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Archives of Obstetrics and Gynaecology Review Article

Circulating Cell-Free RNA: A New Perspective for Endometrial Cancer

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Abstract

In order to implement the knowledge of cancer to monitor its evolution and setting, in the last decade, new minimally invasive and repeatable samples collection have been developed such as liquid biopsy. Cancer biomarkers originating from tumors can represent the molecular status of the tumor or its metastases which release them directly into body fluids or indirectly due to disruption of tumor/metastatic tissue. These biomarkers are detectable in liquid biopsy.

Recently, circulating RNAs (cfRNAs) are increasing their importance as biomarkers in liquid biopsy for cancer patients both for tumor characterization and development and for setting a monitoring personalized therapy. Circulating RNAs are represented by a broad range of subtypes, such as microRNA (miRNAs), long non coding RNA (lncRNAs), messenger RNAs (mRNAs), transfer RNAs (tRNAs), snRNAs (small nuclear RNAs), snoRNA (small nucleolar RNAs), piwiRNAs (piRNA) and circRNAs (circular RNAs). In endometrial cancer only some of these cf-RNAs have been investigated, no studies have been performed on snoRNAs, piRNAs, snRNAs and tRNA, the most analyzed one is represented by cf-miRNA in plasma. These findings open an immediate use of selected cf-miRNA as biomarker in liquid biopsy of EC and suggest further investigation of the other cf-RNAs to implement the knowledge in this fascinating field.

Keywords: Cell free RNA (cfRNA), Circular RNAs (circRNAs), Liquid biopsy, Biomarker, Endometrial cancer

Background

In order to implement the knowledge of monitoring cancer evolution and setting new targeted therapies for monitoring the development of the neoplasia and the efficacy of treatment, in the last decade new minimally invasive and repeatable samples collection have been developed in synergy with the evolution of imaging evaluation. In particular, the most relevant integrating procedure is represented by liquid biopsy that is represented by the collection of biological fluid by not invasive (saliva and urine) or minimally invasive (blood, ascetic fluid) procedures.

Cancer biomarkers originating from tumors can represent the molecular status of the tumor or its metastases which release them directly into body fluids or indirectly due to disruption of tumor/metastatic tissue [1,2]. These biomarkers are detectable in liquid biopsy, include circulating tumor cells (CTCs), proteins, cell free DNA (cfDNA) and RNA (cfRNA) that recently have acquired interest in cancer for their role in diagnosis and treatment response [1,3].

Circulating RNAs and Cancer

Recently, circulating RNAs (cfRNAs, circulating cell free RNA, ccfRNAs) are increasing their importance as biomarkers in liquid biopsy for cancer patients both for tumor characterization and development and for setting monitoring personalized therapies [4]. Circulating RNAs are represented by, such as for intercellular RNAs, a broad range of subtypes, mainly represented by microRNA (miRNAs), long non coding RNA (lncRNAs) and messanger RNAs (mRNAs). Even though the role of circulating non coding RNAs as miRNA and lncRNA

can be easily understood taking into consideration that can be secreted and persist in biofluid in remarkably stable forms, as "free" or associated to exosomes, less is known concerning the role of circulating mRNA, mainly taking into consideration its instability and the presence of RNase, nevertheless its presence is detected in blood. Like intercellular RNAs, circulating RNAs participate in numerous biological process and express aberrantly under abnormal or pathological status. The quality and quantity changes of circulating RNAs, mainly miRNA, are broadly investigated and associated with the initiation and progression of cancer [5].

In particular, the discovery of circulating miRNA happened in 2008 [6] and during the last decade, several circulating RNAs have been identified in plasma and serum and many articles demonstrated their ability to discriminate healthy individuals from cancer patients affected by breast [7], colorectal [8], gastric [9], lung [10], pancreatic [11], and hepatocellular [12] cancers. Moreover, these findings re-evaluated previous discoveries related to circulating mRNAs, that were found, for the first time in 1999 in plasma of nasopharyngeal carcinoma patients [13]. Interestingly, despite the relatively long history of circulating mRNA discovery, this field has not translated into clinical practice, or captured the imagination of the scientific community in the same way as miRNAs. Instead, the scientific community was fascinated by lncRNAs [14]. Few is known concerning the other non coding RNAs (ncRNAs) in cancers, such as small nuclear RNA (snRNA), small nucleolar RNAs (snoRNAs) and piwi RNAs (piRNAs). In particular, snRNA U2 was found to be increased in the blood of patients with ovarian cancer as well as being linked with the responsive to chemotherapy [15], and six small nucleolar RNAs (snoRNAs) were up-regulated in the plasma of non-small cell lung cancer [16], but no information was reported concerning piRNAs or other forms of ncRNA in biological fluids [14].

Nevertheless, the evaluation of ccfRNA in terms of quantity and feasibility is still not properly set, due to the sensitivity of this biomolecules that is influenced by environmental change and their intrinsic instability, presence of RNase in the blood, weak regulation of RNA stability from sample collection to RNA extraction and the sharing of standardized protocol and the difficulties to manage human samples among clinicians and laboratory focused on the quality of Nucleic acids and the need of patient's care and treatment.

