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Mini Review

Two Sorts of DNA in Eukaryotic Cells: Linear Chromosomal DNA and Circular Extrachromosomal DNA

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Abstract

In addition to Chromosomal DNA (chr-DNA), eukaryotic cells contain Extrachromosomal DNA (ec-DNA), e.g., Mitochondrial DNA (mt-DNA), and a particular class named Extrachromosomal Circular DNA (ecc-DNA). The ecc-DNA structures harbor functional sequences known to be present in the chr-DNA. Ecc-DNA has many sizes, from thousands of base pairs to mega base pairs. Their proportion of the total cellular DNA can be up to 20%, both in cells from healthy people and people with diseases, for example, cancer. A research focus is an exploration of how ecc-DNA is involved in oncology. The possible influence of ecc-DNA on chromosomal sequences in the Human Genome Project gets addressed.

Keywords: ecc-DNA, Genetics, Human Genome, Diseases, Diagnostic Reservoir

Introduction

This article describes certain basics of Chromosomal DNA (chr-DNA) and Extrachromosomal Circular DNA (ecc-DNA). Research indicates genetic activities at the level of ecc-DNA in cells with potential relevance to diseases. This concerns an oncogenic potential.

Human Chromosomes are the source of sequences contained in ecc-DNA

The linear chromosomes apart from mt-DNA in eukaryotes cells contain DNA indicating the organism's blueprint. The protein-coding sequences making up the genes represent less than 2% of the total chr-DNA [1]; careful consideration of the remaining sequences is necessary [2]. The "telomere-to-telomere" project achieved a "...gapless, telomere-to-telomere assembly of a human chromosome" [3].

Gene-coding DNA sequences are not present as a continuous sequence stretch (statements range from hypothesis to a general fact); structured in discrete protein-coding subunit sequences, exons, interspersed among non-protein-coding sequences, introns [4]. Following the "intron-exon architecture of eukaryotic genes," this structure is reviewed as a "genes in pieces" model [5]. Studies are required to determine how this model can be consistently resilient. In addition to the multiple-step formation of "functional" genes with their authentic gene pieces, recombination between nonrelated exons can arise: Recombination and "exon shuffling," after transcription to mRNA generate "new proteins with novel combinations of protein domains" [6,7]. With the continuous improvements of the molecular protocols, the "number of errors when mapping ..." could be reduced [8].

Which mechanisms are responsible for selecting genuine sequences from linear chr-DNA and assembling them into

circular double-stranded DNA, for example, containing functional oncogenes?

Endogenous Chromosomal tools are both able to move positions of Sequences within the Chr-DNA and to Generate Extrachromosomal DNA (Ec-DNA)

Apart from various protein-coding sequences, the remaining chromosomal sequences belong to the "non-coding regions"-not coding for proteins. At least parts of these DNA sequences code for functional tasks: e. g., after transcription, there are diverse forms of RNA that act as regulatory factors. Furthermore, Mobile Genetic Elements (MGE) [9] such as ALU-elements, transposons (jumping genes), and retrotransposons have various functions; e. g., "… how it contributes to human disease" [10].

Certain parts of the "non-coding regions" of the human genome have received attention [11]; for example, sequences such as "enhancers" are involved in the development of cancer [12]. Even genetic ablation of a particular RNA locus could cause a "genetic disorder" that leads to organic malformations [13].

Sequences that exert both structural and functional DNA properties

Endogenous Retroviruses (ERVs) " … are remnants of ancient active retroviruses that infected germline cells" [14]. This process ensures that they get passed on from generation to generation. The ERV-DNA copies became an integrated part of human chromosomes, then named HERV. The genome sequences of HERV-K make up approximately 8% of the human genome [15], about four times more than the protein-coding sequences of "human genes."

Studies indicate that the integrated HERV-K sequences are under genetic and epigenetic control [16]. There seems to be a broad consensus that HERVs cannot replicate [17,18]. But these integrated sequences (selected ones?) can be reactivated: upon exogenous/endogenous impact/s? [19] They have pathogenic potentials, such as inducing (?) / promoting (?) neurodegenerative diseases, autoimmune diseases, and multiple cancers [20-22]. If parts of HERV K's genome are present in ecc-DNA, does their pathogenetic potential contribute to causing diseases from this level?

The control of DNA "activities"

Several epigenetic mechanisms control the activities of genomic sequences: e. g., DNA methylation, histone post-translational modifications, and microRNAs (miRNAs). DNA methylation/demethylation is adding/removing methyl groups to one or two components of DNA, specifically the deoxyribonucleotides adenine and cytosine. DNA methylation/demethylation regulates gene expression. Epigenetic modifications don't alter the underlying chromosomal DNA sequence. The project "Epigenetic Patterns in a Complete Human Genome" explores the full epigenome [23].

Extrachromosomal DNA (ec-DNA)

Besides chr-DNA and mitochondrial DNA, eukaryotic cells contain ec-DNA, generated by excision/copying sequences from the chromosomes; the underlying principles of selecting sequences are unknown. Sequences for functional genes also reside in Extrachromosomal (ec) elements. Extrachromosomal circular DNA (ecc-DNA) in eukaryotic cells has been known for a long time [24].

