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Liquid Biopsy in Patients with Thyroid Carcinoma

Ilze Fridrihsone, Arnis Abolins, Andrejs Vanags,
Dzeina Mezale and Guntis Bahs

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Abstract

Thyroid cancer is a comparatively rare tumor, which affects 1–5% of women and approximately 2% of men, although it is the most common endocrine malignancy worldwide. Furthermore, the incidence of thyroid cancer has been increasing remarkably in the last decades. Currently, diagnosis of thyroid cancer mainly is based on cytological criteria. Although fine needle aspiration is a minimally invasive procedure, complications can occur. Correct diagnosis is mandatory to select patients for surgical intervention and to determine appropriate extent of operation. Overdiagnosis and the associated unnecessary surgery should be avoided as it might also lead to complications. Therefore it is important to practice noninvasive methods not only for early diagnosis of thyroid cancer but also for estimation of prognosis. Liquid biopsy is a promising, noninvasive method that can provide detection of circulating tumor cells (CTCs) as well as circulating nucleic acids such as DNA, mRNA, and microRNA in a blood sample. The aim of the chapter is to highlight the efficacy of liquid biopsy for diagnosis and prognosis of thyroid cancer. The chapter will represent a comprehensive literature review based on recent PubMed publications (mainly 2012–2018).

Keywords: thyroid carcinoma, early diagnostics, molecular diagnostics, liquid biopsy, circulating tumor cells, circulating free DNA, circulating miRNA

1. Introduction

Thyroid cancer is relatively infrequent malignancy, which accounts for about 1–5% of the cancer cases in women and less than 2% in men [1]. Although, it is one of the most common cancer among endocrine malignancies accounting for more than 95% of new cases in the United States [2, 3], according to American Cancer Society, thyroid cancer in the United States in 2019

is estimated to be about 53,070 new cases (40,260 in men and 37,810 in woman), and more than 2000 people will die from the disease [3]. As well as in the rest of the world, the incidence of thyroid cancer has increased threefold over the past 30 years and is expected to increase by 50–60% between 2010 and 2020 [3–5]. The increase in thyroid cancer incidence rates could be explained by thyroid ultrasound screening with improved technical performance of the equipment, as well as better access to medical examination [1, 6].

Although, there are no significant rise in thyroid cancer mortality rates, growing detection of indolent forms may lead to overtreatment of the patients by performing unnecessary thyroidectomies [7]. General postoperative complications, such as fever, hemorrhages, infection, or cardiopulmonary and thromboembolic events, as well as specific complications such as hypoparathyroidism/hypocalcemia and vocal cord/fold paralysis can be seen. In large population-based study, 27,912 patients were included and analyzed. General postoperative complications were observed in 6.5% of the patients and surgery-specific complications in 12.3% [8]. The diagnostic gold standard for thyroid cancer is the evaluation of histological features, although there still are some differential diagnostic difficulties. To avoid complications, a new, more precise diagnostic tool is necessary to diagnose and manage thyroid cancer patients.

Liquid biopsy is a usual blood sampling method, referred to as a noninvasive procedure to detect components of the tumor which circulates in the bloodstream, for example, circulating tumor cells (CTCs), cell-free nucleic acids, exosomes, or tumor-educated platelets (TEPs); thus it can be a promising method in tumor diagnostics prior to surgery, as well as in monitoring of the disease [9].

2. Detection of specific particles by liquid biopsy

As previously mentioned, in liquid biopsy, circulating tumor cells (CTC), cell-free nucleic acids, exosomes, or tumor-educated platelets (TEPs) can be acquired and studied for different purposes [9, 10].

Circulating tumor cells are cells which detaches from primary tumor and deposits in a patient's blood. The described ability of CTC is particularly important to fully understand metastatic process; therefore a large scientific research field on this topic has evolved. It is believed that CTC can survive in the bloodstream because of the undergoing epithelial-mesenchymal transition (EMT). In this process tumor cells gain plasticity and motility which allows to extravasate from primary tumor into blood and intravasate into distant tissues as well. Detection of CTC can give valuable clinical information about patient's medical status—CTC can be used as a prognostic marker in different carcinomas including malignant thyroid tumors. Analysis of these cells can serve in treatment process as well—response to different pharmaceutical drugs in individual patient can be analyzed [9, 10].