The analysis of cfRNAs represents a biomarker that can give complementary information concerning gene expression profiles or epigenetic alterations in comparison to primary tumor tissue [1]. Today, cfRNAs are considered biomarkers because of their abundance, specific tumor profile and ability to regulate gene expression [1,2]. The specific mechanism of cfRNAs release in body liquids is still unknown but, as for cfDNA [1,17], they can be released through apoptosis and/or necrosis [1,18].

The fist cfRNA discovered was the cf-mRNA, in 1999, as mentioned above, in the plasma of nasopharyngeal carcinoma patients [13,18], followed by the discovery in melanoma patients' serum [18,19]. However, cf-mRNAs are fragmented and the least abundant, so their detection is difficult [20]. Moreover, they are very instable because of their sensibility to the degradation induced by RNAses present in blood [18,21,22]. Despite these limitations, the presence of cf-mRNAs was found in oncological patients, characterized by high levels in plasma, and these findings suggest a role in the diagnosis and monitoring of cancer [20].

The most known class of cfRNA is represented by circulating free miRNAs (cf-miRNAs), discovered for the first time in the blood of prostate cancer patients [1,23]. They are more stable than mRNAs due to the fact that they are resistant to degradation and RNase activity at room temperature and insensitive to pH changes [18,23]. Moreover, frequently they are complexed with (lipo) proteins or platelets or contained in exosomes which can protect miRNAs from RNAses digestion [1]. miRNAs are endogenous small ncRNA (non-coding RNA) molecules of 19-22 nucleotides of length that post-transcriptionally regulate protein-coding gene expression and gene silencing, binding to specific sequences on target genes [18,20,24,25]. miRNAs have a tissue-specific pattern and they have an aberrant expression in cancer where they play a role in oncogenic pathways (like Ras and Nf-kB) [18]. For this reason, they can be defined as biomarkers in several cancers (liver, prostate, bladder, lungs, breast, ovary), if they are present in liquid biopsy [18,26]. However, it is important to evidence that the same miRNA can be detected in different type of cancer so, for discriminating different tumors and diagnostic purpose, it is necessary to analyze a specific miRNA panel [18].

Another interesting class of RNAs are Small Nuclear RNAs (snRNAs), molecules of 150 nucleotides of length. They are usually located in the cell nucleus and are involved in mRNA processing, spliceosome assembly and translation process, their name derives from this localization [18,27]. Alterations in snRNA expression could be implied in oncogenic processes [18,28]. Various cell free snRNAs (cf-snRNAs) have been studied in liquid biopsy as cancer biomarkers but the most known is the family defined "U": U2 shows altered levels in serum or plasma of different cancers like pancreatic and colorectal adenocarcinoma [18,29], while U6 is overexpressed in the serum of breast cancer patients [18,30].

Similar but different to snRNAs are Small Nucleolar RNAs (snoRNAs), molecules of 60-300 nucleotides of length [26] involved in post-transcriptional modification of rRNA [18,26,31] and gene silencing [18,32], usually reside in the nucleolus. Mutations and aberrant expression of snoRNAs have been reported in cell transformation, tumorigenesis and metastasis, indicating that snoRNAs may serve as biomarkers and/or therapeutic targets of cancer [33]. Some snoRNAs have been studied and detected in liquid biopsy, principally in lung cancer. In particular, snoRD33, snRD66 and snoRD76 were found to be up-regulated in plasma of patients affected by non-small cell lung cancer [16,18].

Recently, a new class of RNAs have been discovered and investigated: the lncRNAs. They are non-coding transcripts longer than 200 nucleotides [34], physiologically present in different cellular compartments and characterized by a variable stability [18]. They are implied in gene expression regulation, protein biogenesis, chromatin remodeling, carcinogenesis and metastasis [18,34]. Indeed, it was demonstrated that they are involved in some oncogenic pathways like p53, NF-κB, PI3K/AKT, Notch [34]. Moreover, they can be found in body fluids and they are considered as cancer diagnostic and prognostic factors [18,35,36].

In addition, even transfer RNAs (tRNAs) usually considered stable RNA and used as housekeeping in gene expression evaluation, are investigated for their potential role in liquid biopsy for cancer detection. They consist in 73-93 length oligonucleotides involved in amino acids transfer to the site of protein synthesis [20]. Several studies have indicated that tRNAs play a role in modulating cell proliferation and cancer progression [37,38]. Some papers have demonstrated the presence of high level of fragments derived from tRNAs in plasma of liver [39] and colorectal cancer patients in comparison to healthy people [40], highlighting their potential as biomarker for cancer diagnosis [39].

