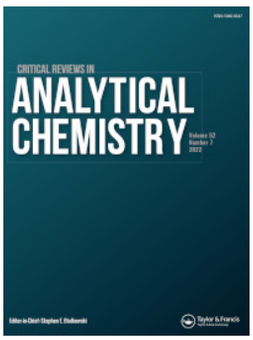

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Potentials of MicroRNA in Early Detection of Ovarian Cancer by Analytical Electrical Biosensors

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ABSTRACT

The importance of nanotechnology in medical applications especially with biomedical sensing devices is undoubted. Several medical diagnostics have been developed by taking the advantage of nanomaterials, especially with electrical biosensors. Biosensors have been predominantly used for the quantification of different clinical biomarkers toward detection, screening, and follow-up the treatment. At present, ovarian cancer is one of the severe complications that cannot be identified until it becomes most dangerous as the advanced stage. Based on the American Cancer Society, 20% of cases involved in the detection of ovarian cancer are diagnosed at an early stage and 80% diagnosed at the later stages. The patient just has a common digestive problem and stomach ache as early symptoms and people used to ignore these symptoms. Micro ribonucleic acid (miRNA) is classified as small non-coding RNAs, their expressions change due to the association of cancer development and progression. This article reviews and discusses on the currently available strategies for the early detection of ovarian cancers using miRNA as a biomarker associated with electrical biosensors. A unique miRNA-based biomarker detections are specially highlighted with biosensor platforms to diagnose ovarian cancer.

KEYWORDS

Analytical sensor; micro RNA; nanotechnology; nucleic acid complementation; ovarian cancer

Introduction

Ovarian cancer has been identified as the fourth common malignancy in the regions of Asia and other continents. It remains the deadliest among all other gynecological cancers, including the cervix[1–3] and uterus. About 70-75% of cases were diagnosed at the stages III and IV, where it extends beyond the pelvis, and 5-year survival rate falls at the lower rate of 11-41%. On the other hand, at early stages (I, II) patient have high chances to cure with the survival rate of 90% when diagnosed early. Weak diagnosis of ovarian cancer included lack of minimally invasive procedure, early detection test, resistance to tumor chemotherapy, and development of symptoms. Chemoresistance assay is still having difficulties to predict the drug-resistance to ovarian cancer.

Ovarian cancer has been named as a silent killer because there is no sign associated with this cancer at the early stages.[4] Worldwide, more than 15,000 women die each year by ovarian cancer, and ranked the disease as the second deadliest cancer for women and the fifth leading cause of cancer death.[5] The deadliest gynecological condition in the United States is ovarian cancer, with more people suffering from this cancer than all other gynecological diseases combined. Ovarian cancer can be successfully treated when diagnosed early, that is why early detection is a key for detecting ovarian cancer. The medical practitioner can diagnose ovarian cancer only after the symptoms such as abdominal bloating, indigestion, or nausea and appetite changes, have been reported at the stage 3. Among gynecological malignancies, ovarian cancer is the primary cause of death. Ovarian cancer progresses asymptomatically, diagnosis is mostly made at incurable stages. Despite several years of study, there is still a shortage of appropriate markers for diagnosis and early detection and screening.

Cell-free microRNAs (miRNAs) have recently been found to circulate in the body fluids of healthy and ill patients, suggesting they may act as a novel diagnostic marker. The current ovarian cancer screening strategies lack efficacy and fail to lower the mortality rate of this cancer.[6] However, there are few initiatives to decide by screening with intravaginal ultrasound, symptom index, and these in combination with biomarkers will help diagnosing earlier ovarian cancer stages. Effective ovarian cancer screening has been developed using a biosensor to improve the efficacy of ovarian cancer. The only professional practitioner can treat it scientifically by their expertise and understanding. We cannot take their skill for granted but as a researcher, can try to help them

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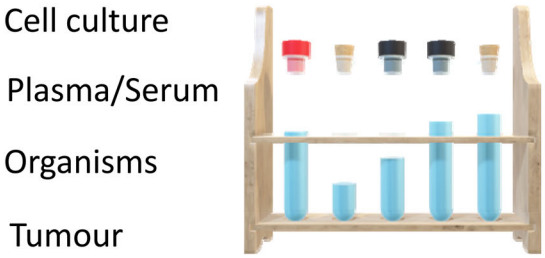

