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REVIEW ARTICLE

MicroRNA-based Biosensors for Early Detection of Cancers

Majid Rezayi[1,2]*, Zahra Farjami[2], Zeinab S. Hosseini[3], Neshat Ebrahimi[4] and Ebrahim Abouzari-Lotf[5]

[1]*Metabolic Syndrome Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran;* [2]*Department of Modern Sciences and Technologies, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran;* [3]*Student Research Committee, Faculty of Medicine, Islamic Azad University of Mashhad, Mashhad, Iran;* [4]*Laboratory of Cedars-Sinai Medical Center, Los Angeles, California, USA;* [5]*Advanced Materials Research Group, Center of Hydrogen Energy, Institute of Future Energy, Universiti Teknologi Malaysia, 54100, Kuala Lumpur, Malaysia*

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Abstract: Small noncoding microRNAs (miRNAs) are known as noninvasive biomarkers for early detection in various cancers. In fact, miRNAs have key roles in carcinogenicity process such as proliferation, apoptosis and metastasis. After cardiovascular disease, cancer is the second cause of death in the world with an estimated 9.6 million deaths in 2018. So, early diagnosis of cancer is critical for successful treatment. To date, several selective and sensitive laboratory-based methods have been applied for the detection of circulating miRNA, but a simple, short assay time and low-cost method such as a biosensor method as an alternative approach to monitor cancer biomarker is required. In this review, we have highlighted recent advances in biosensors for circulating miRNA detection.

Keywords: Biosensor, cancer, diagnosis, biomarker, miRNA, carcinogenicity.

1. INTRODUCTION

Recent findings of the levels of microRNAs (miRNAs) in diseases such as Gastric cancer have emphasized that miRNA can be an ideal biomarker for sensing this type of cancer [1]. Gastric cancer is the second prevalent reason of cancer-related deaths and fourth agent of human malignant disease worldwide [2]. Drug resistance is one of the major reasons of therapy failure in gastric cancer patients. Though the mechanisms of anticancer drug resistance have been approximately investigated, they have not been entirely understood. Drug resistance still is one of the main complications of gastric cancer treatment [3]. Modifications in miRNA expression have been recently proved to donate to drug resistance of gastric cancer. The selective regulation of miRNA expression could partially improve the reaction of gastric cancer cell lines to chemotherapy and considerably enhance the anticancer property of certain drugs. Certainly, many reports propose that drug resistance of cancer cells may be an miRNA connection modulating biological process in clinical trials. Moreover, miRNAs are identified to be somewhat safe and have been more effective in tumor treatment in early preclinical researches as compared to treatments based on RNA interference, which despite being effective, have been discovered to be toxic in preclinical mouse samples [4, 5].

MicroRNAs are small, non-coding, single-stranded ribonucleic acid (RNA) molecules and are composed of 19–23 nucleotides. They have an important role in biological mechanisms such as survival, differentiation and division cellular and also the regulation of gene expression. It is documented that over-expressed miRNAs occur in both oncogenesis and/ or cellular regulation processes [6, 7]. MicroRNAs are perfectly constant in serum/plasma, urine and saliva sample. In several studies, the level of miR-21-5p, miR-132-3p and miR-185-5p was shown to be upregulated in gastric cancer patients [8]. It was reported that both circulating miR-196a/b-3p and miR-106a-5p were overexpressed in metastatic stage. miR-21 as an antiapoptotic factor is overexpressed in various solid cancers and is associated with tumour growth, so it is a diagnostic biomarkers for several types of cancer [4, 9].

For pathological conditions like cancer, expression of miRNA seems to be highly stable, tissue-specific and dysregulated. Therefore, miRNA is a revolutionary biomarker in diagnosis and treatment of cancer [10].

Although laboratory-based methods including qRT-PCR (Quantitative Real-Time Polymerase Chain Reaction), ELISA (Enzyme-Linked Immunosorbent Assay) microarrays, and RNA sequencing have been applied for the detection of circulating miRNAs, these are not ideal techniques compared to point-of-care (POC) devices. Recent advanced research in electrochemical detection assays has strikingly gained ultrahigh sensitivity and selectivity [11-15]. This review summarizes the drawback of current detection methods and also highlights the recent advances in the biosensory methods as point-of-care devices which are capable of measuring circulating miRNAs in various types of cancer.