The last class of cf-RNA discovered and with a putative role of biomarker in liquid biopsy, is represented by circular RNAs (circRNAs). These RNAs are characterized by the covalent linkage of 5' and 3' ends. The circular form makes them biologically stable and resistant to RNAses [18,41,42]. Their main function is gene expression regulation, competing with miRNAs activity; however, they also act as scaffolds, facilitate enzyme-substrate interaction and regulate alternative splicing [18,41,43]. The altered balance between circRNAs and their linear mRNAs promotes aberrant expression of oncogenes and tumor suppressor genes [18,44], participating in the initiation and progression of tumors [41]. There are a few studies about circRNAs in body fluids but they are found in plasma of gastric [18,41,45] and colorectal [41] cancer affected patients.

cfRNA and Endometrial Cancer

The endometrial cancer (EC) is the most frequent cancer that affects the female genital tract [46]. 90% of cases of EC occur in women over 50 years old with a mean age of 65 [47-49]. Although most ECs are diagnosed early, up to 10% of tumors are diagnosed at a late stage with a five-year survival of 16% [47].

In last years, new molecular techniques have led to cancer biomarkers research in liquid biopsy where the scientists can look for CTCs and cell free Nucleic Acids (cfNA). Cell free RNAs (cfRNAs) were found in several types of cancer but what about endometrial cancer? In general, RNAs found in body fluids, are good candidates as cancer biomarkers and their detection presents higher sensitivity and specificity than other molecules of interests (like DNA and proteins) [18]. In particular, some microRNAs (miRNAs) are stable in the majority of body fluids [18,50] consequently, they can be considered an interesting target to be investigated in endometrial cancer patients [51].

The first study analyzing circulating RNAs in liquid biopsy of endometrial cancer, was performed by Torres et al. that evaluated the expression of microRNAs in plasma by RT-qPCR showing high expression levels of miR-99a, miR-100 and miR-199b in plasma samples [51,52]. Subsequently demonstrated, on 34 EC patients, a down-regulation of miR-9 and miR-301b and an upregulation of miR-92a, miR-141, miR-200a, miR-203, miR-449a, miR-1228 and miR-1290 [53]. Regarding the association between miRNAs expression in plasma and clinicopathological characteristics, it was found that miR-9 expression was lower in Grade and miR-449a was upregulated in stage IA [53]. It is to be note that, the majority of the miRNA identified in these studies target a large number of genes involved in PTEN-PI3K-AKT-mTOR pathway, which it is known to play a key role in the development of EC [53,54].

The evaluation of miRNA expression in serum, showed that miR-222, miR-223, miR-186 and miR-204 were up-regulated [51,55]. During the last decade, many studies have been performed on cf-miRNA as reported and summarized by Delangle et al., aimed to associate each of them to specific clinical-pathologic characteristics of tumors, but there is no consensus concerning the association between miRNA expression and clinical pathological features [56].

Regarding cf-circRNAs, there are not many studies about the presence of circRNAs in plasma or serum, a

single study was reported by Ye et al. who analyzed many cf-circRNAs on fresh tissue from grade 3 EC patients [57].

Up to now, the presence of cf-snRNAs, cf-snoRNAs, piRNAs and cf-tRNA was not reported in liquid biopsy of EC.

Concerning cf-lncRNA, several studies are reported: Zhang et al. analyzed the expression of PMS2L2, a lncRNA, in plasma of EC patients. Its levels were correlated with patients' clinical stage and distant metastasis, but no relation was found with patients' age and tumor grades. PMS2L2 results downregulated in EC patients compared to healthy controls, suggesting this cf-lncRNA as a potential diagnostic biomarker in EC [58].

Conclusion

Since the first discovery of circulating miRNAs, there is a large amount of studies focused on their biological functions and the potential use of cfRNA as biomarkers in oncology. The state of art, concerning EC, as reported in this review the most investigated cfRNA is represented by cf-miRNAs.

However, these findings should stimulate the desire to further and deeper investigations in liquid biopsy of ECs and other cf-RNAs biospecimen. Most of them have demonstrated to be able to detectable changes associated to tumor and can represent a promising non-invasive biomarker for early cancer diagnosis, predictor of prognosis, and cancer treatment. However, several issues including technical and non-technical constraints need to be solved urgently. The further understanding of existing form in circulation and biological function, the deeper exploration of the underlying mechanism of release, transport, uptake, and the special status in cell communication are all essential before the breakthrough in the application of circulating miRNA-based cancer therapy [5].

In conclusion, at the moment, the most validated biomarker in liquid biopsy of Endometrial cancer is represented by cf-miRNA even if other studies need to properly define the specific panel associated to EC and focus on diagnostics and prognostics. However, the main goal of this review is to stimulate new findings for the evaluation of novel cfRNAs in order to offer different approaches for the evaluation and monitoring of EC by not by not invasive or minimally invasive (blood, ascetic fluid) procedures.

Authors' Contribution

FS and SP were involved in conceptualizing the manuscript; CS and NC revised the literature; NC and SP complied the manuscript. FM, SP and LA revised the manuscript. All authors approved submission of the manuscript. All authors have read and agreed to the published version of the manuscript.

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