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Review

Higher order chromatin organization in cancer

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ABSTRACT

In spite of our increased understanding of how genomes are dysregulated in cancer and a plethora of molecular diagnostic tools, the front line and 'gold standard' detection of cancer remains the pathologist's detection of gross changes in cellular and tissue structure, most strikingly nuclear dis-organization. In fact, for over 140 years it has been noted that nuclear morphology is often disrupted in cancer. Even today, nuclear morphology measures include nuclear size, shape, DNA content (ploidy) and 'chromatin organization'. Given the importance of nuclear shape to diagnoses of cancer phenotypes, it is surprising and frustrating that we currently lack a detailed understanding to explain these changes and how they might arise and relate to molecular events in the cell. It is an implicit hypothesis that perturbation of chromatin and epigenetic signatures may lead to alterations in nuclear structure (or vice versa) and that these perturbations lie at the heart of cancer genesis. In this review, we attempt to synthesize research leading to our current understanding on how chromatin interactions at the nuclear lamina, epigenetic modulation and gene regulation may intersect in cancer and offer a perspective on critical experiments that would help clarify how nuclear architecture may contribute to the cancerous phenotype. We also discuss the historical understanding of nuclear structure in normal cells and as a diagnostic in cancer.

The earliest described genetic abnormality in cancer was abnormal chromatin, described by Teodor Boveri in 1914 [1]. While he emphasized that chromosomes were altered in mitosis and number, for which he is called the father of cancer genetics, his book also refers to a fuzziness of chromatin. One can see this for oneself in his microscopic plates archived at University of Wurzburg. In fairness, he did not mean what we do in describing chromatin, which was described first by Heintz in the 1920s, yet hints of modern chromatin biology go back a century [2].

As indicated above, one of the most noted changes in cancer cells is abnormal nuclear morphology. In fact, the specific morphological changes displayed by nuclei are often used by pathologists to grade and specify cancer type and stage. The hallmark of cancer is genome dysregulation and, of course, genes can become activated or repressed by a variety of mechanisms involving both local and global changes. However, it remains unclear how genome dysregulation and perturbations in nuclear architecture, both so evidently displayed in cancer, are related. One can think of gene regulation as occurring at different levels. The first level of gene activation/repression occurs when a specific transcriptional regulator becomes available at a discreet developmental time point or via signaling in response to stimuli (hormones, nutrients, ligands, etc.). Another layer of regulation occurs at the level of local chromatin modulation in a process that promotes (or prevents) recruitment of transcription factors/complexes to *cis* elements within a particular locus or regulatory element. The specific local chromatin environment is a consequence of altering the post-translational modifications of histone tails, DNA methylation patterns and/or nucleosome positioning. In cancerous cells, these local chromatin modifications, methylation signatures and gene expression profiles are perturbed and the intersection of these processes in understanding cancer phenotypes is a continuing area of robust investigation. However, the role that overall three dimensional nuclear structure plays in the disease process is not well understood. This 'higher order' level of chromatin regulation occurs at a more global level, involving changes in nuclear localization, associations or larger chromatin regions with repressive compartments, such as the nuclear periphery or pericentromeric heterochromatin, and large-scale changes in DNA structure, such as the formation of DNA loops and/or locus contraction. Most studies to date on the role that nuclear structure plays in gene regulation have been carried out in developmental systems or specific disease models, such as Hutchinson–Gilford Progeria (HGPS) early aging syndrome, that have a clear link to disruption of nuclear morphology by a mutation in a site protein coding gene (e.g. Lamin A in HGPS).

Despite the extraordinarily long history of microscopic evidence linking abnormal large-scale chromatin structure to cancer, remarkably little has been done toward understanding the molecular basis of this relationship. The reasons such large-scale molecular chromatin analyses have not been fully applied to the study of

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cancer are two-fold: (1) it is unclear what the key component(s) are involved the loss of genome structure and (2) the aneuploidy present in cancerous cells is problematic in dissecting the role higher order chromatin structure and scaffolding plays in gene regulation and onset of disease. In this review, we attempt to synthesize research leading to our current understanding on how chromatin interactions at the nuclear lamina, epigenetic modulation and gene regulation may intersect in cancer and offer a perspective on critical experiments that would help clarify how nuclear architecture may contribute to the cancerous phenotype. We discuss the historical understanding of nuclear structure in normal cells and as a diagnostic in cancer, our understanding of epigenetic perturbation in cancer and, finally, how nuclear structure and epigenetics of cancer may be related.

1. Historical perspective of nuclear structure and pathology

The eukaryotic nucleus is now recognized as a highly organized and orchestrated organelle and this structural framework is quite often disrupted cancerous cells. In fact, this disruption is a common diagnostic tool used by pathologists in identifying cancerous cells in an otherwise normal cell population [3]. While much progress has been made in the past few decades on the gene regulatory networks, epigenetic modifications and signaling pathways perturbed by or leading to cancerous phenotypes, less progress has been made in determining the role that nuclear architecture plays in the neoplastic and disease process.

