CHROMATIN AND ITS RELATION TO THE DEVELOPMENT OF OVARIAN CANCER

Abstract

There are modifications in the structure of chromatin that occur as result of chromatin remodeling. These modifications or changes contribute to gene regulation. In the presence of aberrant chromatin remodeling, cells are predisposed to carcinogenesis. Data regarding chromatin remodeling have been able to prove the impact of chromatin remodeling in cancer beyond every doubt, and the occurrence of the phenomenon has brought about the development of new proteins that attack the carcinogenic processes. This review gives a brief overview of the chromatin and its relation to the development of ovarian cancer**.**

Introduction

As observed in eukaryotic cells, there is a typical tight packaging of the genomic DNA into nucleoprotein complex (Moreno *et* al., 2015, pp. 3370–3378) known as the chromatin which is typically described as an aggregation of macromolecules in which elements such as the DNA, RNA, and protein are found. There are two forms in which the Chromatin exists: heterochromatin and euchromatin. This chromatin is known to be involved in important activities such as the regulation of every process relating to genomic DNA (Zhou and Bai, 2019). This packing of the genomic DNA into the chromatin is essential to ensure the unpacked genomic DNA content, which is approximately 2m long can fit perfectly into a small nucleus (only a few microns in diameter, typically 10micrometer). The chromatin comprises nucleosomes which are units containing about 146 base-pairs of DNA that is wrapped 1.65 times around a histone octamer (Fierz and Poirier, 2019, pp. 321-345). Structurally, a chromatin is made up of between 10^3 and 10^6 units of nucleosomes and the nucleosomes are connected by approximately 20-100bp of what is known as linker DNA, causing the structure to form an array. However, this occurrence depends on the organism. The arrays formed by the nucleosomes can become tetranucleosome units which are higher level/order structures. Additionally, the nucleosome arrays can build up to form fibers that exist in several different structures (Luger *et al.,* 2012, pp. 436-447).

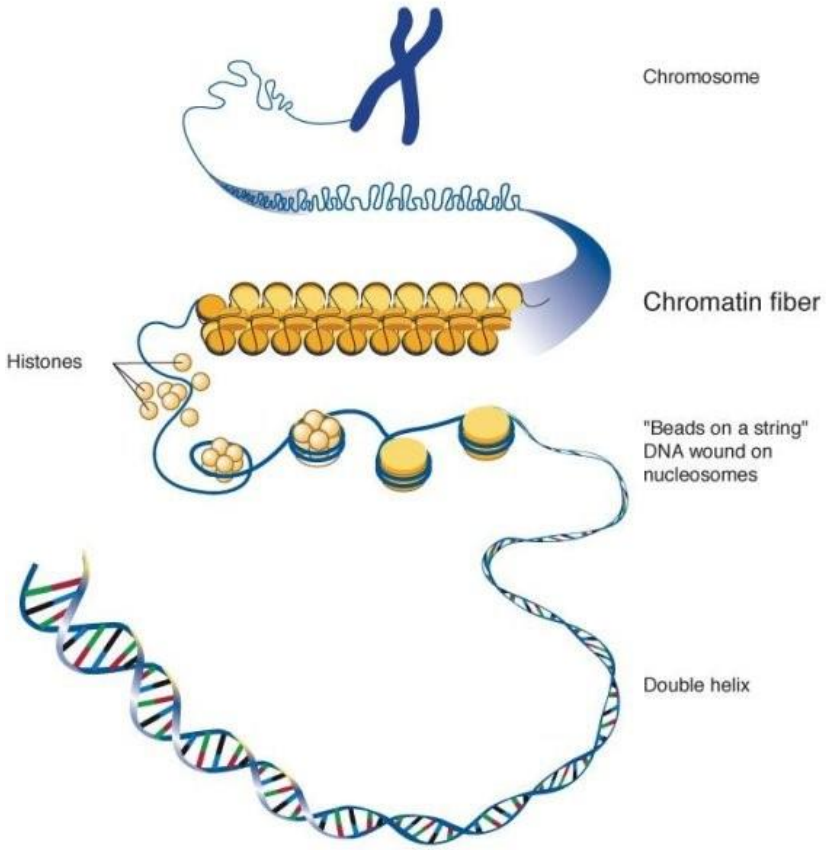


Fig 1. Structure of chromatin has illustrated by the National Human Genome Research Institute

When further analyzed on larger measures, they are observed to form expansive loops known to arbor gene clusters (Dekker and Heard, 2015, pp. 2877–2884). Having an informed knowledge of the structure of chromatins, including both the atomic and genomic structures, as well as the molecular details of the plethora of regulatory protein complexes that are involved provides proper understanding of genome function (Luger *et al.*, 2012, pp. 436-447). The core histones of the chromatin structure have been implicated in many functions through different categories of post-translational modifications (PTMs) (Allis and Jenuwein, 2016, pp. 487–500). Furthermore, these PTMs also occur to a great extent in the linker histones. While there are functions known about linker histones, it remains unclear what their role is in the regulation of chromatin function and structure.