Circulating cell-free nucleic acids (cfDNA, cfRNA, and cfmiRNA) discharge from apoptotic and necrotic tumor cells into the bloodstream. cfDNA originates not only from tumor cells but

also from non-tumor cells after exercise, trauma, or inflammation [10]. Extraction of circulating tumor DNA (ctDNA) alone is a difficult process; therefore mutation in cfDNA particles is being searched and indicates the presence of tumor. A lot of mutation has been analyzed in different types of tumors, for example, epidermal growth factor receptor mutation in non-small cell lung cancer. At present, it is the only liquid biopsy test that has granted and has FDA approval [9, 11].

By liquid biopsy, cfRNA can be analyzed but this particle is not as stable as cfDNA, therefore it is harder to investigate. Another circulating cell-free nucleic acid—microRNAs—is a new and more stable tumor marker in the blood. Exosomes are microvesicles (40–150 nm) that are released in the blood from tumors and normal cells as well. Exosomes contain proteins, DNA, RNA, miRNA, lipids, and metabolites. Tumor-educated platelets (TEPs) are anucleated cell fragments that can be educated by the transfer of tumor-associated particles, mostly RNA [9, 10].

3. Papillary thyroid cancer and liquid biopsy

The large increase in the incidence of thyroid cancer is seen in papillary thyroid cancer (PTC) which represents the major histological type [12]. PTC accounts for 85% of thyroid malignancy [13]. World Health Organization has defined PTC as a malignant epithelial tumor showing follicular cell differentiation and a specific signs of nuclear features which include nuclear enlargement and overlapping, irregular nuclear contours, and nuclear pseudoinclusions or nuclear grooves, as well as optically clear nuclei [14]. PTC measuring 1 cm or smaller in the greatest dimension is defined as papillary thyroid microcarcinoma (PTMC). It is suggested that incidence of PTC increased largely due to an increase in the incidence of PTMC, probably due to thyroid ultrasound screening with improved technical performance of the equipment, as well as better access to medical examination. Foci of PTMC have been reported in up to 22% of surgical thyroid specimens and up to 36% of autopsy series [6, 15].

The main therapeutic method which is used after the diagnosis of PTC is a total or subtotal thyroidectomy with or without radioactive iodine (RAI) and thyroid hormone suppression. Afterward monitorization of disease status is necessary for all the PTC patients which is carried out by measuring levels of serum thyroid-stimulating hormone (TSH) and serum thyroglobulin (Tg) in the blood. Neck ultrasonography is also performed to detect persistent or recurrent PTC nodules in the thyroid gland after treatment [16]. However, if thyroglobulin antibodies (TgAb) are found in the blood, serum thyroglobulin could not be used as a reliable tumor marker because of false negative rate. Furthermore, long time period is needed to observe the changes in the levels of serum TgAb, and this may lead to late diagnosis [17]. Therefore, other biomarkers need to be discovered and used to monitor persistent or recurrent disease.

A new diagnostic tool—liquid biopsy—can be used to analyze circulating tumor cells (CTCs) or circulating epithelial cells (CECs) and circulating cell-free tumor DNA (ctDNA) in the blood

of thyroid cancer patients. This minimally invasive diagnostic method has received a lot of attention over the past years and can also be used to analyze thyroid cancer patients [18]. However, in the case of thyroid carcinoma, only a few studies have been exploring the significance of CTCs in the blood. CTCs are malignant epithelial cells which can separate from primary tumor, invade blood and lymph vessels, and travel through the body to form distant metastases [19]. Salvianti with colleagues proved that quantity of cfDNA with integrity index 180/67 in thyroid cancer patients was higher than in those who had benign thyroid nodules. Therefore, cfDNA could be a suitable marker to diagnose thyroid cancer [20]. On the surface of CTC, epithelial cell adhesion molecule (EpCAM) is found in overexpressed state and therefore can be used for malignant cell visualization from a sample taken by liquid biopsy. Usually EpCAM is overexpressed on tumor cells. The visualization of CTCs can be performed with different antibodies, which allows visualize cells under a fluorescence microscope. In other studies, quantification of various messenger RNAs (mRNAs) is detected in the blood [19].

