Epigenetics in haematologic disorders and role of epigenetic modifiers as targeted therapy of haematologic malignancies.

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ABSTRACT

Epigenetics is concerned with heritable changes in gene expression without alteration of the coding sequence. Epigenetic modification of chromatin includes methylation of genomic DNA as well as modification of chromatin-associated proteins, in particular, histones.

Epigenetic deregulation affects several aspects of tumor cell biology, including cell growth, cell cycle control, differentiation, DNA repair, and cell death. This raises the strong possibility that reversing deregulated epigenetic mechanisms may be an effective treatment for leukemia and cancer.

Keywords: Epigenetic disorders,
Blood diseases,
Epigenetic therapy.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

5'Aza-dC: 5'-aza-20-deoxycytidine.

ADP: Adenosine Di-PhosPhate.

ALL: Acute Lymphoblastic Leukemia.

AML: Acute Myeloid Leukemia.

APL: Acute Prolymphocytic Leukemia.

ATPase: Adenosine Tri-PhosPhatase.

bHLH: basic helix–loop–helix.

bp: base pair.

BRCA1: BReast-CAncer susceptibility gene 1.

C: cytosine

CD: Cluster of Differentiation.

CDK6: Cyclin D–kinase 6 oncogene.

CpG islands: Regions in DNA that contain many adjacent cytosine and guanine nucleotides. The "p" in CpG refers to the phosphodiester bond between the cytosine and the guanine**.**

C-terminal: Carboxy terminal.

DNA: DeoxyriboNucleic Acid.

DNMTs: DNA methyltransferases.

DUB: Deubiquitinating enzyme.

EZH2: Enhancer of Zeste protein-2

FDA: Food and Drug Administration.

H: Histone.

HAT: Histone Acetyl Transferase.

HDAC: Histone Deacetylase.

HKMTs: Histone lysine MethylTransferases.

HMG: High Mobility Group.

HP1: Heterochromatin Protein 1.

HSCs: Hematopoietic Stem Cells.

IC 50: The half maximal inhibitory concentration.

IGF: Insulin-like Growth Factor.

kDa: kilo Dalton.

LSD: Lysine-Specific Demethylase.

MAO: MonoAmine Oxidases.

MBD: Methylated DNA-Binding Domain.

MDS: MyeloDysplastic Syndrome.

MEL: Murine ErythroLeukemia.

miRNA: MicroRNA.

mRNA: messenger RNA.

MLL: Mixed Lineage Leukemia**.**

MOZ: Monocytic Leukemia Zinc finger.

MTA: MethylThioAdenosine.

NAD: Nicotinamide Adenine Dinucleotide.

NHK-1: Nucleosomal Histone Kinase-1.

nm: nanometers.

nt: Nucleotides.

N-terminal: amino terminal.

PAD: peptidylarginine deiminase.

PARP: poly (ADP-ribose) polymerase.

PcG: Polycomb Group.

PP: Protein Phosphatase.

PRMTs: Protein Arginine MethylTransferases.

PTMs: Post Translational Mutations.

RARα: Retinoic Acid Receptor α.

Rb: RetinoBlastoma tumor-suppressor gene.

RNA: RiboNucleic Acid.

RNAi: RNA interference.

SAHA: SuberoylAnilide Hydroxamic Acid.

SAH: S-adenosylhomocysteine.

SAM: S-adenosylmethionine.

SCL: Stem Cell Leukemia.

SENP: Sentrin-specific protease.

siRNA: small interfering RNA.

S phase: synthesis phase.

SUMO: Small Ubiquitin-like MOdifier**.**

T: Thymine.

trxG: Trithorax Group.

TSA: trichostatin A.

Ulp: Ub1(ubiquitin-like protein)-specific protease.

WRN: Werner's syndrome.

