CirDNA Metabolism and Biological Role

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Circulating DNA has already proven itself as a valuable tool in translational medicine. However, one of the overlooked areas of circulating DNA research is its association with different proteins, despite considerable evidence that this association might impact DNA's fate in circulation and its biological role. The colorful history of circulating DNA (cell-free DNA and cell surface bound DNA, which hereafter will be referred to as cirDNA) research and attempts of its use in the field of oncology went from being skeptically discarded to becoming a valuable tool in clinical oncology.

cirDNA blood cell cfDNA ctDNA liquid biopsy

1. Immunostimulatory Characteristics of Circulating DNA

DNA is a macromolecule with immunostimulating properties. This immune response stimulation is based on its double-stranded structure, certain motifs of some sequences, and molecular interactions [1][2]. CirDNA can be perceived by the immune system as a molecular fragment associated with damage, which involves it in the antibacterial and antiviral immune response [3]. Indeed, immune cell interaction with dsDNA leads to the significant activation of genes that regulate the secretion of interferons and other pro-inflammatory molecules. Such stimulation leads to a strong inflammatory response mediated by the secretion of cytokines. CirDNA of nuclear, mitochondrial, and bacterial origin has been shown to similarly stimulate coagulation and platelet activation, but has different effects on inflammation and immune system stimulation [4].

CirDNA can activate the immune system both on its own and in combination with other molecules [2][5], and the immunostimulatory effects directly depend on the form of circulation of cirDNA and chromatin [6]. Histones are cytotoxic for the endothelium and can cause macro- and microvascular thrombosis and renal dysfunction. However, circulating nucleosomes activate different biological pathways upon contact with cells, without the strong cytotoxic effect characteristic of freely circulating histones [3][4][5][6]. Moreover, cirDNA in complexes with histones leads to the induction of anti-DNA antibody production, while the action of blood DNases, on the contrary, inhibits this induction.

There is mounting evidence in the literature that the penetration of cirDNA into the cell and the subsequent triggering of inflammatory biological pathways occurs due to the action of a number of DNA-binding proteins, including histones. Some nucleosome-binding proteins (HMGB1, RAGE) are able to regulate the immunostimulatory effects of both free cirDNA and nucleosomes [7][8][9]. Thus, the form of DNA circulation closely correlates with its biological effects.

2. Role of Circulating DNA in Horizontal Gene Transfer

In addition to the participation of cirDNA in intercellular communication, regulation of inflammation, and the immune system response, various forms of DNA circulation have been described to affect pathophysiological processes associated with the development of malignant neoplasms.

The hypothesis about the transforming ability of cirDNA was first proposed in 1965 [10]. Later, this hypothesis was confirmed in a number of studies and led to the formation of the concept of genometastases: ctDNA is able to end up in healthy cells and lead to malignant transformation [11]. The first work in this field was the transformation of NIH/3T3 mouse fibroblasts in the SW480 culture medium via cirDNA [12]. NIH/3T3 not only went through a malignant transformation after the incubation with SW480 media, but also carried a *KRAS* mutation characteristic for SW480. This effect was also described after the incubation of NIH/3T3 with *KRAS*-positive colorectal cancer patients' plasma [13]. Moreover, circulating nucleosomal complexes secreted by tumor cells have also been shown to be capable of transferring genetic information to a recipient cell and transforming them into malignant ones. Wang et al., in a 2018 study, demonstrated that, in response to chemotherapy, apoptotic lung cancer cells released HMGB1-containing nucleosomal complexes that mediated tumor invasion and metastasis via TLR4 and TLR9 [14].

Chen et al. [15] suggest that oncogene-containing ctDNA can behave like an oncovirus and transfect normal cells, leading to metastasis.