Conventional miRNA detection	Electrochemical methods	Optical miRNA methods	Emerging Technologies
Northern blotting	Nanomaterials based ✓ Metal nanoparticle ✓ Quantum dots ✓ CNT ✓ Others	Nanomaterials based ✓ Metal nanoparticle ✓ Quantum dots ✓ Silver nano cluster ✓ Carbo materials ✓ Others	Microfluidic based Next generation sequencing ICP-MS ECL Photoelectrochemical LSPR Paper- based Electrochemical
Q-RT PCR	Enzyme based ✓ HRP ✓ ALP ✓ Others	Amplification based ✓ LCR ✓ RT-PCR ✓ RCA ✓ LAMP ✓ EXPAR	
miRNA microarrays	Strand amplification		

Figure 1. MiRNA detection methods and their sources. Different methods and stretegies are shown with comparison.

with new early screening technologies and assist them in their diagnostic aspect. Figure 1 showed miRNA detection methods and their sources. Different methods and strategies are shown with comparison. Current imaging diagnostic methods derived from the conventional diagnostics for ovarian cancer, which are ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET-CT). Such types of imaging systems required high installation costs, high radiation hazards, and not user friendly. Ultrasound diagnosis is widely used due to low-cost and low-risk of radiation, which are important for ovarian cancer but have limited specificity and sensitivity. CT imaging in the show and pre-operative staging of tumors is better than ultrasound but has some flaws, such as radiation and expensive. MRI has the advantage of multidimensional imaging compared to CT imaging, but it is also costly and not appropriate for the general population screening.

This article focuses on recent strategies for the early detection of ovarian cancer using miRNA-associated electrical biosensors. The circular miRNA that presents in the plasma membrane, serum, and urine using biosensor and validation of molecular approaches have also been discussed in this review. Newer approaches to successful diagnosis of ovarian cancer at the early and curable stages are desperately needed. During diagnosis, the majority of cancer relapsing patients ultimately return to the disease. Identifying useful clinical biomarkers to assess future resistance to any therapy and cancer prognoses would greatly improve the management of ovarian cancer patients. MiRNAs can exist stably in different types of body fluids as well as in the cytoplasm. Circulating cell-free miRNAs have been shown to have the potential to allow earlier cancer detection and to predict the response to prognosis and therapy.

Current approaches for ovarian cancer detection

Imaging analysis

Current approaches that are widely used to detect early-stage ovarian cancer include the use of transvaginal sonography (TVS) and serum biomarker monitoring. TVS is a noninvasive process, but use an imaging system that captures ovary-sized and formed images. TVS also makes ovarian cancer detection simpler. Evaluated large-scale TVS studies to classify early-stage cancer that has provided the combination of observational findings. TVUS (transvaginal ultrasound) is a procedure that uses sound waves to see in the uterus, fallopian tubes, and ovaries by passing an ultrasound wand into the vagina. It will help to identify a mass (tumor) in the ovary, but it still cannot reveal whether a mass is a cancer or benign. TVUS is a tiny tool placed inside the vagina and the system generates sound waves and a computer set-up converts them to an image. Most diagnostic method including TVS cannot rely to predict early onset of malignancy because asymptomatic disease cannot be detected until the disease was spread to specific organ.[7]

Biomarker analysis

The serum biomarker test has been used for the detection of CA125.[8, 9] Normally, a lower concentration of CA125 has

Table 1. Sensitivity limit of ovarian cancer detection in the clinical sample.