EPIGENETIC REGULATION OF GENE EXPRESSION

Introduction

The sequence of the four nucleotides of the genetic code is like an indelible ink that, with rare exceptions, is faithfully transcribed from cell to cell and from generation to generation. But on top of this code lies another one, literally "epigenetic," which is represented by methyl groups added to the DNA base cytosine, as well as covalent changes in histone proteins around which the DNA is coiled as well as other mechanisms. This epigenetic information is more like a code written in pencil in the margins around the DNA. Although the genome largely distinguishes one person from another, the epigenome, or epigenetic information, distinguishes one cell type from another, changing rapidly in early embryogenesis as cells differentiate **(Gosden and Feinberg AP**, **2007).**

The field of epigenetics provides a partial explanation for how, despite their identical DNA sequences, monozygotic twins or cloned animals can differ in their susceptibility to disease **(Esteller, 2008).** The term "epigenetics" was first used by Conrad Waddington in 1939 to describe "the causal interactions between genes and their products, which bring the phenotype into being" **(Waddington, 1939).** Subsequently, Arthur Riggs and colleagues defined epigenetics as "the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence". As used today the term epigenetics has broadened to encompass changes that are both heritable and transient in nature This definition, contrasts with genetics where DNA sequence can be altered through mutations, deletions, recombination, single nucleotide polymorphisms and amplifications among other defects **(Bird, 2007).**

The following review describes our current understanding of the important deregulated epigenetic mechanisms and the preclinical and clinical development of epigenetic and chromatin modifiers in the therapy of these disorders.

Chromosome structure

A chromosome is an organized structure of DNA and protein that is found in cells. It is a single piece of coiled DNA containing many genes, regulatory elements and other nucleotide sequences. Chromosomes also contain DNA-bound proteins, which serve to package the DNA and control its functions. In eukaryotes (nucleated cells), nuclear chromosomes are packaged by proteins into a condensed structure called chromatin. This allows the very long DNA molecules to fit into the cell nucleus. The structure of chromosomes and chromatin varies through the cell cycle. Chromosomes are the essential unit for cellular division and must be replicated, divided, and passed successfully to their daughter cells so as to ensure the genetic diversity and survival of their progeny. Chromosomes **(Figure 1)** may exist as either duplicated or unduplicated; unduplicated chromosomes are single linear strands, whereas duplicated chromosomes (copied during synthesis phase) contain two copies joined by a centromere. Compaction of the duplicated chromosomes during mitosis and meiosis results in the classic four-arm structure. Chromosomal recombination plays a vital role in genetic diversity **(Wikipedia:chromosome, 2010)**.

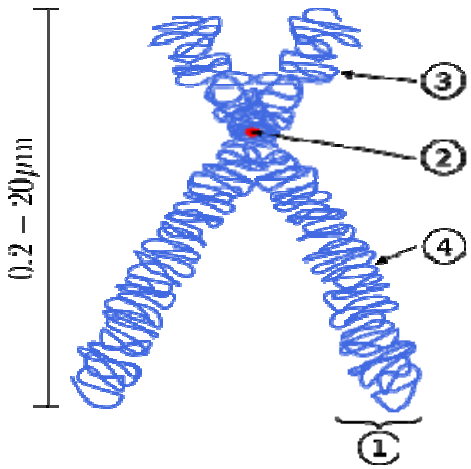

Figure 1: Diagram of a duplicated and condensed metaphase eukaryotic chromosome. (1) Chromatid: one of the two identical parts of the chromosome after S phase. (2) Centromere . (3) Short arm. (4) Long arm **(Wikipedia:chromosome, 2010)**.

Chromosomes can be divided into two types: autosomes and sex chromosomes. Certain genetic traits are linked to sex, and are passed on through the sex chromosomes. The autosomes contain the rest of the genetic hereditary information. All act in the same way during cell division. Human cells have 23 pairs of large linear nuclear chromosomes, (22 pairs of autosomes and one pair of sex chromosomes) giving a total of 46 per cell **(Figure 2).** In addition to these, human cells have many hundreds of copies of the mitochondrial genome. Sequencing of the human genome has provided a great deal of information about each of the chromosomes **(Wikipedia:chromosome, 2010)**.