For a long time, there exist a confusion between chromosomes, chromatid and chromatin because all three of them contain DNA and proteins, however, each of the structures are distinct from each other. As it has been established earlier, the chromatin is made up of DNA and histones packed into tiny fibers. The chromatin is usually further condensed, forming the chromosome. As a result, the chromatin appears as a lower level of DNA organization. On the contrary, chromosomes are higher level of DNA organization. In describing chromosomes, they are a group of condensed chromatins, however single-stranded. The chromosomes typically undergo the process of replication during cell division, this they do in order to preserve the number of chromosomes in

the mother cells to the new daughter cells. While the chromosome is single-stranded, when they are duplicated, they become double-stranded, forming the popular X-shaped structure. The two new strands upon replication are usually identical and intercept at the centromere. The last of the three is the chromatid which is one of the two strands of the X-shaped chromosome. There is a term "sister chromatids" which is used to describe chromatids that are connected by a centromere. These sister chromatids undergo separation during cell division and become what is known as daughter chromosomes in the newly formed daughter cells (Cell news, 2017).

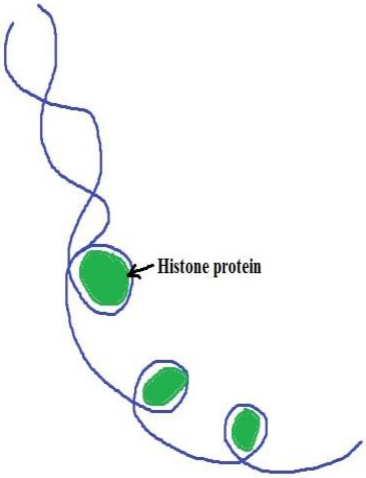


Fig 2A. Chromatin

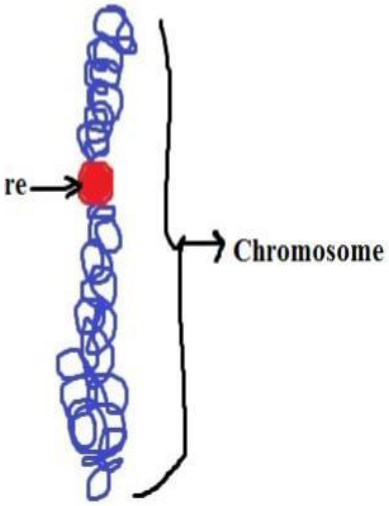


Fig 2B. Chromosome.

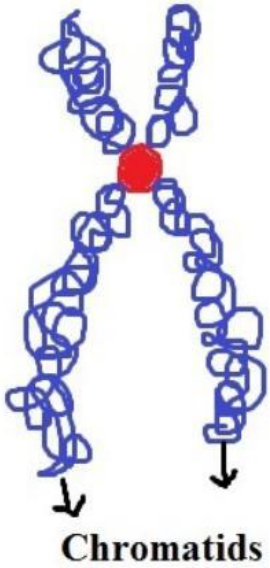


Fig 2C. Chromatids.

