Epigenetic DNA changes and stem cells therapy

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SUMMARY

We are starting to understand the fundamental biological processes which underlie the DNA physical properties. Of particular importance is the field of epigenetics, which literally means 'outside conventional genetics', now used to describe the study of stable alterations in gene expression potential that arise during development and cell proliferation. Epigenetic changes are modifications of DNA, which occur without any alteration in the DNA sequence. Modern directions in the field of epigenetic research aimed to decipher the epigenetic signals that give stem cells their unique ability to self-renew and differentiate into different types. Stem cell-based regenerative medicine holds great promise for repair of diseased tissue. If epigenetic process are reversible, than there is the ability to cure cancer through stem cell therapy.

Key words: *DNA; RNA; Epigenesis, Genetic; Environment; Neoplasms; Antineoplastic Agents; Stem Cells*

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INTRODUCTION

Epigenetics is defined as heritable changes in gene expression that are not accompanied by changes in DNA sequence. Recent analysis of specific epigenetic features of human and mice stem cells have provided important insights into the unique properties of pluripotent and lineage-restricted stem cells. Our rapidly growing understanding of epigenetic processes identifies postsynthetic modification of either the DNA itself or of proteins that intimately associate with DNA as the key mediators (1, 2). These modifications seem to be interpreted by proteins that recognize a particular modification and facilitate the appropriate downstream biological effects.

Many human diseases are caused in whole or in part by environmental factors. It has long been accepted that environmental chemicals can cause many of these diseases through changes in the genome (i.e., genetic effects). Brain damage, cancer, spinal cord injury, heart damage, hematopoiesis, baldness, missing teeth, deafness, diabetes, infertility, et ct. Genome undergoes major epigenetic alterations during mammalian development and embryonic stem cells differentiation. The molecular mechanisms that provide control of these epigenetic events are essential components of stem cell biology, and for establishing and conveying gene expression patterns during cellular differentiation and tumorogenesis as well.

Elucidating epigenetic mechanisms promise to have important implications for advances in stem cell research and nuclear reprogramming and may offer novel targets to combat human diseases, potentially leading to new diagnostic and therapeutic ways in medicine. If epigenetic process are reversible, than there is the ability to cure cancer through stem cell therapy.

Four types of epigenetic pathways have been identified: DNA methylation, histone modification, nucleosome remodeling, and non-coding RNA-mediated pathways. DNA methylation and histone modification are the most studied pathways. Methylation of cytosines within the CpG dinucleotide by DNA methyltransferases is involved in regulating transcription and chromatin structure, controlling the spread of parasitic elements, maintaining genome stability in the face of vast amounts of repetitive DNA, and X chromosome inactivation. Nucleosome remodeling and RNA-mediated pathways will be discussed.

ATP-DEPENDENT NUCLEOSOME REMODELING

The term 'nucleosome remodeling' subsumes a large number of energy-dependent alterations of canonical nucleosome structure, catalyzed by dedicated ATPases in large multiprotein complexes. The importance of these factors for gene regulation and other processes with chromatin substrate has emerged from genetic studies. Mechanistic analyses of nucleosome remodeling by different enzymes provided a diverse, almost confusing phenomenology of ATP-dependent derangement of nucleosomes in vitro, suggesting that different remodeling machines follow different strategies to disrupt histone-DNA interactions (3).

It has been a long-standing challenge to decipher the principles that enable cells to both organize their genomes into compact chromatin and ensure that the genetic information remains accessible to regulatory factors and enzymes within the confines of the nucleus. The discovery of nucleosome remodeling activities that utilize the energy of ATP to render nucleosomal DNA accessible has been a great leap forward. In vitro, these enzymes weaken the tight wrapping of DNA around the histone octamers, thereby facilitating the sliding of histone octamers to neighboring DNA segments, their displacement to unlinked DNA, and the accumulation of patches of accessible DNA on the surface of nucleosomes. It is presumed that the collective action of these enzymes endows chromatin with dynamic properties that govern all nuclear functions dealing with chromatin as a substrate. The diverse set of ATPases that qualify as the molecular motors of the nucleosome remodeling process have a common history and are part of a superfamily. The physiological context of their remodeling action builds on the association with a wide range of other proteins to form distinct complexes for nucleosome remodeling (Figure 1).