Chromatin is the complex of DNA and protein found in the eukaryotic nucleus, which packages chromosomes. The structure of chromatin varies significantly between different stages of the cell cycle, according to the requirements of the DNA. During interphase (the period of the cell cycle where the cell is not dividing), two types of chromatin can be distinguished:

- Euchromatin, which consists of DNA that is active, e.g., being expressed as protein.
- Heterochromatin, which consists of mostly inactive DNA. It seems to serve structural purposes during the chromosomal stages. Heterochromatin can be further distinguished into two types:
    - *Constitutive heterochromatin*, which is never expressed. It is located around the centromere and usually contains repetitive sequences.
    - *Facultative heterochromatin*, which is sometimes expressed.

Individual chromosomes cannot be distinguished at this stage – they appear in the nucleus as a homogeneous tangled mix of DNA and protein.

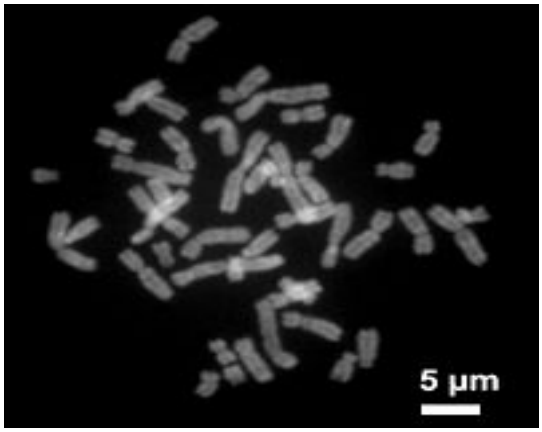

Figure 2: Human chromosomes during metaphase **(Wikipedia:chromosome, 2010)**.

In the early stages of mitosis or meiosis (cell division), the chromatin strands become more and more condensed. They cease to function as accessible genetic material (transcription stops) and become a compact transportable form. This compact form makes the individual chromosomes visible, and they form the classic four arm structure, a pair of sister chromatids attached to each other at the centromere. The shorter arms are called *p arms* (from the French *petit*, small) and the longer arms are called *q arms* (*q* follows *p* in the Latin alphabet). This is the only natural context in which individual chromosomes are visible with an optical microscope. During divisions, long microtubules attach to the centromere and the two opposite ends of the cell. The microtubules then pull the chromatids apart, so that each daughter cell inherits one set of chromatids. Once the cells have divided, the chromatids are uncoiled and can function again as chromatin. In spite of their appearance, chromosomes are structurally highly condensed, which enables these giant DNA structures to be contained within a cell nucleus **(Wikipedia:chromosome, 2010)**.

Nucleosome core particle organization and histone structure

The nucleosome core particle represents the first level of chromatin organization. Core histones (The main protein components of chromatin) play structural roles in chromatin assembly and compaction by forming the nucleosome. Nucleosome is a heterotypic tetramer (H3–H4)2 with two associated dimers (H2A– H2B) in the form ([H2A–H2B] [{H3-H4}2] [H2A–H2B]). Associated with this octamer are about 147 bp of DNA wrapped in 1.7 superhelical turns **(Figure 3).** Nucleosomes are connected by a DNA linker of variable length that forms a 10-nm beads-on-a-string array and are associated with the linker histone H1. The next level of chromatin organization is the 30-nm fiber, which is composed of packed nucleosome arrays recently found to be arranged as a two-start helical model, and mediated by core histone inter-nucleosomal interactions **(Dorigo et al., 2004).**

