Biology

Liquid Biopsy

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Abstract

Nowadays, non-invasive diagnostic methods are becoming more and more incorporated into clinical practice. Non-invasive simply means, no introduction of instruments into the human body is involved, which results in lower health risks. These methods are faster, less painful and much more comfortable for the patient, when compared to invasive ones. The question is, can this non-invasive approach be used in cancer diagnostics?

1 Molecular characteristics of cancer

Cancer is a multifactorial disease, associated with genetic changes, especially in genes which control cell growth and division. Typically, gain of function mutations in proto-oncogenes and loss of function mutations in tumor suppressor and DNA repair genes. Affected cells, then, undergo neoplastic transformation, which leads to changes in cell proliferation, growth and differentiation [1,2]. Cancerous cells are less differentiated, uncontrollably proliferating and thus are somehow "immortal".

2 Standard biopsy

In oncology, standard biopsy is a procedure that involves removing a sample of cancer tissue or cell sample. This procedure is invasive, time-intensive, can't be repeated often and does not represent tumor heterogeneity. Heterogeneity exists not only between primary tumor and distant metastasis but also within the same tumor, so it is called intratumor or spatial heterogeneity [3]. Obtaining a complex genetic profile is limited by tumor accessibility and by clinical complications associated with this invasive procedure [4]. Other disadvantages of surgical biopsy are pain, health risk, discomfort for a patient and economic aspects. The procedure itself may also increase the risk of "seeding" malignant cells along the tract of a biopsy needle [5]. Nevertheless, a tissue biopsy is still "gold standard" for tumor characterization, but is there any other way to identify the genetic profile of a particular tumor? Yes, there is an easier, non-invasive way called liquid biopsy.

3 Cell-free DNA

Before explaining something about liquid biopsy, we need to know what cell-free DNA (cfDNA) is. It is degraded, fragmented, non-cellular DNA released into our bloodstream. In healthy individuals, cfDNA concentrations tend to range between 1 and 10 ng/ml^{-1} in

plasma [6,7]. Increased concentration of cfDNA is common in non-malignant, pathological processes such as trauma [8], myocardial infarction [9], ischaemic stroke [10] and even during exercise [11]. But these fragments are not only derived from our own somatic cell. Cell-free DNA was found in patients with organ transplantation, derived from a transplanted organ (donor derived cell-free DNA =dd-cfDNA) [12], during pregnancy, as short, fragmented DNA of the fetus (cell-free fetal DNA = cffDNA) [13] and in cancer patients, DNA fragments originating in the tumor (circulating tumor DNA = ctDNA) [14]. Especially ctDNA is our DNA of interest when speaking about liquid biopsy.

4 History of cell-free DNA

Cell-free DNA was firstly identified in 1948 by Mandel and Métais in the blood of healthy individuals [15]. Almost 30 year later, Leon et.al. pointed out increased concentration of cfDNA in cancer patients [16]. More than a decade later, Stroun et al. demonstrated the presence of neoplastic characteristics in the circulation. They found that the circulating DNA showed some double-stranded instability that is specific for tumor DNA [17]. Definitive proof, that cfDNA in the plasma can be of tumor origin, was achieved after Sorenson et al. and Vasioutkin et al. detected KRAS mutations in the circulation of pancreatic cancer patients [14] and NRAS mutations in patients with acute myeloid leukemia[18].

5 Liquid biopsy

Liquid biopsy is an alternative to standard, surgical biopsy and gives us information about tumors through a simple blood sample. Blood of oncology patients contains cancer-derived material circulating in the bloodstream, circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA). CTCs are cells released by primary tumor into peripheral blood [19] and ctDNA is shed into the bloodstream by tumor cells undergoing apoptosis or necrosis or via active secretion. Apoptosis generates DNA fragments (cfDNA, ctDNA) of 70-200 bp, but mainly ~166bp (and multiples of it) corresponding to nucleosomal DNA, while necrosis creates much larger fragments. Especially, fragments longer than 10,000 bp are likely to originate from necrotic cells. In general, non-cellular DNA fragments are shorter in oncology patients than in healthy individuals. [6, 13, 20-22].

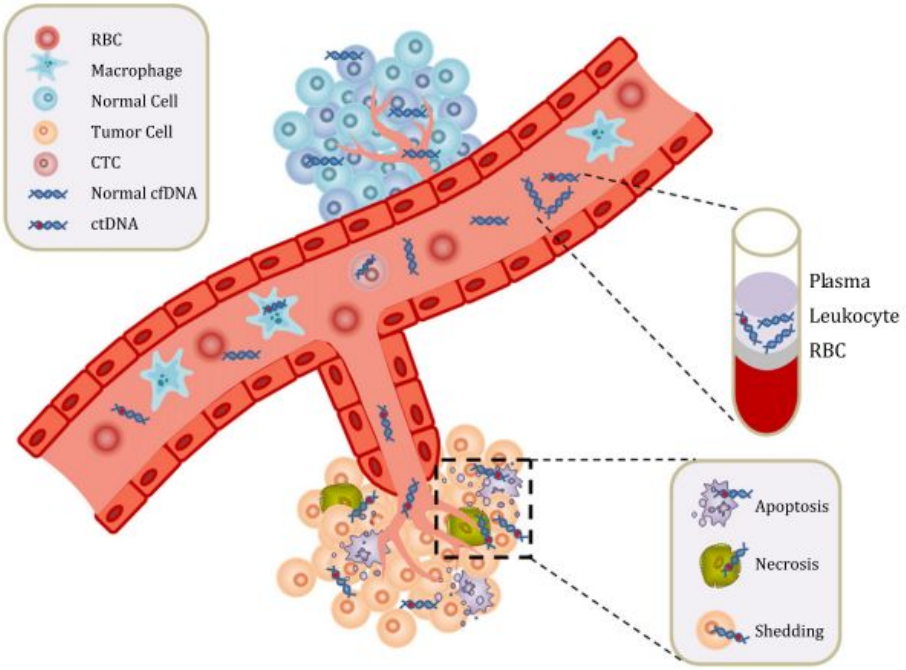

Figure 1: CTCs, normal cfDNA and ctDNA are found in plasma of cancer patients (Credit: Hahn et.al., 2019)

Circulating tumor DNA is found in the cell-free fraction together with cfDNA. Separation ctDNA from normal cfDNA is based on the presence of oncogenic mutations. These mutations are present in malignant cells only, not in normal, somatic cells of the same individual. Fraction of ctDNA can greatly vary from 1% to 90%, depending on tumor size, proliferative stage, level of vascularization, type, duration and response to treatment, etc. [22, 23]. ctDNA contains molecular information about the tumor and therefore it could have a potential as a molecular marker for the cancer diagnostics and monitoring.

In healthy individuals, DNase is responsible for degradation of cfDNA in the bloodstream, but in cancer patients the activity of this enzyme is lowered [24]. This means that ctDNA can be isolated and diagnosed using genomic and molecular biology methods. In oncology, liquid biopsy may find its clinical application in various clinical settings, including cancer diagnosis, prognosis, therapy monitoring and detection of minimal residual disease. When compared to standard tissue biopsy, it can overcome heterogeneity and give us more complex information about tumor genetic profile, metastasis and cancer recurrence. However, analytical and clinical validity of liquid biopsy must be rigorously demonstrated before it can be fully realized in clinical practice.

For references, click here.