RNA-MEDIATED PATHWAYS

Genes can also be turned off by RNA when it is in the form of antisense transcripts, noncoding RNAs, or RNA interference. Chromatin remodeling guided by non-coding RNA contributes mechanistically to the establishment of chromatin structure and to the maintenance of epigenomic memory. Antisense non-coding RNA has been implicated in the silencing

of tumor suppressor genes through epigenetic remodeling events. Studies that have investigated transcriptional activity revealed an unexpected level of complexity in the mammalian transcriptome (4–7).

Chromatin remodeling guided by non-coding RNA (ncRNA) contributes mechanistically to the establishment of chromatin structure and to the maintenance of epigenetic memory (8). Various ncRNAs have been identified as regulators of chromatin structure and gene expression. The widespread occurrence of antisense transcription in eukaryotes emphasizes the prevalence of gene regulation by natural antisense transcripts. Recently, antisense ncRNAs has been implicated in the silencing of tumor suppressor genes through epigenetic remodeling events (Figure 2). Characterization of the antisense RNAs involved in the development or maintenance of oncogenic states may define ncRNAs as early biomarkers for the emergence of cancer, and could have a significant impact on the development of tools for disease diagnosis and treatment.

The RNA-Ago1 complex then targets the nascent promoter-associated RNA, which is transcribed by RNA polymerase II (RNAPII) in the sense direction. Subsequently, the putative silencing complex, which may consist of the PRC2 Polycomb complex (composed of KMT6 [Ezh2], SUZ12 and EED), HDAC1, the DNA methyltransferases Dnmt3A and Dnmt1 and the histone methyltransferases KMT1C (G9a) and/or KMT1A (Suv39h1), is recruited to the promoter. Recruitment of the silencing complex may be mediated through the interaction of Ago1 with Dnmt3a, or directly (H3K9me2 and H3K27me3, respectively) of the nucleosomes proximal to the promoter target site. Histone methylation may be mediated by the candidate lysine methyltransferases KMT6 (for H3K27) and KMT1C (for H3K9) and/or KMT1A (alternative H3K9 methyltransferases are shown in white), leading to the formation of heterochromatin at the target promoter.

EPIGENETIC AND STEM CELL THERAPY

While epigenetic changes are required for normal development and health, they can also be responsible for some disease. Epigenetic alterations are more reversible than genetic events, and there is great potential in the development of "epigenetic therapies".

Disrupting any systems that contribute to epigenetic alterations can cause abnormal activation or silencing genes (9). Such disruptions have been associated with cancer, syndromes involving chromosomal instabilities, and mental retardation (10, 11).

A few examples are given below to illustrate the importance of epigenetic pathways in disease development (12, 13).

Cancer

Aberrant gene function and altered patterns of gene expression are key features of cancer. Growing evidence shows that acquired epigenetic abnormalities participate with genetic alterations to cause this dysregulation. Epigenetic alterations participate in the earliest stages of neoplasia, including stem-precursor cell contributions, and discuss the growing implications of these advances for strategies to control cancer. Since epigenetic silencing processes are heritable, they can play the same roles and undergo the same selective processes as genetic alteration in the development of a cancer. Epigenetic changes have been observed in virtually every step of tumor development and progression.