Biological Importance of the Chromatin

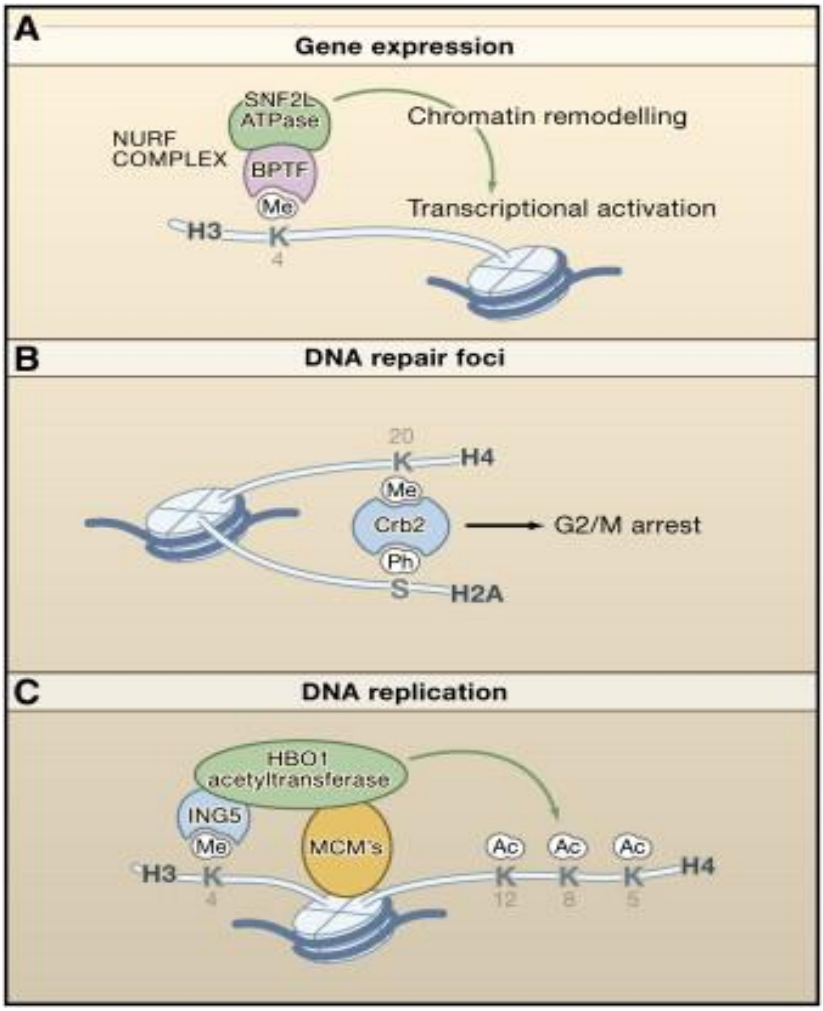
DNA Packaging

One of the most important and essential function of the chromatin is to condense the long strands of the DNA into the nucleus. This is because the length of a complete DNA far exceeds the size of the nucleus where is located (Cell news, 2017). The term "Packing ratio" defines the extent to which the DNA is packed together. As described earlier, in order to achieve the best possible packaging ration, the DNA is not directly packed into the chromatin, there are different levels of packaging involved. First on that list is typically achieved when the DNA is wound round the nucleosome. This level of packaging produces a packaging ratio of approximately 6. The packaging continues by the "wrapping of beads" observed during the interphase stage of the chromatin as well as in mitotic chromosomes. When this packaging level is achieved, the structure arrives at packing ratio (PR) of approximately 40. The fiber is finally arranged in loops, scaffolds

and domains, attaining a PR of around 1,000 in the interphase stage of the chromatin while that of the mitotic chromosomes reaches up to 10,000 packaging ratios.

Transcription Regulation

During the process of transcription, the genetic information that is stored in the DNA is read by proteins. The information is then transcribed into RNA which will end up being translated into proteins. In situations where the chromatin becomes too tight and as a result blocks access to the read proteins, this will block the process of transcription from happening. As it was mentioned earlier that there are two forms of chromatin, however, only one, the Euchromatin can carry out transcription.



The other form, the heterochromatin, is packed too tightly and as a result does not allow for the DNA to be read by proteins. There are other occurrences that can also hinder the process of transcription. These include fluctuations between open and closed chromatin. These fluctuations can stop transcription, or because what is known as transcriptional bursting. While this is so, there is a possibility of other factors coming to play. Some of these proposed factors include the association and dissociation of transcription factor complexes with chromatin. (Cell news, 2017).

Fig 3. Functional consequences of histone modification.

Source: *Tony Kouzarides, 2007.*

Chromatin and DNA Repair

When DNA is packaged into chromatin, it predisposes every single DNA-based processed in the cell to different barriers. The high dynamic arrangement of proteins and DNA can cause modifications to the structure and shape of the chromatin. When there is a damage to the DNA, there is chromatin at that damage site usually relaxes and this relaxation gives room for the proteins involved in repair to bind to the DNA and repair it (Cell news, 2017).

Chromatin and Histone Modification

Modifications to the structure of the chromatin relies greatly on modifying the histone residues in the structure through the addition of phosphate and acetyl chemical groups. Additionally, there could be an addition of methyl chemical groups. When the structures of histone residues are altered or modified, it can predispose important interactions such as the nucleosome-DNA or nucleosome-nucleosome interactions to changes: it could either open or condense the structure of the chromatin. A typical example of this is H4K16ac which causes a reduction in the interaction between the H4 tail and the H2A acidic pockets in the structure. These changes in the interaction goes further to hinder the higher-order nucleosomal folding, thereby making the chromatin structure take up an open conformation (Robinson *et al.*, 2008, pp. 816–825). Moreover, there are some histone modifications that can occur when there is a damage to the DNA and this kind of modification can cause changes to the interactions between the chromatin and non-histone proteins, helping to recruit repair factors and as a result contributing to the initiation and subsequent termination of checkpoint (House *et al.*, 2014, pp. 1-18). There are many different sites in the structure of histones in which modifications can occur. According to findings, there are more than 60 different residues on histones in which there have been modifications. While this is the case, it still doesn't accurately represent the number and extent of modifications that can occur on histones (House *et al.*, 2014, pp. 1-18).