Method	Description	Disadvantages	Biomarker	Sensitivity limit	Detection range	Detection time	Specificity limit	References
TVS (transvaginal ultrasound)	Pelvic examination, transvaginal ultrasonography, abdominal ultrasonography, and exploratory or diagnostic laparoscopy when evaluating a pelvic mass, commonly used preoperatively to help predict the potential for malignancy	Can't tell if a mass is a cancer or benign	- cancer antigen 125 (CA125)	Frequently repeated Sensitivity: varied between 34% and 94%	–	One week	Reliably high in most of the review studies	[12–14]
Serum CA125	Cancer antigen 125 (CA125), also known as mucin-16 (MUC16), is the most widely used tumor marker in ovarian cancer, and considered the "gold standard" Serum level of CA125 is used to monitor response to chemotherapy, relapse, and disease progression in ovarian cancer patients	not sufficient to differentiate between epithelial ovarian cancer	Tumor biomarker CA125	Frequently repeated, low sensitivity (50-62% for early-stage epithelial ovarian cancer) and limited specificity (94-98.5%)	–	–	Microchip ELISA coupled with a cell phone is portable as opposed to traditional ELISA, and this method can facilitate the detection of ovarian cancer at the point-of-care (POC).	[8,9]
Enzyme-linked immunosorbent assay (ELISA)	Serological detection. Detection of biomarkers derived from hyperplasia of epithelial tissue by enzyme-linked immunosorbent assay (ELISA) proves to be a practical way of early diagnosis of ovarian cancer	ELISA is commonly performed in a laboratory setting Cannot be used in a clinical setting for on-site consultation	HE4 from urine was first absorbed on the surface; the primary and secondary antibodies were subsequently anchored on the surface via immuno-reaction; and addition of substrate led to color development because of enzymatic labeling	color intensity was analyzed by an integrated mobile application	–	–	–	[15]
Chemiluminescent immunoassay (CLiA)	Optical fiber based chemiluminescent immunosensor for detection of the native autoimmune response to GiPC-1, a PDZ containing protein involved in regulation of G-protein signalingTested for the presence of IgM anti-GiPC-1 autoantibodies in their serum using the two methods.	Semi quanitative assay	Anti-GiPC-1 IgM optical fiber immunoassay can be created by covalent modification of the core of an optical fiber tip with GiPC-1 protein. C Chemiluminescent ELISA are reliable systems for high and intermediate levels of IgM containing serum concentrations	–	–	–	Detected 18% and 27%	[7]
Electronic nose (E- nose sensor)	Women with suspected ovarian masses and healthy subjects had volatile organic compounds analysis of the exhaled breath using e-nose. E-nose analysis was performed on breath samples	–	Data collected were analyzed by Principal Component Analysis (PCA) and K-Nearest Neighbors' algorithm (K-NN).	the model performance in the prediction was of 89% for sensitivity	–	–	86% for specificity when the strict class prediction was applied	[16]
Localized surface plasmon resonance (LSPR) biosensor	A noninvasive and more sensitive method for detection of EIE4 is very important for the early screening and detection of ovarian carcinoma detection of EIE4 in urine This new biosensor was effective for detection of EIE4 in urine of early ovarian cancer patients, without label and purification, convenient, low-cost, and label-free, and sensitivity	EIE4, as a tumor marker, is smaller than albumin, with concentration in urine inferior to serum and unstable	–	a detection limit of 1 pM	-	-	Wide dynamic range of 1 pM to 10,000 pM	[11]

