ATPase lobe[36].Crystallization helped in revealing the structure of the ATPase domain in association with the tandem chromodomains in ScCHD-1. The Snf2 family has an ATPase module that consists of two Rec A

domains inducing ATP dependent DNA translocation. The chromodomains block the access to a DNA binding surface between the RecA domains, implying that Reconfiguration is of utmost importance for the Rec A domains to interact with DNA. In absence of Nucleosome substrates, the chromodomains inhibit the action of DNA binding and also block the activation of the ATPase domain. The Chromodomain-ATPase junction, when disrupted leads to failure in distinguishing the difference between nucleosome and naked DNA[37].

5. ROLEOF CHD1 & CHD2 PROTEINS IN CHROMATIN REMODELING AND GENE REGULATION

Chromo domain helicase DNA-binding protein 1/2 which are the members of the SNF2-like family of helicase-related enzymes, expressed ubiquitously in all types of tissues in humans with the highest expression in adult tissue in the thyroid, ovary, lung, and cerebellar hemisphere of the brain [13]. CHD is a chromatin-remodeling enzyme that modulates chromatin structure and is also involved in cell cycle regulation, cell differentiation, and development [38]. Generally, non-covalent changes in histone: DNA interaction is catalyzed by chromatin remodeling enzymes, which in turn alters the structure of nucleosome [7]. As discussed earlier the functional domains of subfamily 1 include 2 chromodomains at the N-terminus, an ATPase/helicase core domain, and a DNA-binding domain.

5.1 Chromatin remodeling & regulation of gene expression:-

The core ATPase domain has a DNA-dependent ATPase activity which is poorly stimulated by chromatin without the accessory domains and cannot remodel chromatin alone and other domains of CHD subfamily 1 are necessary for chromatin remodelling. [39]. The DBD of CHD1 doesn't have strong DNA sequence-specific binding characteristics. That's why it comes into contact with extranucleosomal and nucleosomal DNA by DNA binding domain which helps CHD1 to bind broadly throughout the genome with stability. This binding helps to withstand the physical forces that are generated during the movement of nucleosome. The two chromodomains (CDs) at the N-terminus region of CHD2 have an auto-inhibitory role, it decreases the DNA binding and ATPase activity, however, these two domains are necessary for chromatin remodeling. After binding with histones, the auto-inhibitory activity is omitted from the CDs [22]. During transcription, CHD1 and CHD2 were recruited directly at the transcription start site (TSS) along with transcription machinery. In the active region, chromatin architecture is regulated by these two proteins. CHD1 and 2, disassemble nucleosomes which increases promoter accessibility. CHD1 & CHD2 have overlapped site recognition regions. Despite this overlapped site-specificity they often co-localize and these proteins have different affinities at those overlapping sites [40]. CHD2 shows high-affinity binding, which is supported by the c-terminal DNA-binding domain and partly directed by H3K4 methylation [22].

5.2 Histone marker specific recognition:-

CHD1 and 2 both recognizes active promoter with H3K4me2/me3 marker, enhancer with H3K4me1/me2 markers, and bivalent chromatin regions of the poised promoter with both active H3K4me3 and repressive H3K27me3 histone markers. Developmentally regulated genes in stem cells mainly contain this bivalent property [41][22].In vitro, the dual chromodomains of CHD1 in mammals specifically recognize H3K4me3 nucleosomes and tail peptides [42][29].In developmentally regulated genes which have poised promoters with bivalent histone markers recruit CHD2. CHD2 then interacts with the transcription factors coupled to RNA polymerase and replace histone H3 with H3.3 which creates a more permissive chromatin state and allows gene expression by activating gene transcription of promoter and enhancer[41].