Not only mRNA but also microRNA could be detected in CTCs [21]. Biochemical alterations of cancer cells are largely supported by noncoding RNA (ncRNA) dysregulation in the tumor site. Noncoding RNAs lack an open reading frame and do not have protein-coding ability. Based on the size of the functional RNA molecule, regulatory ncRNAs are classified as long ncRNAs and small ncRNAs or microRNA (miRNA) [22]. miRNAs are small, evolutionary conserved, single-stranded, noncoding RNA molecules (approximately 22 nucleotides in length) that bind target mRNA to regulate gene expression [23, 24]. MicroRNAs are involved in various physiological and pathological functions, such as apoptosis, cell proliferation, and differentiation, which indicate their functionality in carcinogenesis as tumor suppressor genes or oncogenes [25]. Up- or downregulation of miRNA can influence the tumorigenic outcome depending on the role(s) of the target genes on vital signaling processes [26].

The most often upregulated miRNAs in papillary thyroid cancer (PTC) are miR-146b, miR-222, miR-221, and miR-181b. Overexpressed miR-146b targets retinoic acid receptor beta (RAR β) and causes reduced expression of this gene leading to increased tumor aggressiveness and extrathyroidal invasion. miR-221 and miR-222 target tumor suppressor and cell-cycle regulator p27. Reduced expression of p27 results in increased proliferation of tumor cells. These processes are related to aggressive behavior of tumor, extrathyroidal invasion, and the presence of lymph node invasion [21, 27–29]. miR-181b also is overexpressed in PTC compared to normal thyroid tissue. miR-181b inhibits expression of cylindromatosis (CYLD) gene which acts as tumor suppressor and normally induces cellular apoptosis [28, 30].

The most often downregulated miRNAs in PTC are miR-145, miR-451, miR-613, and miR-137. miR-145 acts a tumor suppressor in thyroid cancer, and downregulation leads to cancer growth through several pathways [28]. miR-451 acts as a tumor suppressor by targeting the PI3/AKT pathway. Downregulation of miR-451a is associated with tumor aggressiveness and the presence of extrathyroidal invasion [27, 28]. miR-613 is involved in PTC cell proliferation and invasion [28, 29]. In PTC, miR-137 expression is downregulated leading to increased cellular proliferation, invasion, and migration [28] (**Table 1**).

Diagnosis	Type of miRNA	Type of regulation	Outcome	References
Papillary thyroid cancer	miR-146b	Upregulated in cells	Aggressive behavior and extrathyroidal invasion	Rossi et al. [21] Rodriguez-Rodero et al. [27] Boufraqueh et al. [28] Chruscik et al. [29]
	miR-222	Upregulated in cells	Aggressive behavior, extrathyroidal invasion, and lymph node metastasis	Rossi et al. [21] Rodriguez-Rodero et al. [27] Boufraqueh et al. [28] Chruscik et al. [29]
	miR-221	Upregulated in cells	Aggressive behavior, extrathyroidal invasion, and lymph node metastasis	Rossi et al. [21] Rodriguez-Rodero et al. [27] Boufraqueh et al. [28] Chruscik et al. [29]
	miR-181b	Upregulated in cells	Decreased apoptosis of cancer cells	Boufraqueh et al. [28]
	miR-145	Downregulated cells	Regulates cancer growth	Boufraqueh et al. [28]
	miR-451	Downregulated cells	Tumor aggressiveness	Rodriguez-Rodero et al. [27] Boufraqueh et al. [28]
	miR-613	Downregulated cells	Increased cellular proliferation and invasion	Boufraqueh et al. [28] Chruscik et al. [29]
	miR-137	Downregulated cells	Increased cellular proliferation, invasion, and migration	Boufraqueh et al. [28]

Table 1. Expression of microRNA in PTC.