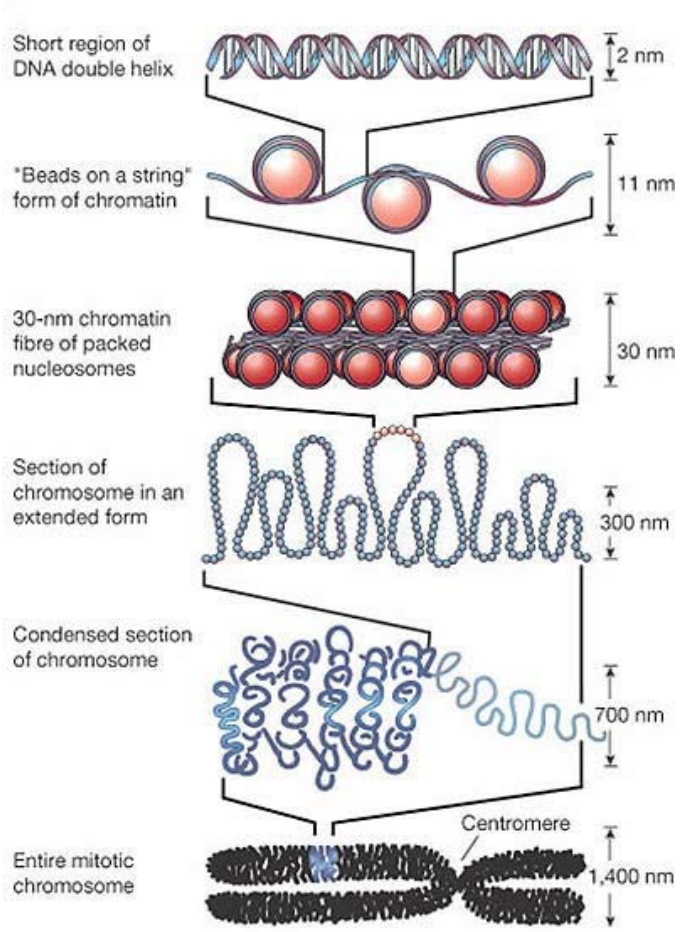

Figure 3: The structure of an eukaryotic chromosome **(Pearson, 2003).**

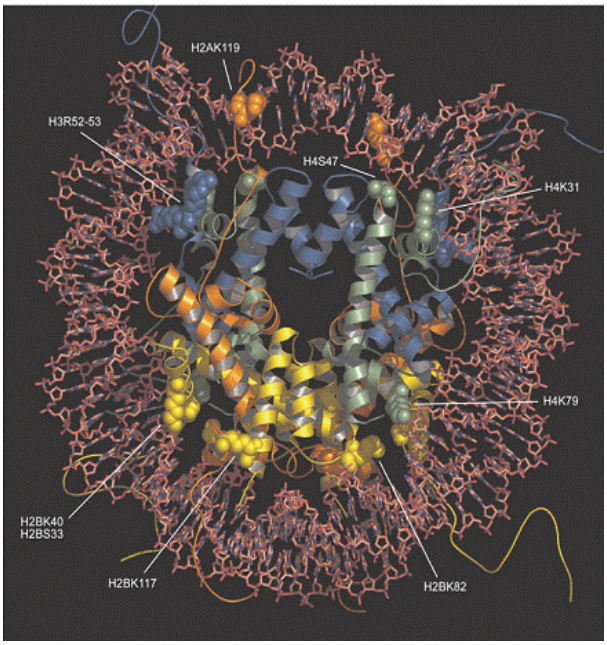

Figure 4: The nucleosome core particle structure. The colours represent different histones H3 (blue), H4 (light green), H2A (orange) and H2B (yellow); the histone modifications are shown as spheres **(Dorigo et al., 2004).**

Each of the core histones contain the histone fold domain, composed of three α-helices connected by two loops, which allow heterodimeric interactions between core histones known as the handshake motif. Additionally, each core histone also contains an N terminal tail **(Figures 4, 5)** that is subjected to a wide variety of Post Translational Mutations (PTMs). The core histone tails play important roles in nucleosome stability, and may contribute to define the condensed state of the chromatin fiber and higher order structures by facilitating nucleosome assembly or disassembly **(Zheng and Hayes, 2003).**