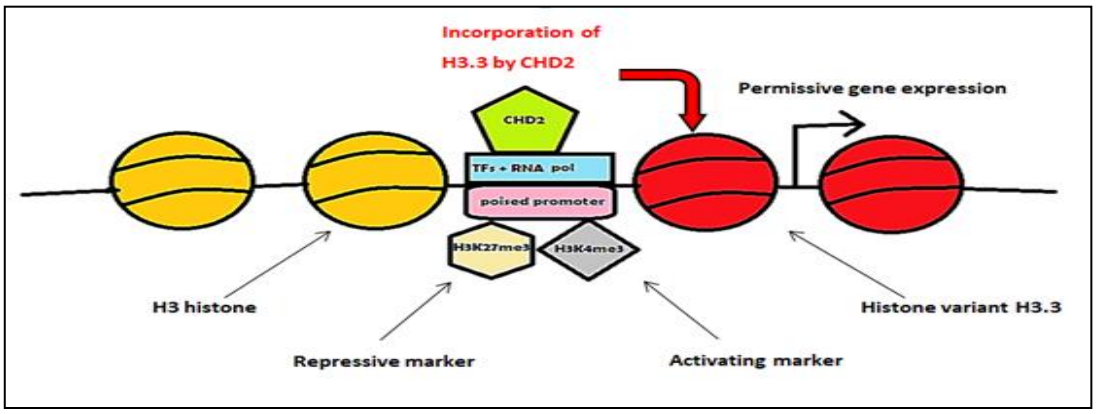

Fig5. CHD2 is recruited to poised promoters which has bivalent histone modifications, interacts with transcription factors (OCT3/4, NKX2-1, and MyoD) and remodels chromatin by incorporating H3.3 that creates permissive chromatin state whereby transcription can occur.

5.3 Interaction with histone variants:-

The role in chromatin remodeling of CHD subfamily 1proteins was first identified in muscle cell differentiation by Co-immunoprecipitation (co-IP) experiment in C2C12 cells, which is a mouse skeletal muscle cell line. It was found that CHD2 with the help of its two chromodomains interacts with H3.3, the most common non- centromeric variant of histone H3 that varies from the canonical H3 by just 4 to 5 amino acids. While the mutant form of CHD2 that has no chromodomain regions, does not interact with H3.3 [43]. CHD1 also regulates deposition of H3.3 in nucleosome similar to CHD2[44].The incorporation of histone variants into chromatin, modulates chromatin dynamics. The replacement of H3 by H3.3 does not alter the overall structure and stability of the nucleosome but adjusts higher-ordered chromatin folding, which changes it to an open chromatin conformation[45][46]. Variant H3.3 maintains the balance of open state and condensed state chromatin conformation. Loss of H3.3, forms over-condensed and miss -segregated chromosomes in the two-cell stage, with correspondingly high levels of aneuploidy [47]. On the other hand, CHD1 not only incorporate H3.3but also interacts with H2A.Z, which is a variant of canonical H2A. H2A.Z is recruited at the open promoter region of active gene by Chromatin Remodeling Complex Swr1. This recruitment inhibits RNAPII activation and pause gene transcription. H3K4me2 is recognised by Chd1 remodeler, and CHD1 disassembles the H2A.Z-containing nucleosome at the promoter. This in turn activates the gene again [48].

5.4 Interaction with other transcription factors:-

In mammals, CHD1is recruited at the 5' end of the active genes. During pre-initiation complex formation CHD1 is recruited to the active promoter. This recruitment is dependent on a subunit of mediator complex. CHD1 also interacts with Transcription factor II Human (TFIIH) and with subunits of the polymerase-associated factor1 (PAF1) which in turn activates activator-dependent transcription initiation with elongation[49].Therefore, CHD1 interacts with multiple factors that are associated with transcriptional activation and plays an important role in gene expression in mammals[50].It was experimentally observed that in mouse embryonic stem cells, CHD2 interacts with the transcription factor OCT3/4 and in human cortical interneurons; CHD2 interacts with homeobox protein Nkx2-1 (NKX2-1) which is also a transcription factor. NKX2-1 regulates genes that have a role in the development of the brain, lungs, and thyroid gland. CHD2 along with NKX2-1 binds and induces the expression of 3 specific genes PAX2, ZIC1, and SPRY1 which are important for interneuron development. Moreover, in CHD2 mutated cells overexpressed NKX2-1 is not sufficient to induce the expression of those 3 target genes. Thus the role of CHD2 is necessary for the expression of neuro-developmentally regulated genes [41]CHD2 regulates different differentiation programs such as muscle cell differentiation.CHD2 interacts with MyoD, a muscle-specific transcription factor, and together bind with the promoter of the myogenic gene. Upon interaction with the promoter, CHD2 facilitates H3.3 deposition at myogenic loci and