That chromatin is organized in the nucleus is not a new idea. While Carl Rabl (1853–1917) was the first to propose the seminal concept of higher order chromosomal organization (Rabl configuration of chromosomes), Theodor Boveri (1862–1915) was the first to use the term "chromosome territory" (CT). In his 1909 publication, Boveri described chromatin movements and organization in three observational hypotheses [4,5]: first, chromosome territory (CT) arrangements are stably maintained during interphase. Second, this stability is lost during prometaphase and there are greater movements of CTs. Finally, while mother and daughter nuclei do not share similar CT proximity patterns, the daughter nuclei do exhibit symmetry with each other and the general radial CT positioning between mother/daughter nuclei is maintained. Although Boveri was able to make great strides in understanding of nuclear dynamics (although the meaning of chromatin itself came later with Heintz), he was reliant on fixed materials and inferior microscopic instrumentation. The most compelling evidence for CTs did not arrive until the 1970s and 1980s, when the ability to study individual chromosomes and loci in the intact nucleus became possible. These studies confirmed much of Boveri's findings, including the idea of CTs and general nuclear organization. Functional assays of nuclear architecture have only occurred in the past decade or two, described in more detail later in this review [4].

In spite of our increased understanding of how genomes are dysregulated in cancer and a plethora of molecular diagnostic tools, the front line and 'gold standard' detection of cancer remains the pathologist's detection of gross changes in cellular and tissue structure, most strikingly nuclear dis-organization [3,6,7]. In fact, for over 140 years it has been noted that nuclear morphology is often disrupted in cancer. In the 1860s, Lionel S. Beale of King's College Hospital examined unstained sputum from a patient with cancer of the pharynx and observed nuclear morphology variations in the cancerous cells [8]. George Papanicolaou developed a stain that enables visualization of many cytoplasmic and nuclear structural features of cells in the 1930s, and applied the stain to cervical cells to test for cancer – the so-called 'Pap test' [9]. Even today, nuclear morphology measures include nuclear size, shape, DNA content (ploidy) and 'chromatin organization'. Given the importance of nuclear shape to diagnoses of cancer phenotypes, it is surprising and frustrating that we currently lack a detailed understanding to explain these changes and how they might arise and relate to molecular events in the cell. Of course, nuclear size and shape is determined by the dynamic nucleoskeleton components and interacting chromatin and RNA. As such, it is an implicit hypothesis that perturbation of chromatin and epigenetic signatures may lead to alterations in nuclear structure (or vice versa) and that these perturbations lie at the heart of cancer genesis.

2. Nuclear scaffolding at the INM – form and function

At the protein level, the nuclear periphery in mammalian cells is comprised of a unique set of inner nuclear membrane (INM) proteins and the nuclear lamins [10,11]. Mammalian INM proteins include lamin B receptor (LBR), lamina associated peptide 2 (Lap2) and Emerin (among many others). These and other INM proteins, including proteins extending into the cytoplasm (e.g. the SUN domain proteins) interact with the nuclear lamina, which is made up of a filamentous meshwork of proteins: lamins A/C and B. Many of the INM proteins (e.g. LBR, Emerin, and Lap2) are able to interact with transcriptional repressors as well as signaling molecules. LBR has been shown to complex with heterochromatin protein 1α (HP1α) and nucleosomes through the core histones H3 and H4. Emerin and Lap2β, which both contain a LEM (Lap2β, Emerin, MAN1) domain, interact with Barrier to Auto-integration Factor (BAF), germ-cell-less (GCL), retinoblastoma protein (Rb) and HDAC3 [12–17]. Finally, the lamins themselves have been implicated in interactions with chromatin. Given the sub-localization of these scaffolding/regulatory proteins in the nuclear volume, much recent work has focused on the role these proteins play in gene regulation.

Chromatin itself is organized into structural domains likely by association with distinct nuclear compartments enriched in regulatory or structural proteins, such as the INM/lamina proteins described above [11,18–23]. Growing evidence suggests that gene activity is modulated by interactions with these sub-nuclear compartments. For instance, late replicating genes and gene-poor chromosomes tend to be located at the nuclear periphery, while early replicating genes and gene-rich chromosomes are more centrally disposed, suggesting that many inactive genes are located at the periphery of the nucleus [24,25]. However, the nuclear periphery has been shown to function in both gene silencing and activation [19,26]. These studies, while quite informative, provide only circumstantial evidence into the potential function of nuclear structure on genome function.

In order to describe more functional assays, let's focus on one example of a well-studied locus that undergoes changes in nuclear scaffolding and positioning, the Immunoglobulin Heavy chain locus (IgH). It has been shown in murine cells, using 3D-ImmunoFISH, that germ-line (not recombined) immunoglobulin heavy chain loci (IgH) are preferentially localized to the nuclear lamina in hematopoietic progenitors, T lineage cells and non-B cells (such as fibroblasts) but centrally positioned in pro-B cells, where they are active [27–30] (for a graphical description of techniques used in nuclear structure analyses, see Fig. 1). Additional studies have correlated the transcriptional activation of many other mammalian genes with their repositioning away from the nuclear periphery, including muscle specific genes and adipose genes, leading to the hypothesis that the nuclear periphery may be a repressive compartment [31–34]. Intriguingly, it was also demonstrated that localization of the IgH locus to the nuclear periphery is not just cytological, but reflects real molecular contact over a large region with components of the INM/lamina compartment in a Lamin Associated Domain (LAD – see below) [35,36]. Upon locus activation, using