Table 1 Categories of Modifications that have been identified on Histones

Chromatin Modifications	**Residues Modified**	**Functions Regulated**
Acetylation	**K**-ac	Transcription, Repair, Replication, Condensation
Methylation (lysines)	**K**-me1 **K**-me2 **K**-me3	Transcription, Repair
Methylation (arginines)	**R**-me1 **R**-me2a **R**-me2s	Transcription
Phosphorylation	**S**-ph **T**-ph	Transcription, Repair, Condensation
Ubiquitylation	**K**-ub	Transcription, Repair
Sumoylation	**K**-su	Transcription
ADP ribosylation	**E**-ar	Transcription
Deimination	**R > Cit**	Transcription
Proline Isomerization	**P-cis > P-trans**	Transcription

Adapted from Tony Kouzarides. 2007.

Majority of the identified modifications have been reported as dynamic modifications and there are enzymes which have been identified to act by removing these modifications. However, there is an exception to this which is the methylation of arginines (Tony Kouzarides, 2007, pp. 693-705). While these modifications have been regarded as dynamic, there is still no demethylating activity detected, rather, it is a deamination process which has been shown to associate with removal of methyl-arginines. This ultimately showed that the process of deimination can work against the methylation of arginine. Furthermore, there exists no enzyme responsible for the conversion of peptidyl citrulline to arginine, however, the possibility of this exist based on evidence, on the basis of the short-lived form of citrulline on promoters. In another case, the isomerization of proline can be reversed because majority of isomerases are known to have inherent ability to serve as catalysts for the formation of *cis*- and *trans*-proline.

There are many enzymes responsible for the modification of histones. Among them, the methyltransferases and kinases remain the group with the highest specificity. This probably explains why the most characterized modification is methylation. On the contrary, phosphorylation of histones has not been characterized as much there is the need for activation of discrete signaling pathways in order to take note of occurring modifications. There are some situations where other factors affect the specificity of enzymes involved in histone modification. These factors include the possibility of preferential specification of nucleosomal versus free histones by the complexes in which these enzymes are located Furthermore, proteins that work with the enzyme may influence the histone residue to modify (Metzger *et al., 2005*, pp. 436-439).

Functions of the Mechanisms Involved in Histone Modification

Two functional mechanisms of histone modifications are well described. First on the list is the "unraveling" of chromatin by disrupting nucleosome connections, and the second one involves non-histone proteins. The second function has received the greatest attention to date. A group of proteins is urged to bind or blocked from chromatin based on the makeup of modifications on a certain histone. Enzymatic actions (e.g., remodeling ATPases) are carried by these proteins, which further change chromatin. The processes governed by changes (transcription, replication, and repair) entail multiple phases, as such it necessitates the recruitment of an ordered set of enzyme activity (Tony Kouzarides, 2007, pp. 693-705). Each of these phases could necessitate a new form of chromatin-remodeling activity, as well as a different changes to attract them.

The higher-order structure of chromatins may be influenced by modifications through modifying how histones interact with DNA or histone contacts in nearby nucleosomes. Lysine is basically charged; however, it is neutralized by the modification, and this makes acetylation to best unfold chromatin of all the known changes. Although this function is difficult to monitor in vivo, biophysical study suggests that internucleosomal interactions are critical for stabilizing chromatin higher-order structure. As a result, any change in the charge of histone will very certainly have structural implications for chromatin physical form. Furthermore, thanks to the recent development

of ways that helps to make recombinant nucleosomes undergo modifications at specific places, this question can now be answered in vitro.

Another modification that may have substantial implications for chromatin compaction via charge changes is phosphorylation. Although the involvement of this alteration in mitosis, apoptosis, and gametogenesis has not been proven rigorously in vitro, evidence of its role in these processes suggests that it does (Krishnamoorthy *et al.*, 2006, pp. 2580-2592).

