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Fundamentals of extrachromosomal circular DNA in human cells - Genetic activities as regards cancer promotion alongside chromosomal DNA

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Abstract

In addition to chromosomal DNA (chr-DNA) and mitochondrial DNA, eukaryotic cells contain extrachromosomal DNA (ec-DNA). Analysed extrachromosomal circular DNA (ecc-DNA) accounts for up to 20% of the total cellular DNA. Ecc-DNAs contain coding and non-coding sequences originating from chr-DNA and mobile genetic elements (MGEs). MGEs include sequences such as transposons, which have the potential to move between different and the same DNA molecules, thereby, for example, causing rearrangements and inactivation of genes. Ecc DNAs have aroused interest in diseases such as malignancies and diagnostic procedures relating to this. A database to collect ecc-DNA has been established. Investigations are needed to find possible differences in sequences of chr-DNA after sequencing the whole cellular DNA (WCD), namely: chr-DNA plus ec-/ecc-DNA compared to chr-DNA, which is separated from ec-/ecc-DNA. Standards for sequencing protocols of WCD have to be developed that also reveal the sequences of ecc-DNA; this concerns “single-cell genomics” in particular.

Keywords: Extrachromosomal Circular DNA; Oncogenes; Cancer Promotion; Whole-Genome Sequencing (WGS); Database.

1. Facts on ec-/ecc-DNA

It is a long-known fact that eukaryotic cells, besides chr-DNA and mitochondrial DNA (mt-DNA), contain extrachromosomal DNA (ec-DNA) or extrachromosomal circular DNA (ecc-DNA) [1, 2, 3]. Microvesicles [4] and “extracellular vesicles” [5] containing ec-DNA constitute a particular situation. Mainly, research has focused on explicitly termed extrachromosomal circular DNA (ecc-DNA) in tissues of healthy human subjects [6] and plasma [7, 8] of human origin. Moreover, research has focused on oncogenes [9] in cells containing ec-/ecc-DNA with sequences that code for them [10].

The size of ecc-DNA ranges from about 2 kbp to ≥ 20 kbp [11], 35 ± 15 kbp [12] up to 1–5 Mbp [13]. The sizes may depend on the source of cells: the smaller ones up to 100 kbp in healthy cells [12, 14]. Their share of the total cellular DNA varies from approximately 2% to 20% [12, 15], depending on the subjects, tissues, and cells tested. The estimated number of ecc-DNA molecules ranges from at least 100 per cell and up to “thousands in cancers and cell lines” [16] depending on cells or tissues studied. Numerous reports indicate that tumor cells contain the highest share of ecc-DNA of the whole cellular DNA (WCD). Sparse information is available on the generation of ec-/ecc-DNA [17], in particular, concerning the transfer of chr-DNA derived sequences coding for oncogenes to ecc-DNAs [18].

Regarding the development of ec-/ecc-DNA from chr-DNA, there are various indications, such as “abnormal repair mechanisms”, “genomic rearrangements ... involving circular DNA”, and “self-joining” in the cited references. Models of formation are proposed, focusing on cancer pathogenesis [19] and following “environmental stress” [20]. For these

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processes, mobile DNA [21] or mobile genetic elements (MGE) may provide linking mechanisms between chr-DNA and the generation of ec-/ecc-DNAs. Because of their known and proven arsenal of corresponding elements (see below), they can contribute to transpose/move DNA sequences between different locations and join them to functional units.

2. Facts about DNA sequences contained in ec-/ecc-DNA

The different types of sequences in the ecc-DNA fraction indicate that they are derived from chr-DNA [3, 22]. This also applies to different types of sequences, which are referred to as MGE. MGE is a collective term for various genetic elements, such as Alu elements [23] and transposons. Estimates on their share of the WCD range from 50 to 80%. MGE stands for DNA sequences with transposable potential known for their ability with the help of encoded enzymes upon stimulation to cut out sequence sections of chr-DNA and reintegrate them — randomly or purposefully? — at different locations of the chr-DNA (level 1) or into ec-/ecc-DNA (level 2) and vice versa (“jumping genes”); these kinds of rearrangements could possibly influence adjacent sequences containing regulatory or coding sections.

Reports support the interpretation that stressful situations [24] on cells can cause various genetic changes. The stress events focus on the effects such as environmental processes and carcinogens, leading to changes in the chr-DNA, such as genomic aberrations and instabilities. Here, a broad consensus concerns the activation of proto-oncogenes to form oncogenes [9, 25]. The activation sequels and the metabolic consequences have drawn interest in the context with ecc-DNA.

Furthermore, sequences of a different kind exist with the potential to transpose or to regulate:

Sequences of human endogenous retroviruses (HERV) share about 8% of the human genome, and some of them are active [26]. Hints suggest “Tumor microvesicles contain retrotransposon elements ...” [4] and findings indicating that HERV-K elements may also be contributing to variations within the human genome [27].

Viral DNA sequences, with potential regulatory function due to the individual pattern of methylation, are present in the ecc-DNA fraction of peripheral blood mononuclear cells (PBMCs) of healthy subjects [12].