4. Follicular thyroid cancer and liquid biopsy

Follicular thyroid carcinoma (FTC) is the second most common type of thyroid cancer, comprising 10–15% of all thyroid carcinomas [4, 13, 31]. Although FTC is the second most common type, its incidence has decreased over the past few years [31]. World Health Organization has defined FTC as a malignant epithelial tumor showing follicular cell differentiation in which the diagnostic nuclear features of PTC are absent. Lesions are usually encapsulated and show invasive growth pattern [14]. The diagnosis of FTC is a difficult dilemma in cases when capsular, vascular, or extrathyroidal invasion or metastasizing is not straightforward [32, 33]. A lot of studies have researched biomarkers in liquid biopsy for PTC; however, limited amount of information is found about FTC. One of those studies explored miRNA dysregulation and tried to found miRNA markers for diagnostic purposes in the case of FTC [34]. Dettmer with colleagues found upregulation of miR-182/-183/-221/-222/-125a-3p and a downregulation of miR-542-5p/-574-3p/-455/-199a. They concluded that distinction between FTC and hyperplastic nodules can be done by the use of dysregulated miRNA in the tissues. The miRNAs found

Diagnosis	Type of miRNA	Type of regulation	Outcome	References
Follicular cancer	miR-199a-5p	Downregulated in cells	Increased cellular proliferation	Rodriguez-Rodero et al. [27] Boufraqueh et al. [28]
	miR-197	Upregulated in cells	Increased cellular proliferation	Rossi et al. [21] Boufraqueh et al. [28]
	miR-346	Upregulated in cells	Increased cellular proliferation	Rossi et al. [21] Boufraqueh et al. [28]

Table 2. Expression of microRNA in FTC.

in follicular thyroid cancers (FTC) are also frequently present in other subtypes of thyroid cancer [27]. miR-199a-5p is downregulated, but miR-197 and miR-346 are upregulated in FTC leading to increased cancer cell proliferation [28] (**Table 2**).

5. Medullary thyroid cancer and liquid biopsy

Medullary thyroid cancer (MTC) accounts for only 5% of thyroid cancers; it is responsible for approximately 13% of all thyroid cancer-related deaths [4]. MTC is a malignant thyroid tumor showing evidence of C-cell differentiation [14]. One of the recently studied miRNAs in medullary thyroid cancer is miR-21, which is downregulated especially in the aggressive forms [27]. miR-129-5p also is significantly downregulated in MTC compared to normal tissue leading to increased cellular invasion and migration [28] (**Table 3**).

Diagnosis	Type of miRNA	Type of regulation	Outcome	References
Medullary thyroid cancer	miR-183	Upregulated in tissue	Aggressive behavior and metastatic disease	Rodriguez-Rodero et al. [27] Accardo et al. [35]
	miR-375	Upregulated in tissue	Aggressive behavior and metastatic disease	Rodriguez-Rodero et al. [27] Accardo et al. [35] Boufraqueh et al. [28]
	miR-21	Downregulated in tissue	Aggressive behavior and metastatic disease	Rodriguez-Rodero et al. [27] Accardo et al. [35] Boufraqueh et al. [28] Pennelli et al. [36]
	miR-129-5p	Downregulated in tissue	Decreased cellular apoptosis and increased cell migration	Rodriguez-Rodero et al. [27] Boufraqueh et al. [28]

Table 3. Expression of microRNA in MTC.

6. Anaplastic thyroid cancer and liquid biopsy

Anaplastic thyroid carcinoma (ATC) is aggressive thyroid tumor which consists of undifferentiated follicular thyroid cells [14].

Many miRNAs have been found to be dysregulated in thyroid cancer, but only a few miRNAs are exclusively dysregulated in anaplastic thyroid cancer (ATC) [28]. Loss of miR-200 expression in ATC results in epithelial-mesenchymal transition (EMT) that represses the epithelial features of cancer cells and disrupts the cell-cell adhesion mediated by the loss of E-cadherin. This process enables cells to migrate and invade [26–28].