2. TRADITIONAL DETECTION METHODS AND EMERGE OF SENSOR-BASED TECHNIQUES :

Nucleic acid-based biomarkers provide potential assistance in early diagnosis of cancer. Recent evidences demonstrated that miRNAs as detection markers carry key information about the pathophysiological status of cancer patients as has been found in numerous methods such as microarrays, bead-based arrays, northern blotting and quantitative real-time PCR [16] (Fig. **1**). The principle of miRNA microarrays is based on the Watson–Crick base of nucleic acids bond. Microarrays document simultaneous discovery of hundreds of miRNAs. The classical procedures (such as ELISA) for the diagnosis of cancer may need some hours or even days from when examinations are ordered to when consequences are received. These procedures can be boring, time consuming and frequently need extra care and costly instruments [17]. This particularly makes the initial diagnosis of cancer more problematic for the cancer patients who are admitted to an emergency department. So, measurement of carcinomatous indicators is serious in helping the diagnosis

*Address corresponding to this author at the Metabolic Syndrome Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran: E-mail: rezaeimj@mums.ac.ir

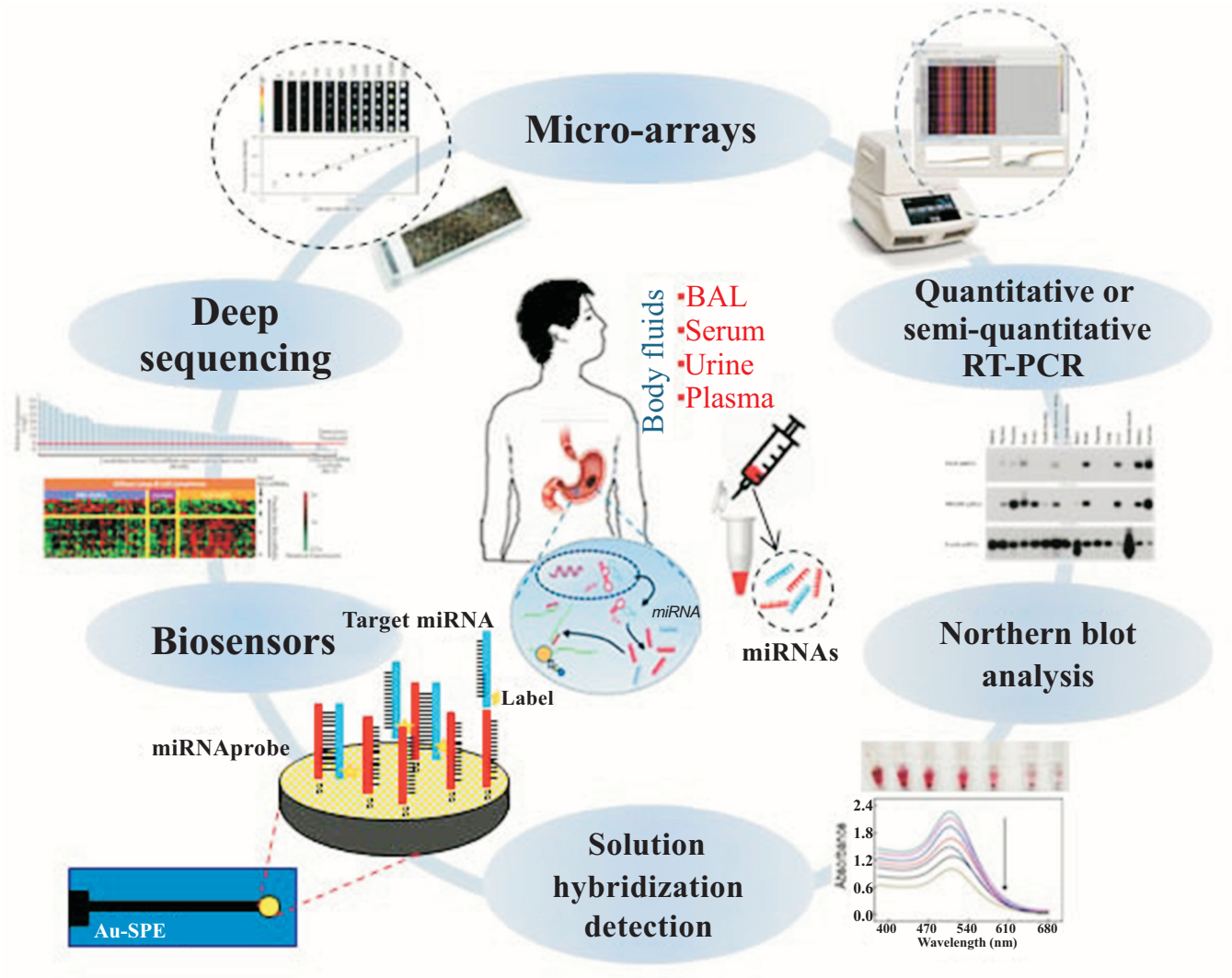

Fig. (1). Pre-cancer diagnosis methods based on circulating miRNA biomarkers.

of cancer. An additional, sensitive and fast technology platform is immediately required to fulfill fast diagnosis requirements in cancer marker discovery during early stages of the disease [17]. Now, numerous research studies have been conducted in the discovery of cancer markers, which mostly comprised of spectrophotometric or optical procedures [18], fluorescence immunoassay [19], chemiluminescence analysis [20, 21], electrochemical techniques [22, 23], radioimmunoassay, capillary electrophoresis and chromatographic analysis [24, 25].

As mentioned above, the discovery of cancer biomarkers has a significant role in clinical diagnoses especially in asymptomatic individuals and for evaluation of treatment. Numerous immunoassay approaches are developed for the discovery of cancer biomarkers. The use of electrochemical immunoassay is probably the most promising way to elucidate some of the challenges relating to sensitivity, quickness and selectivity [26].