3. Ecc-DNA associated with diseases focussing on cancer

Apart from sequences of “chromosomal tandem repeats” [3], there are others with coding, promoting, and regulative potential assigned to ec-/ecc-DNA, which are known in the context of the development of tumors.

Thus far, the knowledge gained from research into ec-/ecc-DNA has deepened the understanding of how tumors (all?) may develop. The ecc-DNA in tumor cells contains sequences coding for oncogenes and is expressed in transcriptomes [28, 29, 30, 31]. Furthermore, this concerns indications for “poorer outcomes” for various kinds of cancer patients [32], autoimmune disease [33], the involvement of ecc-DNA in “neurodegenerative” processes [34], and their “accumulation” in aging cells [13].

Although not systematically studied, it is believed that the composition and sequences of ecc-DNA change over time, causing extraordinary heterogeneities, and that it is also dependent on the tissue. Whether the high amount of ecc-DNA in cancer cells and “aging cells” is due to self-replication has not been sufficiently clarified yet [19]. Beyond the involvement of cancer-promoting properties, do ec-/ecc-DNAs have regular functions in the metabolism of cells controlled by a particular type of genetic communication? An indication for this situation may be the individual patterns of methylation of sequences of the 5'-NCR of the HCV contained in the ecc-DNA of healthy subjects' PMCs [12].

4. Ecc-DNA and whole-genome sequencing (WGS)

The ongoing processes for reorganizing the chr-DNA sequences are caused both by internal programming and probably the interactions between ec-/ecc-DNA segments and chr-DNA.

Based on the respective facts, the following situations should be considered paradigmatically. WCD subjected to sequencing protocols contains both chr-DNA and ec-/ecc-DNA; in particular, in cells of cancer tissue ec-/ecc-DNA containing coding sequences regarding the promotion of cancer are enriched. If ec-/ecc-DNA sequences after the WGS are not displayed, the results may be missing crucial information for medical purposes. Therefore, given such significant sequences, protocols for cell genomics have to be addressed. Does the exclusive use of WCD for WGS consider ecc-DNA reliably besides chr-DNA sequences? For example, regarding: i) the human genome itself, ii) for basic research, as well

as for iii) targeted medical purposes, such as finding sequences, single ones or more being functional in clusters for certain genes that are or could be expected to be involved in diseases, diagnostics and therapy [35].

A special field of research is already based on single-cell genomics [36, 37]. However, caution is advised without considering: i) different amounts of ecc-DNA per cell, ii) their various genetic information, and iii) the use of single-cell diagnostics. The genetic heterogeneity of ecc-DNA in different cells, e.g., cancer cells, provides an unknown interpretation potential depending on the randomly caught cell(s). Single-cell genomics not considering the ecc-DNA are not appropriate to allocate the “clonal diversity to chromosomal and/or ecc-DNA [38].

5. Challenges

There are genetically active sections within the ec-/ecc-DNA fractions, such as those sequences promoting tumors. Consequently, the basic information obtained so far should enable the development of diagnostic tools, and concerning medical aspects such as therapy [39]. Depending on the subject/patient tested, if the results of WGS do not designate corresponding sequences of ecc-DNA, relevant information about the particular malignancy may be lost.

6. Clarifications are needed

Regarding the human genome, articles on “genes in pieces” based on the intron/exon postulates should also indicate whether the studies on human genomes have considered the possible influence of the known high shares of ec-/ecc-DNA [40] and their “reintegration” into the linear genome [29].

Given the ongoing alteration of genomes, the elaborated results after sequencing the WCD by WGS protocols seem to be just snapshots of just existing sequences of the chr-DNA. This situation applies to human “reference chromosomes” [41]. A protocol for the “...detection of ecc-DNA from NGS data” has been developed [42].

In particular, this holds for studies on cancer cells not considering ec-/ecc-DNA and the “single-cell” genomics [38]. A database for sequences in ecc-DNA has been established [43].

7. Conclusion

Ecc-DNAs can contain oncogene-coding sequences relevant for the promotion of cancer; ecc-DNA may be suitable to gain indications for the clinical-stage, therapeutic decisions, and drug resistance. Ecc-DNA should be considered in the respective diagnostics for patients with suspected cancer. The information obtained in this way reflects the most current and individual status.

Investigations are required to gain clarity as to whether sequences contained in the ec-/ecc-DNA fraction can affect the interpretation of sequences provided by sequencing the whole DNA (WDS) extracted from cells. Depending on the results, binding standards should be developed for protocols that determine sequences from human chr-DNA and ec-/ecc-DNA. Only experimental controls with proven facts meet scientific standards.

Abbreviations

Alu sequence elements, characterized by the restriction enzyme Alu I; ec-DNA, extrachromosomal DNA; ecc-DNA, extrachromosomal circular DNA; chr-DNA, chromosomal DNA; HCV, hepatitis C virus; HGP, human genome project; LINE: long interspersed element; MGE, mobile genetic elements; 5'-NCR, 5'-non-coding-region of the HCV-genome; PBMC, peripheral blood mononuclear cells; SINE: short interspersed element; WCD, whole cellular DNA; WGS, whole-genome sequencing

Compliance with ethical standards:

Disclosure of conflict of interest

No conflict of interest.

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