Chromatin outside the cell: the new paradigm in biology

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Abstract

Since the discovery of the structure of DNA by Watson and Crick in 1953, "DNA inside the nucleus" has been the dominant biological paradigm which has spawned the complex science of molecular biology and genomics. Although this reductionist approach has been a commercial success, produced an enormous quantity of information and given us intricate insights into cellular functioning, it has provided little understanding of human health and disease and to that extent has been largely unproductive. This is primarily because this DNA-centric molecular approach has entirely ignored physiology. We know now that there is a huge amount of DNA in the form of extra-cellular cell-free chromatin (cfCh) that exists in the extracellular compartment of the body, including in circulation, that is derived from the billions of cells that die in the body every day. cfCh is fragmented but has extraordinary and diverse local and systemic biological functions which places cfCh in the realm of physiology. cfCh has the ability to integrate into genomes of healthy cells to damage their DNA and trigger apoptotic and inflammatory responses. DNA damage and inflammation are integral to ageing and ageing-related disorders such as cardio-vascular diseases, diabetes, stroke and neurodegenerative disorders. Cancer is another example which may be initiated and propagated via cfCh by its ability to bring about DNA damage, genomic instability and inflammation. Our recent finding that patho-physiological effects of cfCh can be abrogated by the use of appropriate cfCh inactivating agents suggests therapeutic possibilities. The above considerations lead me to propose that "DNA (chromatin) outside the cell" should now be considered the central paradigm in biology replacing the currently accepted model in which the DNA resides inside the nucleus. 109-1012 cells die in the adult human body daily and much of the fragmented chromosomes in the form of cell-free chromatin (cfCh) are released into the extracellular compartment of the body, including into the circulation. Our research has shown that cfCh have extraordinary and diverse local and systemic damaging effects on host cells that may form the basis of many human maladies. cfCh can freely enter into healthy cells, accumulate in their nuclei, trigger a DNA damage repair response and integrate into their genomes by an unique mechanism. Genomic integration of cfCh leads to dsDNA breaks, inflammation, chromosomal instability, senescence and apoptosis of recipient cells. These pathologies are integral to ageing and ageing-related disorders such as cardio-vascular diseases, diabetes, stroke and neurodegenerative disorders. cfCh induced inflammation and DNA damage may play a key role in infection and sepsis as well as autoimmune disorders and cancer. cfCh isolated from cancer patients can readily transform NIH3T3 cells by inducing DNA damage, inflammation and chromosomal instability and up-regulating multiple hallmarks of cancer. The transformed cells form tumors when inoculated into immune-deficient mice. Cell-free DNA isolated from sera of cancer patients are inactive, suggesting epigenetic mechanisms underlying the oncogenic process. When cancer cells are injected intravenously into mice, they die rapidly upon reaching distant organs to release cfCh particles which localize in nuclei of target cells to induce dsDNA breaks, inflammatory cytokines and up-regulating cancer hallmarks. Fluorescent phosphorylated H2AX signals and those of NF?B and cancer hallmarks are frequently activated simultaneously in the same target cells suggesting that such cells have a high propensity for oncogenic transformation. These findings suggest that cfCh from dying cancer cells can transform cells of the microenvironment both locally and in distant organs providing a novel mechanism of tumor invasion and metastasis. Together with core histones, which make up the nucleosome, the linker histone (H1) is one of the five main histone protein families present in chromatin in eukaryotic cells. H1 binds to the nucleosome to form the next structural unit of metazoan chromatin, the chromatosome, which may help chromatin to fold into higher-order structures. Despite their important roles in regulating the structure and function of chromatin, linker histones have not been studied as extensively as core histones. Nevertheless, substantial progress has been made recently. The first near-atomic resolution crystal structure of a chromatosome core particle and an 11 Å resolution cryo-electron microscopy-derived structure of the 30 nm nucleosome array have been determined, revealing unprecedented details about how linker histones interact with the nucleosome and organize

higher-order chromatin structures. Moreover, several new functions of linker histones have been discovered, including their roles in epigenetic regulation and the regulation of DNA replication, DNA repair and genome stability. Studies of the molecular mechanisms of H1 action in these processes suggest a new paradigm for linker histone function beyond its architectural roles in chromatin. The epigenome determines heritable patterns of gene expression in the absence of changes in DNA sequence. The result is programming of different cellular-, tissue- and organ-specific phenotypes from a single organismic genome. Epigenetic marks that comprise the epigenome (e.g. methylation) are placed upon or removed from chromatin (histones and DNA) to direct the activity of effectors that regulate gene expression and chromatin structure. Recently, the cytoskeleton has been identified as a second target for the cell's epigenetic machinery. Several epigenetic 'readers, writers and erasers' that remodel chromatin have been discovered to also remodel the cytoskeleton, regulating structure and function of microtubules and actin filaments. This points to an emerging paradigm for dual-function remodelers with 'chromatocytoskeletal' activity that can integrate cytoplasmic and nuclear functions. For example, the SET domain-containing 2 methyltransferase (SETD2) has chromatocytoskeletal activity, methylating both histones and microtubules. The SETD2 methyl mark on chromatin is required for efficient DNA repair, and its microtubule methyl mark is required for proper chromosome segregation during mitosis. This unexpected convergence of SETD2 activity on histones and microtubules to maintain genomic stability suggests the intriguing possibility of an expanded role in the cell for chromatocytoskeletal proteins that read, write and erase methyl marks on the cytoskeleton as well as chromatin. Coordinated use of methyl marks to remodel both the epigenome and the (epi)cytoskeleton opens the possibility for integrated regulation (which we refer to as 'epiregulation') of other higher-level functions, such as muscle contraction or learning and memory, and could even have evolutionary implications.

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