A biosensor as a point-of-care diagnostic strategy, has an important role in the initial stage of cancer [27-30].

A biosensor via biological target miRNAs and as a transducer is able to measure the signal of recognition reaction. According to the type of transducer, the miRNA biosensors are categorized into electrochemical, optical and magnetic [31] which were explained by examples from recently published papers (Table **1**).

3. ELECTROCHEMICAL DETECTION METHODS

Attractive features of biosensor approaches led to recent development of biosensor-based detection methods for circulating miRNA assays, which were classified into either amperometric or voltammetric measurements. According to an investigation by Kilic *et al*., an electrochemical miRNA detection method for the diagnosis of breast cancer was employed. In this way, coupling agents including N- (dimethyl amino) Propyl-N'-ethyl carbodiimide hydro chloride (EDC) and N-hydroxysulfosuccinimide (NHS) attached onto the cell lysates and/or detection probes onto the pencil graphite electrode (PGE). The detection limit of this evaluation was 6 pmol for mir21 from cell lysates [32]. In another study by Azimzadeh *et al*. in 2015, an electrochemical nanobiosensor based on the detection of miRNA 155 in plasma for early diagnosis of breast cancer was fabricated. In this biosensor, the graphene oxide and gold nano-rod were applied for modifying the surface of the electrode. According to the results, the signal of this electrochemical biosensor had a linear association with miRNA 155 concentration and the detection limit was calculated as 0.6 fM. This nanobiosensor had high specificity and the ability to separate complementary miRNA and non- complementary ones, and single-, three-base mismatch miRNA. So, this biosensor is useful for early detection of breast cancer without the need for separation of the

Table 1. Comparison of biosensory technologies for diagnostics of miRNAs in various cancers.