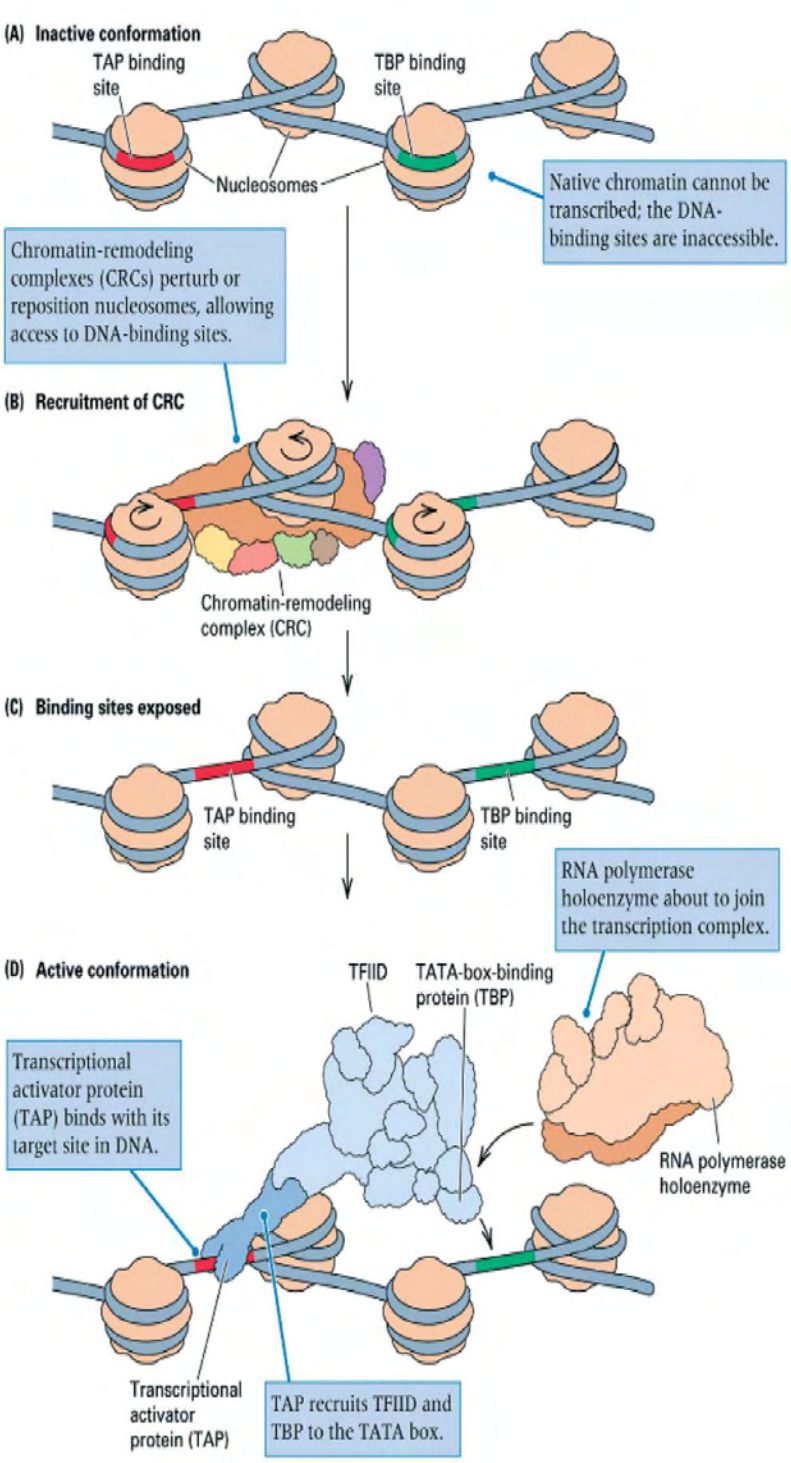

Figure 1. Function of chromatin-remodeling complexes. (A) Native chromatin may conceal key DNA-binding sites. (B) A chromatin-remodeling complex either repositions the nucleosomes along the DNA or chemically modifies the histones. (C) DNA-binding sites become accessible. (D) The transcription complex is recruited to the site. (From: Genetics: Analysis of Genes and Genomes, 6th Edition, Hartl, Jones 2005. Jones and Bartlett Publishers).