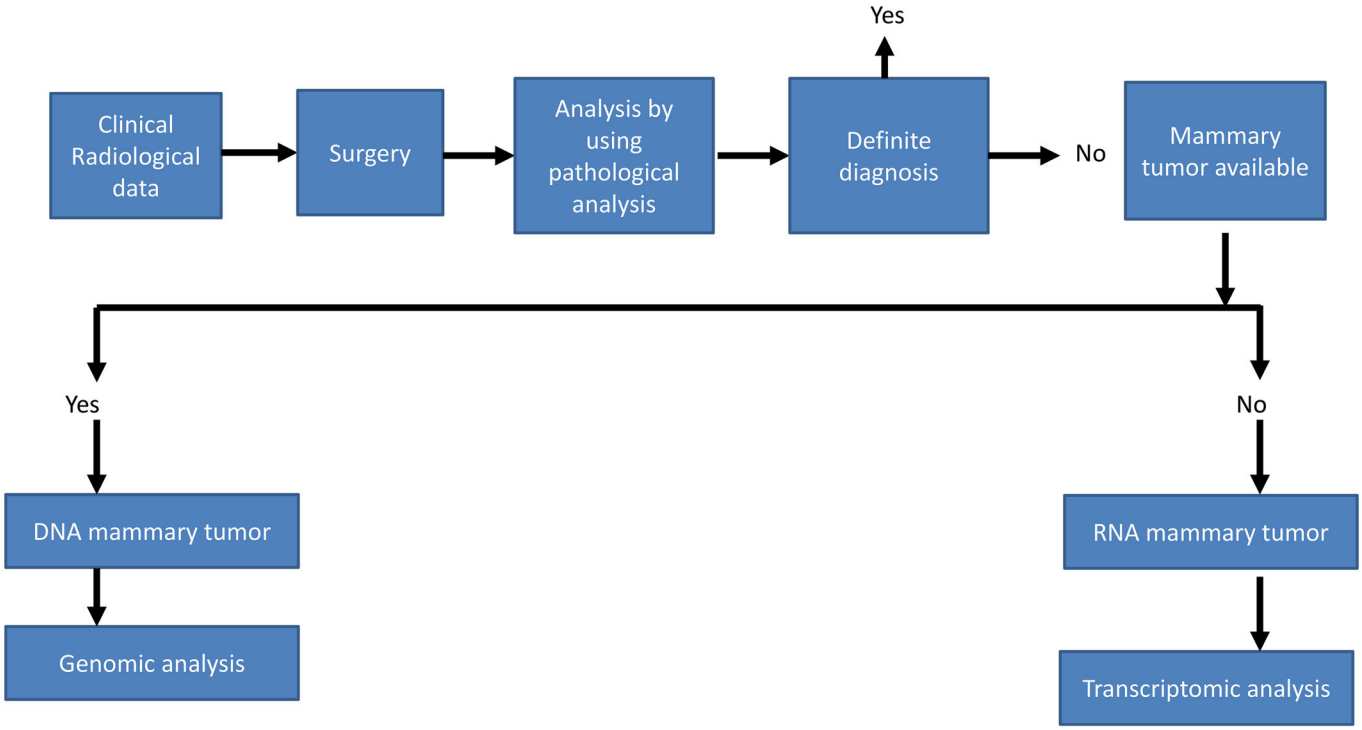

Figure 2. The proposed diagnostic method to detect ovarian tumorsin patients that was related to previous history of breast cancer.

Table 2. The comparison between mRNA and miRNA.

	Messenger ribonucleic acid (mRNA)	Micro ribonucleic acid (miRNA)
Configuration	coding RNA	Small noncoding RNA
Sequence partition	transcribed from genome exon	Intronic part of the transcriptome
Biogenesis	directly transcribed from DNA and can detect the amino acid sequence of a peptide	miRNAs are short nucleotide sequences (20-25 nt) that serves as a regulator for gene expression
Length	3x(number of amino acids in the protein it codes for) nucleotides, which can be hundreds of nucleotides	19-24 nucleotides long
Function	carries the "message" or sequence of nucleotides for protein synthesis, from DNA in nucleus to the ribosomes on ER	Smaller than most other forms of RNA
Action	allows decoding of genetic information	allow silencing of gene regulation
Storage	Need to be stored at —70 °C	Can be stored at 4 °C
Stability	Not stable	Stable

Q3

Table 3. Common miRNA detection by using a molecular approach.

Method	Advantages	Disadvantages	Reference
Northern blotting	• Identify related gene expression by detecting the RNA expression • A semi-quantitative mRNA analysis by combination with an RNA marker • Does not need to pre amplify miRNA samples • Low tech • Cheap • Semi-quantitative	• Sensitivity is low • The requirement of the sample is large • Sample degradation • Carcinogenic labeling	[23,24]
Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)	• Better quantification of circulating microRNAs by RT-qPCR • Sensitivity up to 1 nucleotide difference	Cannot identify novel miRNA	[25]
Microarray	Key technology conducting miRNA speech spectrum analysis and simultaneously high-throughput identification of a range of miRNAs	• Selectivity performance • Lower specificity than qRT-PCR	[26]

been found in our bodies. In patients with ovarian cancer, the CA125 level is higher than the usual level that indicates the presence of ovarian cancer. CA125 is a protein present in the blood, collected from the arm, and then sent to the laboratory to conduct a simple test that will determine the amount of CA125 in the blood sample. The CA125 amount is lesser than 35 units per milliliter (u/ml) in most healthy women. In some cases, women usually have high levels of CA125 in the blood and for the critical reason, the test is not accurate. CA125 has been proved to be successful in predicting malignancy. For

Table 4. Therapeutic miRNA for detection of ovarian cancer.