miRNA	Cancer	Sample	Method	Biosensor Platform	Label	Detection Limit	Detection Range	Sequence	Refs.
miRNA 21	Breast cancer	293-HEK cell line	DPV	PGE/ EDC/ NHS	Biotin	6 pmol	NM	5′-UAGCUUAUCAGA CUGAUGUU-GA-3′	[30]
miRNA 155	Breast cancer	plasma	DPV/ CV	GCE/ GO/ GNRs	Oracet Blue	0.6 fM	2.0 fM to 8.0 pM	NM	[31]
miRNA 155	Breast cancer	serum	CV/EIS/SWV	Au-SPE	NM	5.7 aM	10 aM-1.0 nM	5′-UUAAUGCUAAUCGUGAUA GGG GU-3′	[32]
miRNA 21, miRNA 155, miRNA 196a and miRNA 210	multiple pancreatic carcinoma	serum	CV	SPGE(16-channel)	NM	10 fM	NM	miRNA21: UAGCUUAUCA-GACUGAUGUUGA miRNA155: UUAAUG-CUAAUCGUGAUAGGGGU miRNA196a: UAGGUAGUUU-CAUGUUGUUGGG miRNA210: AGCCCCUGCCCACCGCACACUG	[34]
miRNA 21	Breast cancer	plasma	DPV/ CV/EIS	GCE/ MWCNTs	Label free	84.3 pM	0.1 to 500.0 pM	-	[35]
miRNA 155	Some cancers	serum	DPV	GCE/ AuNPs	MB	3.3 fM	10 fM–1 nM	5′-UUAAUGCUAAUCGUGAUAGG GGU-3′	[50]
miRNA 155	inflamma-tion and most of cancer	serum	QD	GQDs	NM	0.14 pM	1 fM to 100 pM	5′-UUAAUGCUAAUCGUGAUAGGGG-3′	[36]
miRNA 21	Breast cancer	serum	EIS	MGCE / Cps	Label free	60 aM	-	-	[37]
miRNA 21	Some cancers	plasma	CV	GC	Label free	0.20 fM	0.20 fM and 1.0 nM	5′-rUAGCUUAUCAGACUGAUGUUGA-3′	[38]
miR-199a-5p	Breast cancer	serum	EIS / CV	GCE/ GO/ GNR	NM	4.5 fM	15 fM to 148 pM	5’-CCCAGUGUUCAGACUACCUG UUC-3’	[39]
mir-155	Some cancers	serum	Amperometry	GCE/Nafion/ thionine/ Pd-NPs	Label free	1.87 pM	5.6 pM−0.56 μM	50-UUA AUG CUA AUC GUG AUA GGG GU-30	[33]
miRNA-21	Various cancer	serum	Fluorescence	NM	NM	500pM	NM	5′-UAGCUUAUCAGAC2UGAUGU UGA-3′	[42]
miRNA-21	Breast cancer	Lysates from three different cultured cell	Fluorescence	NM	NM	3.55pM	40pM-40 nM	5′-UAGCUUAUCAGAC2UGAUG UUGA-3′	[45]
miRNA-215	Gastric cancer	gastric cancerous tissues	Fluorescence	PDA micro-tube	NM	8 pM	0.01 to 10 nM	5′-UGCCUGUCUACACUUGCUGUGC-3′	[41]
Let-7b	some cancers	Hela cells	Fluorescence	NM	NM	3.2 pM	10 pM–10 nM	NM	[51]
miRNA-21	Some cancers	Serum	Colorimetric	AuNPs/ capture probe		0.1 fM	0-25 fM	NM	[46]
miRNA-224	Some cancers	A549 cell lysate	Colorimetric	E-LFSB	NM	7.5 pM	7.5 pM to 75 nM	5′-AAA AUG GUG CCC UAGUGA-CUACA-3′	[47]

(Table 1) Contd.....

miRNA	Cancer	Sample	Method	Biosensor Platform	Label	Detection Limit	Detection Range	Sequence	Refs.
MiRNA 155	Breast cancer	MCF-7 and HEK 293 cell lysates	Fluorescence	CdTe QDs surface	NM	12.0 pM	20.0 and 100.0 pM	NM	[40]
miRNA-14	some cancers	HepG2 & 22Rv1 cell lines	Fluorescence	NM	NM	1.03 pM	1 pM–10 μM	NM	[43]
miRNA-21	some cancers	serum	Fluorescence	NM	NM	33 pM	1–50 nM	5′-UAGCUUAUCAGACUGAUG UUGA-3′	[44]
miRNA 155	Breast cancer	serum	MoS_2 field-effect transistor	Field-effect transistor	Label free	0.03 fM	0.1 fM to 10 nM	5'-UUAAUGCUAAUC GUGAUAGGGGU-3'	[49]

HEK: human embryonic kidney, **DPV:** Differential Pulse Voltammetry**, PGE:** pencil graphite electrode, **EDC:** N-(dimethylamino)propyl-N'-ethylcarbodiimide hydrochloride, **NHS:** N-hydroxysulfosuccinimide, **GCE:** glassy carbon electrode, **GO:** graphene oxide, **GNRs:** gold nanorods, **CV:** cyclic voltammetry, **EIS:** impedance spectroscopy, **SWV:** square wave voltammetry, **Au-SPE:** gold-screen printed electrode, **SPGE:** screen-printed gold electrode, **MWCNTs:** Multi-walled carbon nanotubes, **MB:** metylen blue **QD:** quantum dot, **GQDs:** graphene quantum dots, **MGCE:** magnetic glass carbon electrode, **Cps:** capture probes, **GE:** gold electrode. **GNR:** gold nanoroad, **CdTe QDs:** cadmium telluride quantum dots, **PDA:** polydiacetylene, **E-LFSB:** enzyme-amplified lateral flow strip biosensor