Hypermethylation of CpG islands can cause tumors by shutting off tumor-suppressor genes. In fact, these types of changes may be more common in human cancer than DNA sequence mutations (4). Too little DNA methylation (hypomethylation) is believed to initiate chromosome instability and activate oncogenes (14). A malignant cell can have 20–60% less

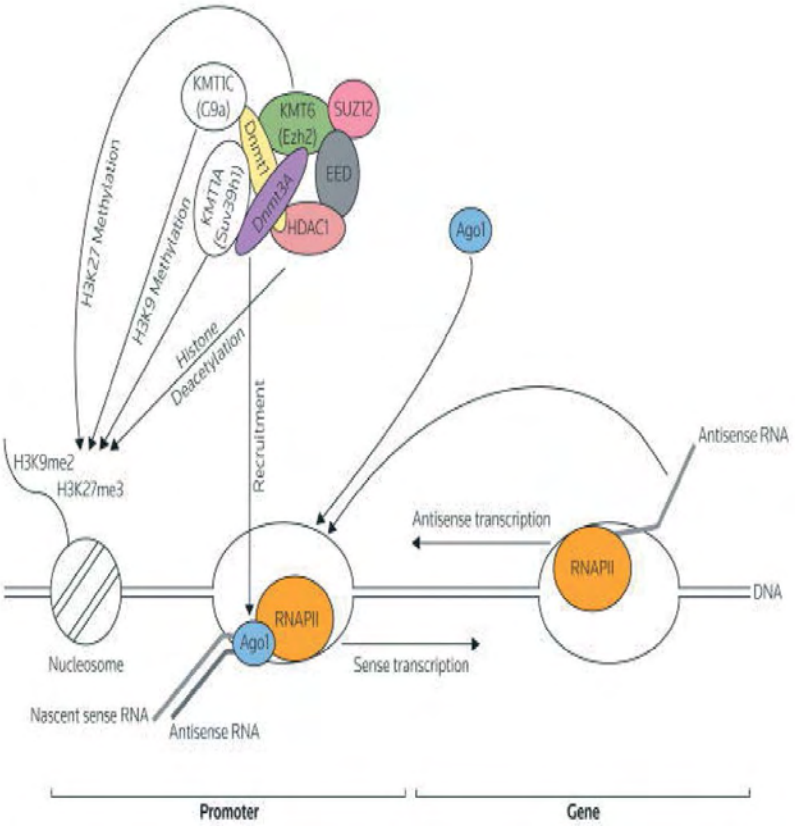

Figure 2. A model for the antisense non-coding RNA-mediated initiation of transcriptional gene silencing in human cells Antisense RNA associates with the argonaute 1 (Ago1) protein. (From Malecova, *Curr Opin Mol Ther. 2010*).

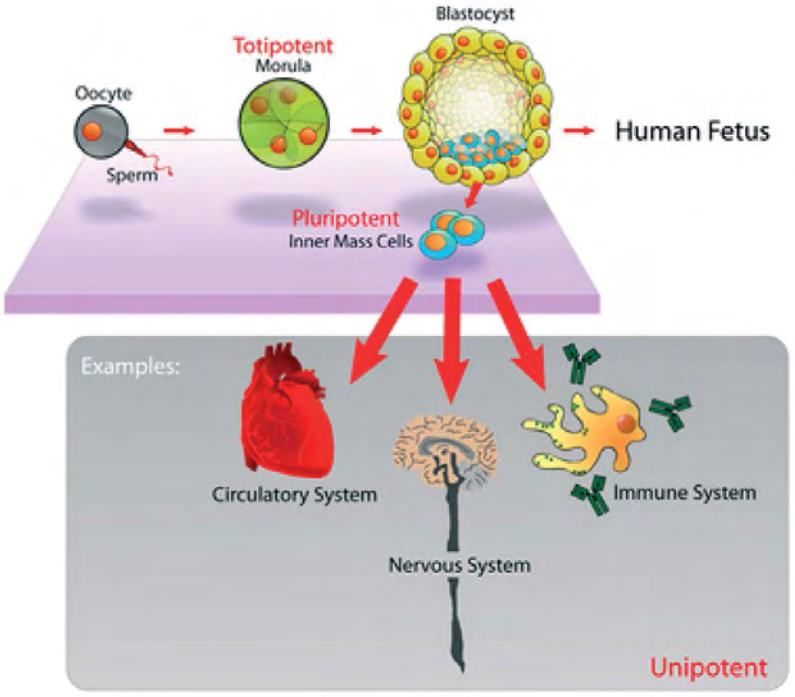

Figure 3. Pluripotent, embryonic stem cells originate as inner cell mass (ICM) cells within a blastocyst. These stem cells can become any tissue in the body, excluding a placenta. Only cells from an earlier stage of the embryo, known as the morula, are totipotent, able to become all tissues in the body and the extraembryonic placenta

genomic methylation than its normal counterpart. Conversely, too much DNA methylation (hypermethylation) may initiate the silencing of tumor suppressor genes. Medical researchers are evaluating epigenetic markers as a means for early cancer diagnosis and prediction of clinical outcome. Therapeutics based on epigenetic strategies is also being considered for cancer treatment and prevention (15, 16).