Overexpression of the miR-17-92 cluster results in downregulation of tumor suppressor PTEN. This process potentiates the activation of AKT/mTOR growth and survival signaling. Another important tumor-suppressive pathway targeted by miR-17-92 is the TGF β signaling pathway [28]. Increase of miR-17-92 leads to a loss of the tumor inhibitory effect of TGF β in thyroid cancer cells, which enhances cell proliferation [26–28].

Diagnosis	Type of miRNA	Type of regulation	Outcome	References
Anaplastic thyroid cancer	miR-30 family	Downregulated in cells	Protect cancer cells from apoptosis and autophagy	Sasanakietkul et al. [26] Rodriguez-Rodero et al. [27] Boufraqueh et al. [28]
	miR-125a, miR-125b	Downregulated in cells	Promote tumor invasion	Sasanakietkul et al. [26]
	miR-138	Downregulated in cells	Increase with the progression of histological dedifferentiation and malignant behavior	Sasanakietkul et al. [26] Rodriguez-Rodero et al. [27]
	miR-200 family	Downregulated in cells	Increase the invasive potential of tumor	Sasanakietkul et al. [26] Rodriguez-Rodero et al. [27] Boufraqueh et al. [28]
	miR-17-92 cluster	Upregulated in cells	Promote tumor growth and invasion	Sasanakietkul et al. [26] Rodriguez-Rodero et al. [27] Boufraqueh et al. [28]
	miR-146a, miR-146b	Upregulated in cells	Dysregulated cell differentiation and invasion	Rodriguez-Rodero et al. [27] Sasanakietkul et al. [26]
	miR-221, miR-222	Upregulated in cells	Promote tumor growth and invasion	Rodriguez-Rodero et al. [27] Sasanakietkul et al. [26]
	miR-4295	Upregulated in cells	Increased cell migration and invasion	Rodriguez-Rodero et al. [27] Sasanakietkul et al. [26]

Table 4. Expression of microRNA in ATC.

miR-30 plays a role in thyroid cancer differentiation and progression. Overexpression of miR-30 family members leads to a change in cell morphology and decreased vimentin expression in cancer cells [28]. This is suggesting that miR-30 family members are involved in cancer progression by regulating the EMT process [26–28] (**Table 4**).

7. Poorly differentiated thyroid cancer and liquid biopsy

Poorly differentiated thyroid carcinoma (PDTC) is malignant epithelial cell tumor derived from follicular cells that shows limited characteristics of follicular cell differentiation and is morphologically and behaviorally intermediate between differentiated thyroid carcinomas and anaplastic thyroid carcinoma [14]. miR-23 and miR-150 are downregulated, but miR-146b, miR-221, and miR-222 are upregulated in poorly differentiated thyroid cancer ensuring more aggressive behavior (**Table 5**).

Diagnosis	Type of miRNA	Type of regulation	Outcome	References
Poorly differentiated thyroid cancer	miR-23	Downregulated in tissue	Promote tumor relapse	Sasanakietkul et al. [26] Boufraquech et al. [28]
	miR-150	Downregulated in tissue	Enhance cancer-specific mortality	Sasanakietkul et al. [26] Boufraquech et al. [28]
	miR-146b	Upregulated in tissue	Promote thyroid tumorigenesis	Sasanakietkul et al. [26]
	miR-221, miR-222	Upregulated in tissue	Induce the tumor angiogenesis	Sasanakietkul et al. [26]

Table 5. Expression of microRNA in PDTC.

Author details

Ilze Fridrihsone^{1*}, Arnis Abolins¹, Andrejs Vanags², Dzeina Mezale¹ and Guntis Bahs³

Address all correspondence to: ilze.fridrihsone@rsu.lv

1 Department of Pathology, Riga Stradins University, Riga, Latvia

2 Department of Surgery, Riga Stradins University, Riga, Latvia

3 Department of Internal Diseases, Riga Stradins University, Riga, Latvia

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