promotes differentiation of myoblasts into muscle cells. While loss of CHD2 halts the myotube formation as well as decreases myogenic gene expression. [43].

6. CHD1 ASSOCIATED PATHOLOGY

CHD1 was initially discovered as a DNA-binding protein which regulates the gene expression by altering the chromatin structure, and is considered as the founding member of CHD family [31][15]. Recently it has been shown that subfamily 1 CHD proteins have distinct impact on cell proliferation, DNA damage repair and pluripotency. Mutations in most of the genes encoding CHD proteins have been demonstrated to lead a variety of cancerous and developmental diseases. The first demonstration for CHD family proteins playing a functional role in pathogenicity was in CHD7 in patients with CHARGE syndrome [51].and the first demonstration of cancerous role was when the subfamily 2 member CHD5 was identified as tumour suppressor which is frequently deleted in a range of cancer[52]. Recent discoveries have proved that, like CHD5/7, CHD1 &2 are also muted or inactivated in various cancers and other diseases which we will discuss here

6.1 Estrogen Receptor Positive Breast Cancer:-

The most common malignant disease in woman is breast cancer and 70% of human breast cancer is ER+ breast cancer, which is as the name suggests, caused by Estrogen and its receptor (ERα and ERβ).Estrogen is a steroid hormone that regulates the growth and proliferation of breast tissue by activating the Estrogen receptors, nuclear transcription factors and gene expression. Estrogen signalling plays an important role in this ER+ breast cancer[53]. Here we will discuss how chd1 is linked with ER+ breast cancer. To understand this, at first microRNAs (miRNAs) should be understood well. Unlike mRNAs, miRNAs are noncoding RNAs consists of 20-25nucleotides that degrades mRNA as well as inhibits translation by base pairing with mRNA at 3' untranslated region. This is reported that miRNA has both oncogenic and tumour suppressor activities. MicroRNAs play important roles in controlling breast cancer. It has been reported that, miR26a and miR26b, micro RNAs of miR26 family, are down regulated in breast cancer and therefore these are known as tumour suppressor miRNAs. MicroRNA26a expression is reduced when Estrogen is added [54][55].From the results of functional screening it was suggested that CHD1, GREB1 & KPNA2 are the three genes that are involved in Estrogen promoted cell proliferation and are also targeted by miR26a. Experimentally it was observed that the expression of miR26a/b suppress cell growth in human breast cancer by different pathways, among of which, one is degradation of CHD1 transcript. It has been proved that CHD1 is responsible for estrogen-stimulated proliferation of breast cancer cells and depletion of CHD1 promotes cell growth inhibition. Thus CHD1 is oncogenic at least in the context of ER+ breast cancer.