There are several proteins that are attracted to specific changes have been found. The discovery of numerous proteins able to identify H3K4me has revealed that their function is to bind enzymatic activity to chromatin. A PHD domain in BPTF which is found in the NURF chromatin-remodeling complex, binds H3K4me3. After cells are exposed to DNA-damaging chemicals, the PHD-finger protein ING2 tethers the restrictive mSin3a-HDAC1 histone deacetylases complex to highly active genes which have specific patterns of proliferation (Pena *et al.*, 2006, pp. 100-103). This discovery reveals a novel mechanism for actively shutting from the H3K4-methylated genes that are heavily transcribed.

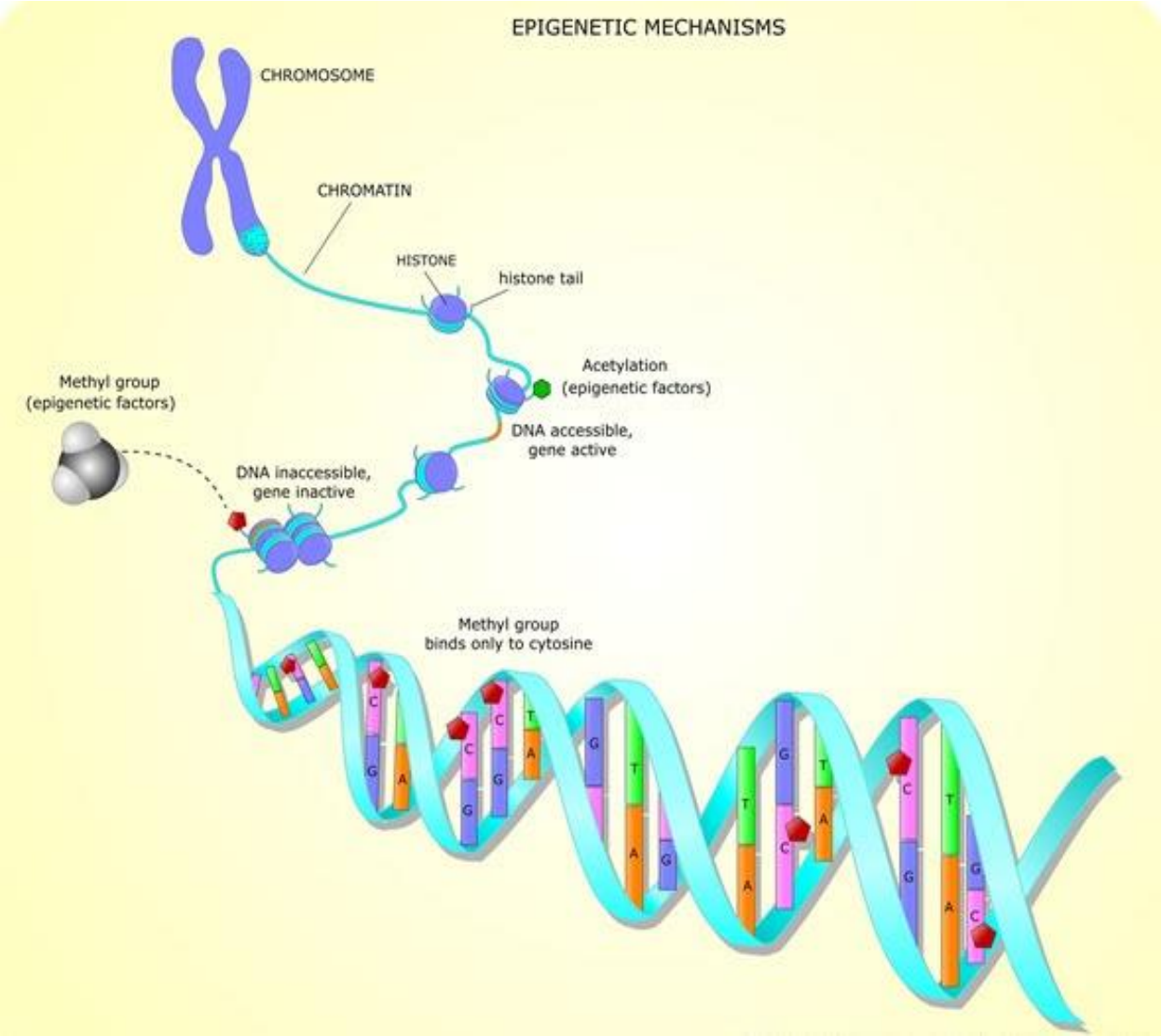


Fig 4. Histone modification

Source: news-medical.net

Chromatin Remodeling and Cancer

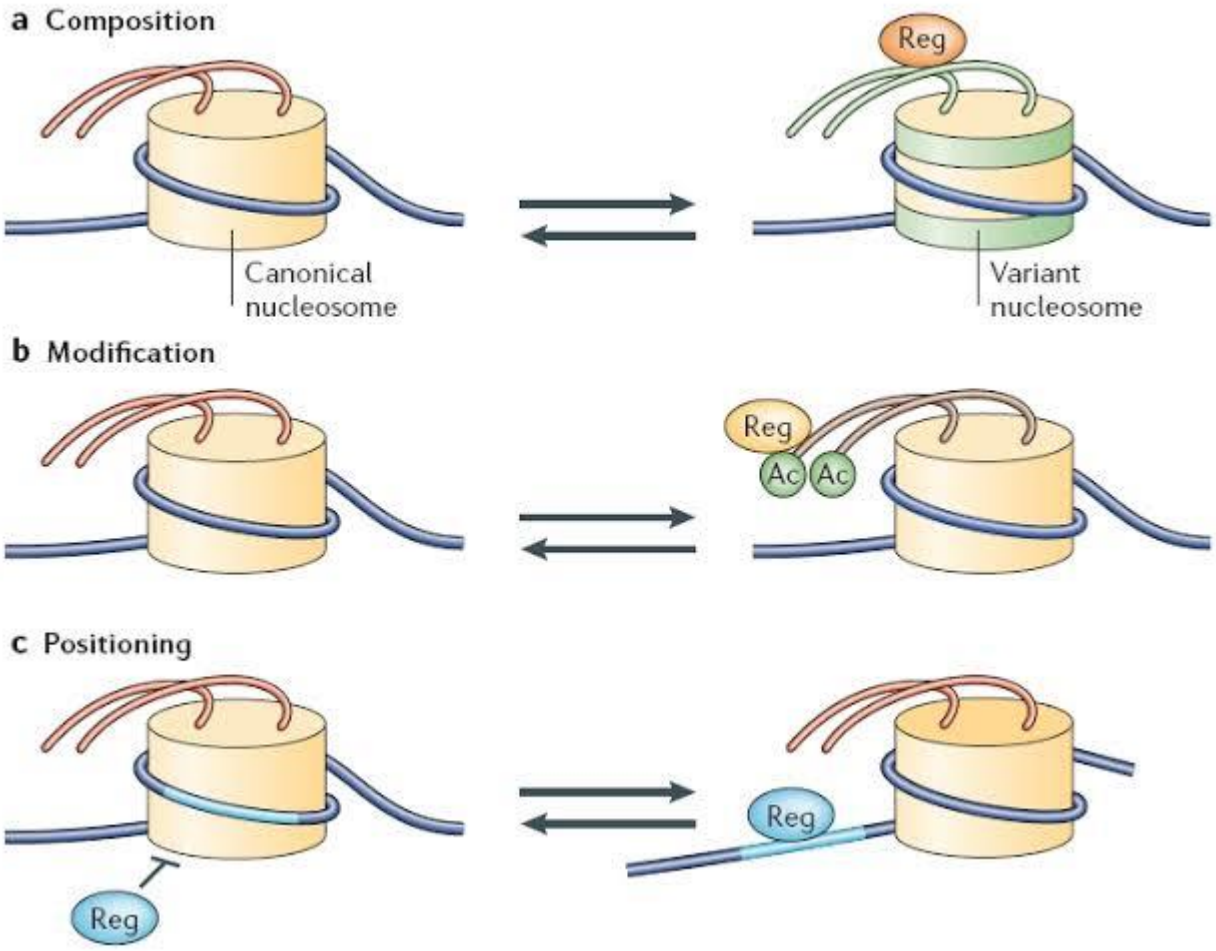


Fig 5. Chromatin Remodeling

Source: nature.com

The dynamic change of chromatin architecture to give condensed genomic DNA access to proteins involved in regulatory transcription, and hence govern gene expression, is known as chromatin remodeling (Teif and Rippe, 2009, pp. 5641–5655). Covalent histone modifications by specific enzymes, and ATP-dependent chromatin remodeling complexes, which either move, expel, or restructure nucleosomes, are the two main mechanisms for chromatin remodeling. Nucleosomes are intrinsically repressive due to their close contacts, as they oppose most other DNA-binding proteins by restricting access and modifying the shape of the DNA duplex (Nodelman and Bowman, 2021, pp. 73-93). Additionally, the histone core is extensively employed for storing epigenetic information via PTMs which is crucial for supplying short- and long-term memory which ultimately facilitates the differentiation of allows cells and their subsequent response to the

environment. Chromatin remodelers are required by nucleosomes in order to dynamically change their locations and composition.