sample, RNA extraction and amplification [33]. Cardoso and co-workers for the diagnosis of breast cancer developed a sensitive and modified Au-screen printed electrode (Au-SPE) platform for the detection of miRNA-155. The detection range and LOD (limit of detection) of this biosensor were 10 aM to 1.0 nM and 5.7 aM, respectively [34]. Also, in a different study by Wu *et al*., a label-free and amperometric biosensor for the detection of miR-155, based on electrocatalytic activity towards H_2O_2, was employed. The electrode platform was a conductive self-assembled multilayer of nafion, thionine and palladium nanoparticles and showed good sensitivity and selectivity towards mir-155 with a linear range and LOD of 5.6 to 5.6×10^5 pM and 1.87 pM, respectively [35].

Zeng *et al*. introduced an ultrasensitive electrochemical biosensor for the detection of multiple pancreatic carcinoma (PC) associated miRNA biomarkers such as miRNA21, miRNA155, miRNA196a and miRNA210. They used a platform based on the screen-printed gold electrode to improve the efficacy of detection. This PCR-free biosensor provided a way for early detection of pancreatic cancer [36]. Rafee pour *et al*. fabricated a label-free electrochemical biosensor that could detect miRNA in a range from 0.1 to 500.0 pM with a low detection limit of 84.3 fM [37]. In another study, a sensitive electrochemical based graphene quantum dot biosensor by immobilization of horseradish peroxidase (HRP) for detection of miRNA 155 in real samples was proposed by Hu *et al.* The response of biosensor towards miRNA155 was in the range of 1 fM to 100 pM with a detection limit of 0.14 [38]. Zhang *et al*. presented a free-immobilization electrochemical biosensor for the detection of circulating miRNA 21. This work was based on the capture probes (Cps) and duplex-specific nuclease assisted target recycling (DSNATR). The detection limit of this biosensor was 60 aM [39]. MicroRNA-21 in plasma as a diagnosis biomarker in most types of cancer was applied. A composite platform of pyrrolidinyl peptide nucleic acid (acpcPNA)/polypyrrole (PPy)/silver nanofoam (AgNF) immobilized on a gold electrode could detect miRNA-21 between 0.20 fM and 1.0 nM, with a very low detection limit of 0.20 fM. [40]. Mansouri M. *et al*. developed a novel electrochemical biosensor based on glassy carbon electrode (GCE) with graphene oxide (GO) and gold nanoroad (GNR) for the detection of MiRNA-199a-5p. Using Electrochemical Impedance Spectroscopy (EIS) and Cyclic Voltammetry (CV), the range of this study was from 0.1 fM to 10 nM with the LOD of 0.03 fM [41].

4. FLUORESCENCE-BASED DETECTION METHODS

Recently, fluorescence technique was applied for monitoring and sensitive detection of miRNA with signal amplification. The fluorometric methods are suitable choices for quantitative diagnosis of miRNAs due to their high sensitivity. But one of the drawbacks of these methods is the use of expensive labeled probes and also boring preparation [42].

Borghei *et al*. used fluorescence resonance energy transfer (FRET) for the detection of miRNA 155 in breast cancer, with an LOD of 12.0 pM. Also, it was reported as an excellent approach to detect the miRNA in HEK 293 and MCF-7 cell lysates [43].

In 2018, Wang *et al*. published a paper for the diagnosis of mir-215 in gastric cancerous. In this study, oligonucleotides receptors were immobilized on a single polydiacetylene (PDA) microtube platform and then target miRNAs via sandwich complex were co-hybridized with both Au nanorod (AuNR) and oligonucleotides. The proposed biosensor showed a linear response from 0.01 to 10 nM with a detection limit of 8 pM [44]. In the next work, Ma *et al*. presented a fluorescence-based method for the detection of miRNA-21 by using of 2-aminopurine (2-AP)-labeled probe and exonuclease. In this study, LOD was 0.5 nM [45].