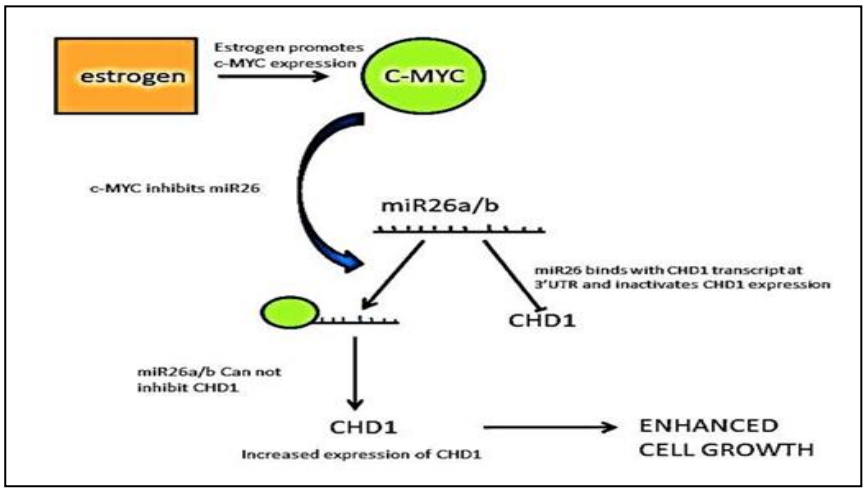

Fig6. Pathway of CHD1 expression regulation in ER+ breast cancer cells

c-MYC is an estrogen responsive gene and has been reported to suppress microRNAs including miR-26a/b. Unregulated c-MYC and CHD1 expressions were observed in human breast cancer. From this above reports Sheng Tan and his colleagues identified a pathway by which estrogen suppresses miR26a by utilizing c-MYC and promote CHD1 expression in ER+ breast cancer cells [56].

6.2 Prostate cancer:-

The most common cancer and the second leading cause of cancer death among men in the U.S.Ais prostate cancer. At the very first stage the tumour growth is very slow and as the time passes it becomes highly aggressive. Patients with localized prostate cancer have urinary and sexual dysfunction. It has been reported that in 13% to 26% of prostate cancers, have a deleted 5q21 chromosomal band. The deleted 1.3mb part of that band contains RGMB & CHD1 gene. Homozygous alteration or deletion of CHD1 is the second most common genetic event in prostate cancer after PTEN deletion [57].CHD1 is recruited to chromatin upon double strand break induction and is required for repositioning or ejection of nucleosome adjacent to the double strand break site. During double strand break CHD1is recruited to nucleosome and makes an open complex by remodelling it. This exposed DNA of the open complex serves as a substrate for CtIP (CtBP Interacting Proteins)- dependent DNA end resection and formation of single stranded DNA occurs. These newly formed single stranded DNA then coated with replication protein A (RPA) which prevents the formation of secondary structure. These steps are required for subsequent homologous recombination events. But these steps are not required for non- homologous end joining(NHEJ) because this process don't need end resection and this makes NHEJ a CHD1 independent process. Depletion of CHD1 reduces the recruitment of CtIP to the chromatin upon double strand break induction. Thus loss of CHD1 decreases the error free homologous recombination mediated double strand break repair process and increases the error prone NHEJ mediated DSB repair. That's why DNA became unstable and these DNA repair defects are discovered in subset of prostate cancer [58][59]. CHD1 and androgen receptors are required for AR-dependent transcription activation for those genes that are androgen responsive in prostate cancer. There are several AR- responsive tumour suppressor genes, like – NKX3-1, FOXO1 & PPARγ which are present downstream of CHD1 and are regulated by this CHD1 gene by positive control. Thus CHD1 acts as a tumour suppressor in prostate cancer [57].

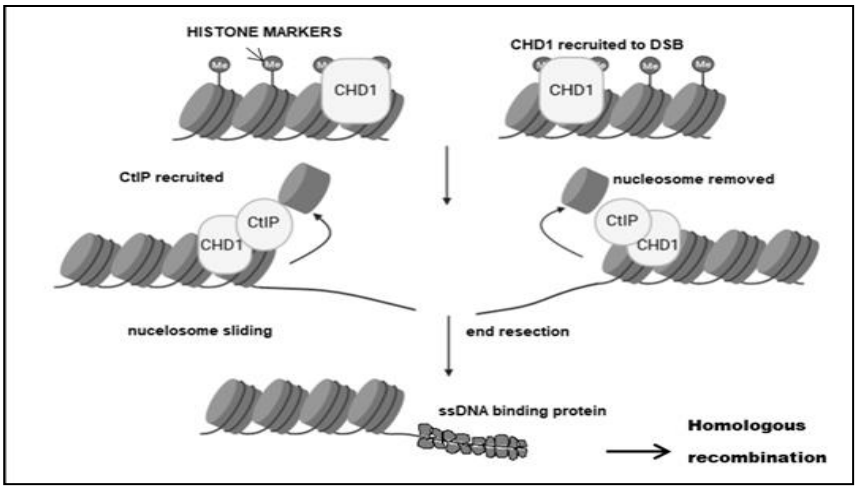

Fig.7Function of CHD1 in HR repair pathway (histone markers are not drawn from second step)