Aging

DNA methylation decreases as cells age. Identical twins are epigenetically indistinguishable early in life, but have substantial differences in epigenetic markers with age (17). This observation suggests an important role by the environment in shaping the epigenome. It has been shown that the process of aging involves some epigenetic pathways that have been identified in the process of carcinogenesis.

Because so many diseases, such as cancer, involve epigenetic changes, it seems reasonable to try to counteract these modifications with epigenetic treatments. These changes seem an ideal target because they are by nature reversible, unlike DNA sequence mutations. The most popular of these treatments aim to alter either DNA methylation (18) or histone acetylation.

Inhibitors of DNA methylation can reactivate genes that have been silenced. Two examples of these types of drugs are 5-azacytidine and 5-aza-2′-deoxycytidine (4). These medications work by acting like the nucleotide cytosine and incorporating themselves into DNA while it is replicating. After they are incorporated into DNA, the drugs block DNMT enzymes from acting, which inhibits DNA methylation.

Drugs aimed at histone modifications are called histone deacetylase (HDAC) inhibitors. HDACs are enzymes that remove the acetyl groups from DNA, which condenses chromatin and stops transcription. Blocking this process with HDAC inhibitors turns on gene expression. The most common HDAC inhibitors include phenylbutyric acid, SAHA, depsipeptide, and valproic acid (4).

STEM CELL AND THERAPY

Stem cells are biological cells found in all multicellular organisms that can divide (through mitosis) and differentiate into diverse specialized cell types and can self-renew to produce more stem cells. In mammals, there are two broad types of stem cells: embryonic stem cells, which are isolated from the inner cell mass of blastocysts, and adult stem cells, which are found in various tissues. In adult organisms, stem cells and progenitor cells act as a repair system for the body, replenishing adult tissues. In a developing embryo, stem cells can differentiate into all the specialized cells (these are called pluripotent cells) (Figure 3).

There are three accessible sources of autologous adult stem cells in humans:

1. Bone marrow, which requires extraction by *harvesting*, that is, drilling into bone (typically the femur or iliac crest),
2. Adipose tissue (lipid cells), which requires extraction by liposuction, and
3. Blood, which requires extraction wherein blood is drawn from the donor (similar to a blood donation), passed through a machine that extracts the stem cells and returns other portions of the blood to the donor.

Stem cells can also be taken from umbilical cord blood just after birth. Of all stem cell types, autologous harvesting involves the least risk. By definition, autologous cells are obtained from one's own body, just as one may bank his or her own blood for elective surgical procedures.

Highly plastic adult stem cells are routinely used in medical therapies, for example in bone marrow transplantation. Stem cells can now be artificially grown and transformed (differentiated) into specialized cell types with characteristics consistent with cells of various tissues such as muscles or nerves through cell culture (19, 20). Embryonic cell lines and autologous embryonic stem cells generated through therapeutic cloning have also been proposed as promising candidates for future therapies (21). It is of enormous interest to investigate relationship between cancer research and origin of oncogenes and tumor-suppressor genes as an archaeal origin (22).

