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Review

Heterochromatin instability in cancer: From the Barr body to satellites and the nuclear periphery

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ABSTRACT

In recent years it has been recognized that the development of cancer involves a series of not only genetic but *epigenetic* changes across the genome. At the same time, connections between epigenetic regulation, chromatin packaging, and overall nuclear architecture are increasingly appreciated. The cell-type specific organization of heterochromatin, established upon cell differentiation, is responsible for maintaining much of the genome in a repressed state, within a highly compartmentalized nucleus. This review focuses on recent evidence that in cancer the normal packaging and higher organization of heterochromatin is often compromised. Gross changes in nuclear morphology have long been a criterion for pathologic diagnosis of many cancers, but the specific nuclear components impacted, the mechanisms involved, and the implications for cancer progression have barely begun to emerge. We discuss recent findings regarding distinct heterochromatin types, including the inactive X chromosome, constitutive heterochromatin of peri/centric satellites, and the peripheral heterochromatic compartment (PHC). A theme developed here is that the higher-order organization of satellites and the peripheral heterochromatic compartment may be tightly linked, and that compromise of this organization may promote broad epigenomic imbalance in cancer. Recent studies into the potential role(s) of the breast cancer tumor suppressor, BRCA1, in maintaining heterochromatin will be highlighted. Many questions remain about this new area of cancer epigenetics, which is likely more important in cancer development and progression than widely appreciated. We propose that broad, stochastic compromise in heterochromatin maintenance would create a diversity of expression profiles, and thus a rich opportunity for one or more cells to emerge with a selective growth advantage and potential for neoplasia.

1. Introduction

Our review of heterochromatin instability in cancer will begin by placing the "heterochromatic compartment" in the context of overall nuclear organization. Genomic DNA exhibits a higher-order organization within a complex, compartmentalized nuclear structure; this in turn is intimately associated with a series of epigenetic modifications that distinguish heterochromatin from euchromatin. In mammalian cells, much of our knowledge of how heterochromatin is formed and maintained comes from studies of X-chromosome inactivation in female cells, where it forms the condensed Barr body. Pathologists have long noted cancer-associated changes in gross nuclear appearance (reviewed in [1,2]) but in recent years it was the loss of the heterochromatic Barr body, noted commonly in aggressive breast cancers, that prompted a more in-depth investigation into heterochromatin loss in cancer (reviewed in [3]). We will consider the nature and regulation of heterochromatin in normal mammalian cells, as understood from X-chromosome silencing, and how this led to a role for BRCA1 in satellite heterochromatin. A series of findings contribute to an exciting but still very much emerging story, which begins with the disappearing Barr body and leads to the misregulation of centromere-associated satellite heterochromatin and the peripheral heterochromatic compartment in cancer. Our emphasis is primarily on the heterochromatic compartment, yet we will consider that disruption of heterochromatin can alter the organized euchromatin and nuclear environment more generally.

2. Nuclear heterochromatic versus euchromatic compartments in normal somatic cells

Folding of the genome above nucleosome packaging of the 30 nm fiber remains in many respects a puzzle, but in recent years great advances have been made in our understanding of the higher-order organization of the genome and nuclear structure. To facilitate the coordinated functions of DNA replication, different types of RNA transcription, splicing and export, the nucleus must act as a highly efficient factory, and we now understand it has

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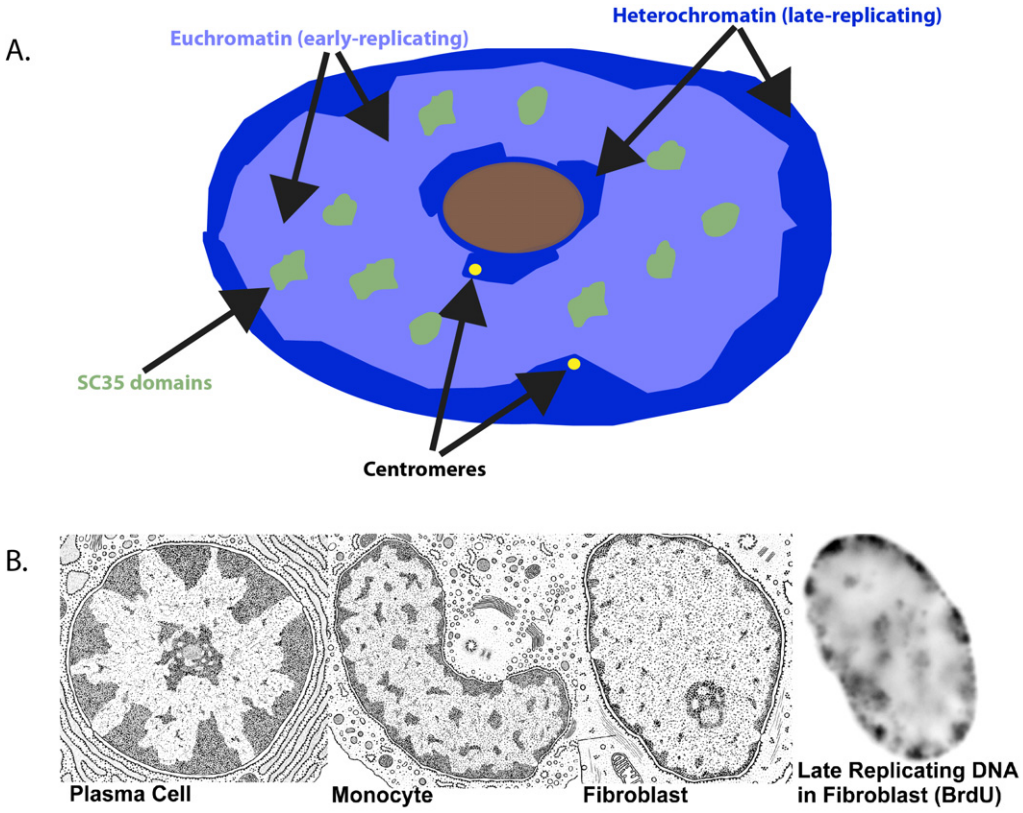