7. CHD2 ASSOCIATED PATHOLOGY

CHD2 gene is located on the 15q26.1, which is associated with different developmental disorders in human. CHD2 is recruited to developmental genes via transcription factors, histone markers and RNA polymerase where it remodels chromatin in a permissive state where it facilitates transcription and promotes differentiation. CHD2 regulate the expression of developmental genes. It has been observed that ZIC1, SPRY1 & PAX2 (neuronal development genes) expressions are reduced when CHD2 is depleted. This proves that CHD2 regulates these genes by binding with their promoters. In inhibitory neuron

models CHD2 knockdown reduces the expression of genes that are responsible for new neuron formation & synaptic transmission [60].In mouse neuronal progenitor cells, ChIP sequencing revealed that CHD2 binds with RE1-silencing transcription factor (REST) which is a transcriptional repressor and suppress neuronal genes in non-neuronal tissues. REST binds with neuron restrictive silencer element and supresses its transcription. REST are found in stem cells which are regulated by many signalling pathways. In non-neural cells, REST binds to the NRSE/RE1 domain and represses the expression of neural-specific target genes thus. High level of REST is found in non-neural cells and low level of REST is found in post natal neuron which causes the expression of neuron specific target genes. Inactivation or down regulation of this REST gene induces abnormal neurogenesis. REST is positively correlated with CHD2 expression. It was observed that REST expression was upregulated CHD2 was overexpressed .This regulation was mediated by CHD2 by binding to the genomic region of REST. Thus CHD2 plays an important role in neurogenesis and haploinsufficiency of CHD2 causes seizures [61][62]. 11 out of 17 mutated CHD2 bearing patients developed multiple seizure types. These seizure types were: 9 patients with GTCS, 3 patients with myoclonic seizure, 3 with focal seizure and 2 patients with epileptic spasms[48].CHD2 along with NKX2-1 transcription factor controls the GABAergic inhibitory neuron development which are identified as the main inhibitory neurons of the central nervous system [60].

8. CONCLUSION

CHD subfamily 1 proteins have been found to regulate the chromatin architecture of various important genes, some of which are important for development, some of them are important for tumour suppression & some are for tumour formation. Although we have progressed a lot, to reveal the role of CHD subfamily 1 in cancer, epilepsy and other developmental diseases, much is still unknown about the molecular functions and target genes of this chromatin remodeler family. Improved animal models will be necessary to understand the mechanism of CHD 1and CHD2 mutation and depletion on different cellular processes. A comprehensive understanding of subfamily 1 regulation in oncogenes, neuronal development and brain function will be able to provide the information about a vast range of phenotypes observed in patients with mutant CHD1/2 genes. In near future, full knock out models will be able to rule out possible gain/loss of functions and dominant negative effects, in which appropriate controls will be playing an important role. Members of subfamily 1 have tumour suppressive characteristics in some contexts, as well as oncogenic properties in others. More understanding about these proteins will be used as cures and will also to predict patient survival in easy and protruding way. Induced Pluripotent Stem Cell (iPSC) based disease models are extremely prominent, will be able to model any given patient mutation in upcoming future, which will help to understand effects and specificity of these proteins easily and precisely.In addition, gene target network analysis, next generation sequencing techniques and analysis tools are the emerging. These in-silico methods will also provide important information about CHD1/2 mediated transcriptional control. These information will help to discover novel treatment options for patients with mutated CHD1/2 proteins[63][64].

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