COMMENTS

One question that arises is the cause of epigenetic changes. The answer to this can not be found in the known biochemical processes in the cell. Studies of the connections between DNA methylation and chromatin structure and the DNA methyltransferase-associated proteins will likely reveal that many epigenetic modifications of the genome are directly connected. The CpG methylated region may recruit further chromatin modulatory activities, such as methyl-CpG binding protein complexes and their associated repressive activities, and finally lock a given region of the genome in a silent state regulating human disease, and tumorogenesis as well. Although the studies describing the stem cells are incredibly promising, it is still far-fetched from their direct applications for human therapies. The multipotency of stem cells is reduced over time due to progressive gene silencing. Do we can knockout tumorogenesis by reversible gene silencing? If this case, than stem cell therapy seems promising candidate for strategies to control cancer. A Johns Hopkins-led team of scientists has produced the first-ever epigenetic landscape map for tissue differentiation from precursor to progeny. Time for healing, not curing genetic diseases is still far from achieving. Just a deeper knowledge of the choice of the appropriate DNA quantum state, can lead to complete recovery.

Conflict of interest

We declare no conflicts of interest

REFERENCES

1 Reamon-Buettner SM, Borlak J. A new paradigm in toxicology and teratology: altering gene activity in the absence of DNA sequence variation. *Reprod Toxicol*. 2007;24:20–30.

2 Weinhold R . Epigenetics: the science of change. *Environ Health Persp*. 2006;114:160–7.

3 Peter BB, Wolfram H. ATP-dependent nucleosome remodeling. *Annual Review of Biochemistry*. 2002;71:247–73.

4 Egger E. Epigenetics in human disease and prospects for epigenetic therapy. *Nature*. 2004;429:457–63.

5 Morris KV, Chan SW, Jaconsen SE, Looney DJ. Small interfering RNA-induced transcriptional gene silencing in human cells. *Science*. 2004;305(5688):1289–92.

6 Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: Insights into functions. *Nat Rev Genet*. 2009;10(3):155–9.

7 Bertone P, Stolc V, Royce TE, Rozowsky JS, Urban AE, Zhu X, et al. Global identification of human transcribed sequences with genome tilling arrays. *Science*. 2004;306(5705):2242–6.

8 Malecova B, Morris KV. Transcriptional gene silencing through epigenetic changes mediated by non-coding RNAs. *Curr Opin Mol Ther*. 2010;12: 214–22.

9 Penagarikano O . The pathophysiology of fragile X syndrome. *Ann Rev Genom Hum G Annual*. 2007;8:109–29.

10 Szyf M. The dynamic epigenome and its implications in toxicology. *Toxicol Sci*. 2007;100:7–23.

11 van Vliet J, Oates NA, Whitelaw E. Epigenetic mechanisms in the context of complex diseases. *Cell Mol Life Sci*. 2007;64:1531–8.

12 Fraga MF, Agrelo R, Esteller M. Cross-talk between aging and cancer: the epigenetic language. *Ann N Y Acad Sci*. 2007;1100:60–74.

13 Goldberg AD, Allis CD, Bernstein E. Epigenetics: a landscape takes shape. *Cell*. 2007;128:635–8.

14 Feinberg AP, Vogelstein B. Hypomethylation distinguishes genes of some human cancers from their normal counterparts. *Nature*. 1983;301:89–92.

15 Jones PA, Baylin S B. The fundamental role of epigenetic events in cancer. *Nat Rev Genet*. 2002;3:415–28.

16 Jones PA, Baylin SB. The epigenomics of cancer. Environmental Diseases from A to Z. *J Environ Sci Heal S*. 2007;128:683–92.

17 Kaati G. Cardiovascular and diabetes mortality determined by nutrition during parents' and grandparents' slow growth period. *Eur J Hum Genet*. 2002;10:682–8.

18 Robertson KD. DNA methylation and chromatin: Unraveling the tangled web. *Oncogene*. 2002;21:5361–79.

19 Becker AJ, McCulloch EA, Till JE. Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. *Nature*. 1963;197:452–4.

20 Siminovitch L, McCulloch EA, Till JE. The distribution of colony-forming cells among spleen colonies. *J Cell Compar Physio*. 1963;62(3):327–36.

21 Tuch BE. Stem cells—a clinical update. *Aust Fam Physician*. 2006;35(9):719–21.

22 Koike H, Yokoyama K, Kawashimi T, Yamasaki T, Makino S-I, Clowney L, et al. GATC methylation by Dam methylase in archaea: its roles and possible transcription regulation by an FFRP. *P Jpn Acad B-Phys*. 2005;81(7):278–90.