ATP-driven chromatin remodelers are critical gatekeepers for maintaining and driving changes in the epigenetic environment. Chromatin remodelers are essential for maintaining normal physiology of cells and cellular identity determination, as evidenced by their participation in a variety of developmental diseases and malignancies.

The SWItch/sucrose non-fermentable (SWI/SNF) complex, which has numerous subunits, including ARID1A and BRG1 is one type of chromatin remodeling complex (Takeda *et al.,* 2016, pp. 607–613). Chromatin remodeling complexes modify chromatin shape by interacting with subunits, and thus regulates gene expression levels via regulating the interaction of double-stranded DNA-containing proteins. Adenosine triphosphate (ATP)-dependent complexes controlling histone-DNA interaction may be used to achieve this shift in accessibility. Intracellular communication is also linked to epigenetics (Huang *et al.,* 2014, pp. 73-88). Epigenetic alterations may cause carcinogenesis, developmental problems, and multifactorial disease since these are critical events in cell proliferation.

A n exclusive examination of genome sequences found changes in genes that codes for remodeling factors in a plethora of human cancers, including those for the SWI/SNF complex, leading to the hypothesis that SWI/SNF complexes are protective of cancer (Oike *et al*., 2013, pp. 849–855). Mutations in SMRCA4/BRG1 have been found in more than 30% of "non-small cell lung cancer (NSCLC)" patients. In ovarian cancer, mutations in the ARID1A gene have been found in 46–57 percent of clear cell carcinoma (Jones *et al.*, 2010, pp. 228–231). Hepatocellular carcinoma (HCC), gastrointestinal adenocarcinoma, and malignant melanoma all have been found to have ARID1A mutations. In serous endometrial cancer, the chromodomain helicase DNA-binding protein 4 (CHD4), which forms the NuRD complex, is overexpressed or mutated, and an overexpression of metastasis-associated protein 1 has been found in breast cancer (Mayes *et al*., 2014, pp. 183–233).

Aberrant chromatin remodeling and ovarian cancer

As a result of the process of cell division being delayed, the "ovarian clear cell carcinoma (OCCC)" appears as a chemoresistant malignancy. The adenofibroma-carcinoma and endometriosis-carcinoma sequences, respectively, are two cancer-causing pathways found in OCCC (Nishikimi *et al*., 2015, pp. 866-871). Although both pathways have genetic underpinnings that are not known, it has been postulated that the ARID1A mutation contributes to the development of OCCC through the endometriosis-carcinoma sequence as opposed to the adenofibroma-carcinoma sequence (Nishikimi *et al.*, 2015, pp. 866–871). Jones and colleagues (2010) discovered ARID1A mutations in 57% OCCC patients, concluding that ARID1A gene is a tumor suppressor which facilitates the inactivation of gene products via the abnormal chromatin remodeling linked with the carcinogenesis of OCCC. ARID1A is a member of the SWI/SNF complex, involved in the regulation of processes such as cell growth, cell cycle regulation, and cell division, and DNA repair (Weissman and Knudsen, 2009, pp. 8223–8230).

In 119 individuals experiencing OCCC, Wiegand et al (2010) discovered that 55 of them had ARID1A mutation. A shortage of BAF250a (encoded by ARID1A) was also observed in 36% of

them. BAF250a confers precision to the SWI/SNF complex and facilitates gene expression regulation. Moreover, ARID1A mutations and BAF250a deficiencies were found in OCCC and neighboring infiltrative tumors but not in faraway lesions. This implies that this mutation and the

resulting BAF250a deficit occur early in endometriosis neoplastic transition (Takeda et al, 2016, pp. 607–613). A prior investigation demonstrated that an ARID1A deficiency, alongside the activation of AKT protein and a histone H2A variation (H2AX), was an early phenomenon in

endometriosis-associated ovarian cancer (EAOC) and infiltrative ovarian cysts (Itamochi et al, 2015, pp. 967–973). In individuals who have stage I/II OCCC, an ARID1A deficiency has also been found as a poor prognostic factor, and could be a valuable biological indicator for prognosis

prediction (Wiegand *et al.*, 2010, pp. 1532-1543).