Extrachromosomal circular DNA (ecc-DNA)

The origin, genetic activities, and their control

Various ways describe the generation of ecc-DNA, such as during apoptotic cell disintegration [25] or repair, [26] originating from the chromosomes [27]. Depending on their size, they may contain protein-coding and properly non-coding sequences and sequences derived from MGE; MGE can mediate intra- or intercellular DNA trafficking [28]. The composition of the sequences may be subject to metabolic pressure. The term "ecc-DNA" describes the full spectrum of circular DNAs in eukaryotes [29]. Their sizes range from a few thousand base pairs to mega base pairs. What are the basics for selecting chr-DNA-derived sequences that become incorporated into ecc-DNA?

What kind of superordinate control regulates "genetic activities" of ecc-DNA [30]? Data hint that epigenetic mechanisms such as DNA methylation control ecc-DNA - an operational regime.

There is growing consensus that faulty DNA methylation might be an essential factor in developing diseases. Research focuses on the links between chr-DNA and ecc-DNA methylation and human diseases such as cancer and various congenital disabilities. So far, much of this research targets cancer and tumor suppressor genes. Is there is an ongoing exchange of sequences between chr-DNA and ecc-DNA?

The proportion of ecc-DNA of whole cellular DNA increases in eukaryotic cells with cell stress or aging and "contribute to intercellular heterogeneity in normal and tumor cells" [31]. Studies have indicated that cells containing ecc-DNA are prone to develop diseases – for example, ecc-DNA with several 100kbp may increase the oncogenic potential of cells [32].

An oncogene is a gene "… that is a mutated (changed) form of a gene involved in average cell growth. Oncogenes may cause the growth of cancer cells "[33] Such mutations provide characteristics

to the sequence that render it gradually/completely resistant to epigenetic control mechanisms.

These findings apply to the connection of ecc-DNA in "oncogene amplification" [34], the generation of tumors [35-39], their pathogenesis [40-42], and their acceleration [43].

Ecc-DNA is particularly abundant in multiple human cancer cells, although its frequency varies among different tumor types. Elevated levels of ecc-DNA have been considered an effective biomarker of cancer pathogenesis. Reports have demonstrated the amplification/evolution of oncogenes and therapeutic resistance genes located on ec-DNA [44-46]. The molecular cascades from proto-oncogenes to functional oncogenes seem ordinary events that may drive intratumor genetic heterogeneity. Are "tumor suppressor genes" effective on "oncogenes" encoded in both chromosomal and extrachromosomal DNA? Specific details have revealed how ecc-DNA "remodel" genomic DNA [47]. ecc-DNA also has implications in degenerative processes in the central nervous system [48]. Their features might provide the basics for developing biomarkers [49] and applications designed for diagnostic purposes [50,51]. Diagnostics based on cell-free, circulating tumor DNA (ct-DNA) in the plasma should be standardized when evaluating samples from the serum/plasma of patients presumed or already confirmed to have cancer [52-54]. Is discrimination required to determine the origin of the selected sequence, namely from chr-DNA (genes in pieces) or ecc-DNA (composed of sequence pieces of chromosomal basis)?

Exceptional case/s?

Ecc-DNA is also detectable in healthy individuals' Peripheral Blood Mononuclear Cells (PBMCs). The investigated samples contain a sequence that is part of the 5′-non-coding region (5'-NCR) of the Hepatitis C Virus (HCV), a single-stranded RNA virus but not a retrovirus! This DNA sequence section is part of the Internal Ribosomal Entry Site (IRES) of HCV [55]. First, it shows individual patterns of methylation (methylomes)-epigenetically controlled? [56]. Second, the methylation patterns of this HCV-DNA sequence section have changed over time. A possible interpretation of this phenomenon is that the changing methylation patterns of this sequence may indicate a particular active control mechanism. The origin of this sequence section of the 5'NCR present in the ecc-DNA may be from a chromosomal basis [57].

The possible impact of ecc-DNA on the Human Genome Project (HGP)

The goals of the HGP are to reveal the DNA sequences of human chromosomes as a basis for gene identification. The constitutive considerations about a human reference genome [58] do not mention ecc-DNA as a possible falsifying factor on the chromosomal sequences. The point is that only the sequestration of ecc-DNA from chr-DNA may warrant pure/genuine chr-DNA sequences. The ECCs plorer protocol [59] may be an appropriate tool for this task. A database for ecc-DNA is available [60]. In consideration of new details regarding ecc-DNA, there are requests for a new human reference genome [61]. Concerning the human genome project using whole cellular DNA: How was the possible mixing of sequences of chr-DNA and ecc-DNA excluded when designing the human reference genome?

Final comment

Ecc-DNA is present in eukaryotic cells, but what induces their generation is unclear: individual genetic rearrangements due to endogenous triggers, exogenous elicitors such as "onco viruses," e. g. EBV? So far, the ongoing research has drawn attention to ecc-DNA because of its involvement in cancer development and possible use in diagnostics. It seems appropriate to consider testing for ecc-DNA with oncogenic coding potential in individuals suspected of having a tumor.

Perspectives: Eukaryotic cells can contain high numbers of ec/ecc DNA with coding sequences. Therefore, the definite separation of ec/ecc DNA from chromosomes must be guaranteed before "genome sequencing" for the HGP to rule out any possible mixing with chromosomal DNA. This occurrence also applies to the re-incorporation of eccDNA into chromosomes [62]. Besides the chromosomes, the eccDNA fraction should be considered when performing diagnostics based on the patient's sequence data.

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