In recent years, researchers have shown an increased interest in investigation on fluorescence methods for the detection of various miRNAs in some types of cancer. But, among them, Zhang suggested an ultrasensitive fluorescence technique for miRNA-14 detection in biological samples via duplex-specific nuclease (DSN) signal amplification. The fluorescence intensity found the concentration from 1 pM–10 μM with a detection limit of 1.03 Pm [46].

As mir-21 is correlated to some types of cancer, Ouyang *et al*. presented a fluorescent biosensor based on MnO_2 nanosheets for the detection of mir-21 in real samples. This sensor was presented as an enzyme-free and low-cost biosensory device and has a promising potential for recognization of mir-21 in cancers with LOD of 33 pM [47]. In another study, Lio *et al*. developed a fluorescent platform based on quencher-free and enzyme-free for simple and sensitive detection of miRNA-21 based on 2-aminopurine probe (2-AP) in combination with signal amplification. The limit of detection and linear range of this sensor were reported as 3.5 pM and 40 pM to 40 nM, respectively. The authors claimed that this sensor can be applied to detect miRNA-21 expression levels in cancer cells [48].

5. COLORIMETRIC BIOSENSORS

The colorimetric-based biosensors are another technique for the identification of miRNAs in real samples. Actually, these methods have been one of the major interesting research subjects due to simple handling of the sample and ability to display color without specific and costly instruments [49].

In 2016, Gao *et al*. modified gold nanoparticle (GNPs) surface by means of DNA probe and Horseradish peroxidase (HRP). Through this colorimetric sensor and in the presence of mir-224, a sandwich structure was formed that could detect mir-224 with LOD

of 7.5 pM, in A549 cell lysate within 30 min [50]. Xia, *et al* presented a converting colorimetric method by immobilizing the probes on gold nanoparticles (AuNPs) and employing of duplex-specific nuclease as signal amplification for detection of miRNA-21. In this project, the detection limit of 0.1 fM and concentration of miRNAs were in the range of 0–25 fM [49].

Wang *et al*. reported the best limit of detection (0.22aM) through the magnetic bead. In this research, a controllable gold electrode based on a homemade electrically magnetic field was applied to detect miRNAs related to oral-cancer in saliva. The junction-probe was used to capture target miRNAs via applying enzymatic catalysis amplification and potential which led to control the temperature on the electrode's surface followed by ultrasensitive detection.

6. TRANSISTOR-BASED SENSORS

Field effect transistor (FET) biosensors have attached much attention due to offering inexpensive, label free and rapid detection. In the recent years, by using 2D materials such as molybdenum disulfide (MoS_2) instead of 1D materials such as nanotubes or nanowires, the sensitivity of these types of biosensors has tremendously increased [51].

This technique has been presented as one of the biomedical technologies for monitoring and sensing biological components. In 2017, Mansouri M. *et al*. fabricated a sensitive detection method for miRNA-155 as a breast cancer biomarker based on MoS_2 FET. MoS_2 FET device presented a comparatively low threshold fluctuation of 48.10 mV/decade and a high movement of 1.98×10^3 $cm^2V^{-1}s^{-1}$ [52]. The concentration range of target miRNA-155 from 0.1 fM to 10 nM and LOD of 0.03 fM were achieved by using the proposed FET biosensor.

CONCLUSION

In this review, we discussed some biosensor detection methods for the diagnosis of miRNA in cancers that can be used for clinical diagnosis. The efficient biosensors as a biomedical diagnosis for rapid detection of cancers through analysis of cellular changes are in high demand. By using the biosensors, the prognosis and treatment methods are improved. In this regard, we are faced with some challenges that a better understanding of the molecular changes in cancer progression can be helpful. One of the major challenges is complexity of cancer cells which plays a central role in regulating gene expression. Genetic analysis and next-generation sequencing are the beneficial ways for the detection of novel cancer-specific biomarkers for the application of biosensors.

In the past years, the biosensor-based method for the detection of miRNAs in various cancers diagnosis has attracted much attention as a high-throughput format.

Although significant efforts on new sensing strategies on promising selectivity, sensitivity, and other properties such as being simple and rapid, there are still considerable challenges to meet some criteria such as portability, robustness, and user-friendly point-of-care methods for the detection of circulating miRNA in cancerous tumor. However, novel technology for miRNA sensing will be developed by advances in various fields such as microfluidics, nanotechnology and biomaterials.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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