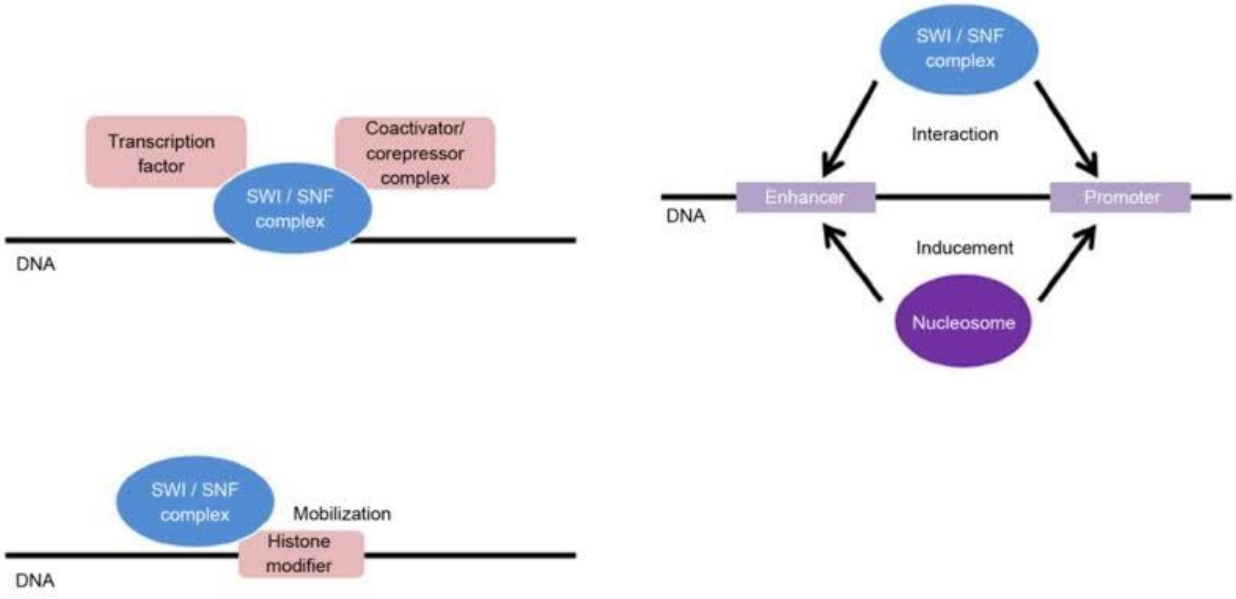


Fig 6. Actions of SWI/SNF complexes on chromatin structure that affect transcriptional regulation.

Source: Okawa *et al.*, 2017

Immunohistochemistry for SMARCA4 expression in lung cancer tumor tissues demonstrated suppression of SMARCA4 in no patients with squamous cell carcinoma, 10% with adenocarcinoma, 31.3 percent with large cell carcinoma, and 36.4 percent with pleomorphic carcinoma (Yoshimoto *et al.*, 2015, pp. 595–602), as well as somatic mutation and deletion of SMARCA4 in these cancers (Weigand *et al.*, 2010, pp. 1532–1543). SMARCA4 is a constituent of the BAF and PBAF complexes, and its mutations and deletion result in incomplete complexes and aberrant subunits, which can lead to gene deregulation and illness (Helming *et al*, 2014, pp. 309–317.).

Therapy targeting aberrant chromatin remodeling

Small molecule inhibitors of the PI3K/AKT signal transduction pathway are extremely sensitive to cancer cells with an ARID1A deficiency. As a result, medicines which block the PI3K/AKT pathway are useful in cancer patients who have an ARID1A deficiency. Therapy aimed at cancer cells' epigenetic regulatory mechanisms is also in the works. Bitler *et al*, (2015) investigated the actions of EZH2 in cancer cells with an ARID1A mutation and discovered that an EZH2 inhibitor specifically suppressed the cellular proliferation with an ARID1A mutant (Bitler *et al.,* 2015, pp.231–238) This indicates that the inactivation of EZH2 could be used to treat cancers that are caused by ARID1A mutations, and EZH2 inhibition has been shown to reduce the number of ARID1A-mutated ovarian tumors in vivo.

An ARID1A in-frame mutation hindered ARID1A trafficking to the cytoplasm, according to Guan *et al*, (2012). The ARID1A protein was subsequently destroyed by the ubiquitin-proteasome system, allowing for the development of cancer. Thus, in cells with an ARID1A mutation, ARID1A degradation might be blocked by targeting the ubiquitin-proteasome pathway, thus restoring the original cancer-fighting activity (Guan *et al.*, 2012, pp. 986–993). As a result, in cancers with ARID1A mutations, ARID1B is indeed a therapeutic target (Helming *et al*., 2014, pp. 251–254.). ARID1A expression detected by immunohistochemistry could be a valuable marker for determining malignancy, prognosis, and therapeutic effectiveness (Nagymanyoki *et al*., 2015, pp. 253–257.).

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