Fig. 1. (A) Diagram depicting the separation of euchromatin (light blue) and heterochromatin (dark blue) domains in a normal human fibroblast nucleus. SC35 domains (green) rich in pre-mRNA metabolic factors reside within an internal euchromatic compartment. (B) Patterns of heterochromatin (darkly staining material) in the nucleus vary depending on cell type (reproduced from Thomas L. Lentz, M.D., *Cell Fine Structure: An Atlas of Drawings of Whole-Cell Structure*, Philadelphia, W.B. Saunders Publishing Company, 1971). This largely peripheral heterochromatin is also late-replicating as shown by Brdu labeling on far right in a human fibroblast nucleus.

specialized domains delegated to its multitude of distinct tasks. Evidence for this functional compartmentalization has been revealed by in situ techniques examining specific DNA or RNA sequences and different types of metabolic complexes directly within the nuclear environment. Chromatin segregates into two distinct but broad nuclear compartments: much of the repressed, densely packaged heterochromatin clusters around the nuclear periphery or nucleolus, to form the "peripheral heterochromatic compartment" (PHC), whereas loosely packaged, active euchromatin fills most of the internal nucleoplasm (Fig. 1A). The pattern of the PHC is cell-type specific and we suggest reflects a higher-order "blueprint" of the epigenome in that cell type (Fig. 1B). This will be an important concept as we consider the degree to which the PHC may be disrupted in cancer.

The large euchromatic compartment is further delineated by a number of distinct sub-compartments and bodies. Nucleoli have long been recognized as prominent structures devoted to the efficient expression of rRNA and ribosomal assembly and other functions [4,5]. Fluorescence techniques have revealed the presence of at least several other major sub-structures, not visible by phase microscopy, such as SC35 domains (speckles), PML bodies and Cajal Bodies, as reviewed elsewhere [6,7]. Numerous SC35 domains facilitate pre-mRNA metabolism by congregating factors that form splicing and export complexes [8]. These are spatially associated with many highly active genes which cluster in the domain periphery [9,10] (Fig. 2A). On a smaller scale, there may also be some clustering of genes at up to 2000 tiny sites enriched for polymerase II, referred to as "transcription factories" [11] that are prevalent at the periphery of SC35 domains and throughout the nucleoplasm. It has been proposed that transcription factories may mediate the preferential association of genes from separate chromosomes [12]. While some evidence suggests that specific genes can interact with each other in a dynamic nucleus, other evidence suggests that much gene interaction is likely due to their affinity for a common structure [13–17]. What is clear is that there is a highly coordinated sequestration of genomic regions, with general segregation of inactive from active chromatin, and clustering of active genes at sites concentrated with RNA metabolic factors.

How is this spatial separation maintained within the nuclear environment? It is known that nuclear positioning including proximity to the lamina and nuclear envelope strongly correlates with transcriptional activity, and studies into the functional basis for this have gained momentum. Lamin associated domains (LADs) are enriched for gene poor sequences, suggesting a repressive chromatin environment within these domains [18]. There is some evidence that targeting to the nuclear lamina can lead to gene silencing as it does in yeast [19], although this is not always the case in mammalian cells [20]. Further indication that sequences within LADs may often be hypomethylated in cancer suggests the regulation of this higher-order structure may be markedly altered in the cancer nuclear environment [21].

In our view, nuclear organization can be thought of as a paradoxical combination of the largely stable, higher-order organization (of nuclear sub-domains and genomic regions) with a highly dynamic shuffling of metabolic factors at the molecular level. We suggest the former reflects the heritable epigenetic program of the cell-type and the latter reflects the constant modulation of function in response to cellular cues. For example, it has been shown that the mobility of factors within SC-35 domains can be quite high [22,23], even though the positioning of these domains in the overall nuclear space is often quite stable (discussed in [24]). In addition, in some cases specific genes show a very constant (near 100%) position