This hypothesis expands the concept of "genometastasis", where the source of oncotransformation is apoptotic bodies' DNA. Moreover, since there are DNA-binding receptors on the cell surface [16][17][18], the authors suggest tissue-specific metastasis formation In addition, it has been suggested that the cirDNA of normal cells (for example, DNA released into the bloodstream by lymphocytes as a result of antigen stimulation) can transfect tumor cells. In particular, integration of the cytokine-coding region containing cfDNA into a tumor cell genome can lead to the expression of various cytokines, such as interleukin 2, interleukin 12, macrophage colony-stimulating factor, etc.; cell-free DNA containing an unmutated oncogene (e.g., *ras* gene) or an unmutated oncosuppressor gene (e.g., wild type *p53* gene) can result in knockout via homologous recombination of the corresponding mutant oncogene or suppressor gene within the cancer cell and, consequently, to apoptosis of the tumor cell or even spontaneous remission of cancer [19].

The authors [15] explain the phenomenon of the presence of cirNA on the blood cell surface in healthy donors by the ability of cirDNA to bind to receptors on the surface of leukocytes [20], and the decrease in its content during the development of a tumor [15] is explained by the absence of a DNA receptor on the surface of cancer patients blood cells, or loss of DNA-binding properties due to mutation. Apparently, cirDNA is a signaling molecule in the bloodstream, and its binding to a specific receptor on the surface of lymphocytes can lead to cell activation and the emergence of an anti-tumor immune response. Thus, mutation of the DNA receptor on the surface of lymphocytes can lead to tolerance of the anticancer immune response.

3. Role of Circulating DNA in Angiogenesis and Blood Coagulation

In addition to invasion and metastasis, several studies have demonstrated the potential involvement of cirDNA as part of nucleosomal complexes in angiogenesis. Nucleosomes contribute to an increase in the expression of IL-8 (which is involved in the early stages of angiogenesis) by binding to the endothelial cell surface with subsequent activation of the NF-κB/Rel-A pathway [21]. These findings might help explain why hypoxic and hypervascular areas are often found in close proximity in tumor tissues, and may also point to a potential role for nucleosomes in disease progression [22]. Further indirect evidence of the circulating nucleosome participation in angiogenesis is their ability to bind heparin-binding angiogenic factors such as FGF-1, FGF-2, VEGF, and TGFβ-1, stimulating angiogenesis in in vitro and in vivo systems [23].

Recently, cirDNA has been shown to participate in blood coagulation [24]. Evidence that supports this asseveration is that purified genomic DNA increases the activation of proteases that participate in the blood clotting pathway, such as the coagulation factors XII and XI. Moreover, cirDNA from activated neutrophils that are part of the NETs trigger blood clotting that relies on FXII and FXI. Furthermore, it has been observed that histones interact with the A1 domain of the von Willebrand human factor, which can propagate platelet adhesion mediated by GPIbα [24].

4. Blood Circulating DNA Clearance

The amount of cirDNA in circulation is determined by the ratio between the release rates and the rates of its internalization, degradation, and elimination [25]. Under the conditions of cancer progression, chronic inflammation, and increased cell death, due to a shift in this ratio towards increased release and insufficient elimination, an increase in the level of cirDNA in the circulation is observed.

CirDNA half-life in the blood has been estimated by different sources to be from several minutes to up to two hours [3][26][27], and depends on a number of factors, including the form of cirDNA circulation, the type and stage of the disease, the effectiveness of treatment, etc. [1][28]. Degradation and elimination of blood cirDNA are carried out via several mechanisms: degradation by blood plasma endonucleases [29], formation of immunological complexes [30], phagocytosis and lysosomal degradation [31][32][33], metabolism by liver cells [33], and direct elimination of nucleosomal complexes in the kidneys [34][35]. The main role of the blood cirDNA degradation is attributed to circulating enzymes such as DNAse I, FSAP, and factor H [3][36][37]. Moreover, blood proteases have been shown to indirectly affect the levels of nucleoprotein complex cirDNA by hydrolyzing proteins and increasing the availability of nucleic acids for blood nucleases: DNA-protein complexes and protruding DNA pieces are cleaved first, followed by further digestion of better-conserved DNA within nucleosome complexes [29][38][39][40].

Thus, despite the study of the cirDNA phenomenon for more than 70 years, many questions, such as its biological role in circulation and the contribution of various forms of DNA circulation to physiological and pathological processes, remain open.

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