miRNA type	Target gene	Function	References
9	MIR-9	MicroRNA gene expression signature in ovarian clear cell carcinoma induced by MIR-9 overexpression	[42]
16	BCL 2	Targeting BCL 2 oncogene, downregulating and cancer cell apoptosis	[43]
18	TRIAP1 and IPMK	MicroRNA-18a prevents cancer of the ovaries by targeting TRIAP1 and IPMK directly	[44]
21	Cisplatin	Mediation of cisplatin resistance in cancerous ovarian cells	[45]
92	Integrin *a5*	Inhibits the peritoneal spread of ovarian cancer cells by inhibiting the expression integrin 5 which has previously been shown to be a key molecule in peritoneal diffusion.	[46]
93	CDKN1A	CDKN1A targeting for the polycystic ovarian syndrome	[47]
101	Brain-derived neurotrophic factor gene	MiR-101 inhibits ovarian carcinogenesis by suppressing brain-derived neurotrophic factor expression	[48]
199	Hepatocyte growth factor receptor	De-regulated in high-grade ovarian carcinomas and associated with the loss of miR-199a-3p	[38]
335	Abdominal Aortic Aneurysms	Manufacture of the interdigitated electrode Sensor and surface analysis the current study used miRNA-335-5p as a method for clarifying the extent of abdominal aortic aneurysm through interactive analyzes on an interdigitated electrode sensor	[49]
141	KLF12/Sp1/survivin axis	Improves anoikis resistance in metastatic progression of ovarian cancer by targeting the axis KLF12 / Sp1 / survivin	[50]
149	A2780	Modulates chemosensitivity of ovarian cancer A2780 cells to paclitaxel by targeting MyD88	[51]
186	ABCB1	Induces sensitivity of ovarian cancer cells to paclitaxel and cisplatin by targeting ABCB1	[52]
191	DAPK1	Inhibits TNF-a induced apoptosis of ovarian endometriosis and endometrioid carcinoma cells by targeting DAPK1	[53]
205	ZEB1	MiR-205 promotes motility of ovarian cancer cells via targeting ZEB1	[54]
216	PARP1	Increases cisplatin sensitivity in ovarian cancer cells by targeting PARP1	[55]
221	Apoptotic protease activating factor-1 gene	Overexpression of miRNA-221 promotes cell proliferation by targeting the apoptotic protease activating factor-1 and indicates a poor prognosis in ovarian cancer	[56]
382	ROR1 gene through regulating EMT	MiR 382 inhibits migration and invasion by targeting ROR1 through regulating EMT in ovarian cancer	[57]
506	Epithelial-to-mesenchymal	MiR-506 inhibits multiple targets in the epithelial-to-mesenchymal transition network and is associated with good prognosis in epithelial ovarian cancer	[58]
613	KRAS	MicroRNA-613 inhibited ovarian cancer cell proliferation and invasion by regulating KRAS	[59]
1271	Cyclin G1	Inhibits ovarian cancer growth by targeting cyclin G1	[60]

different reasons including menstruation, endometriosis, ovarian cysts, and even ovarian cancer, higher CA125 concentration in the blood may increase. CA125 was used to screen ovarian cancer along with TVUS. The ultrasound scan provided the picture that differs with the irregular condition examined. CA125 circulation can be observed in the course of ovarian cancer diagnosis months before the late stage. CA125 was limited in the detection of early ovarian carcinoma when cancer had cramped to the ovaries. This antigen lacks the specificity and sensitivity to be a reliable biomarker for early detection of ovarian cancer. CA125 was usually conducted as the standard diagnostic procedures for ovarian cancer, and their diagnostic values are reduced due to the lack of sensitivity and specificity. Just 50-60% of ovarian cancer patients with stages I and II have CA125.[10]

The sampling method of ovarian cancer in the past studies

The sampling method for detection of ovarian cancer was tedious, and it is crucial to find a noninvasive, sensitive, and specific assay for early screening and detection of ovarian carcinoma.[11] No reliable molecular prognostic biomarkers have been established from the previous studies. The presence of advanced ovarian cancer was using clinical grounds and can be confirmed using pathologically by removing the ovaries. When the disease was advance, the sampling method by using sampling tissuse or ascitic fluid. The routine imaging was not required in the patient that have high potential of ovarian cancer. If the diagnosis was uncertainty, a pelvic ultrasound, CT scan of the abdomen together with pelvis, and TVS (transvaginal ultrasound) were done to confirm the presence of ovarian cancer (Table 1). The proposed diagnostic method to detect ovarian tumors in patients that was related to previous history of breast cancer has been shown in Figure 2. The first screening remains by using pathological analysis. If the primary tumor was not exist which are indicator of majority cases, the transcriptomic analysis should be performed. These results required further analysis by using biomarkers and compared the result. The human epididymis secretory protein 4 (HE4) has been widely studied and considered as a promising tumor marker for early diagnosis of ovarian cancer.[11] HE4 was smaller than albumin with concentration in urine inferior to serum and unstable. Detection of HE4 biomarker of epithelial tissue by enzyme-linked immunosorbent assay (ELISA) showed to be a practical way of early diagnosis of ovarian cancer but it cannot be used in a clinical setting for on-site consultation.

Conventional methods of miRNA detection

To strengthen the currently available biomarker-based detections, miRNA has recently been focused much on diagnosing the diseases due to its higher specificity. Table 3 showed common miRNA detection using the molecular approaches. Northern blotting was considered a golden standard for miRNA detection.[17] In which, gel electrophoresis separated the RNAs in different lengths and transferred to the membrane where the detection probe hybridized with the target miRNA to produce the signal in such a form of chemiluminescence.

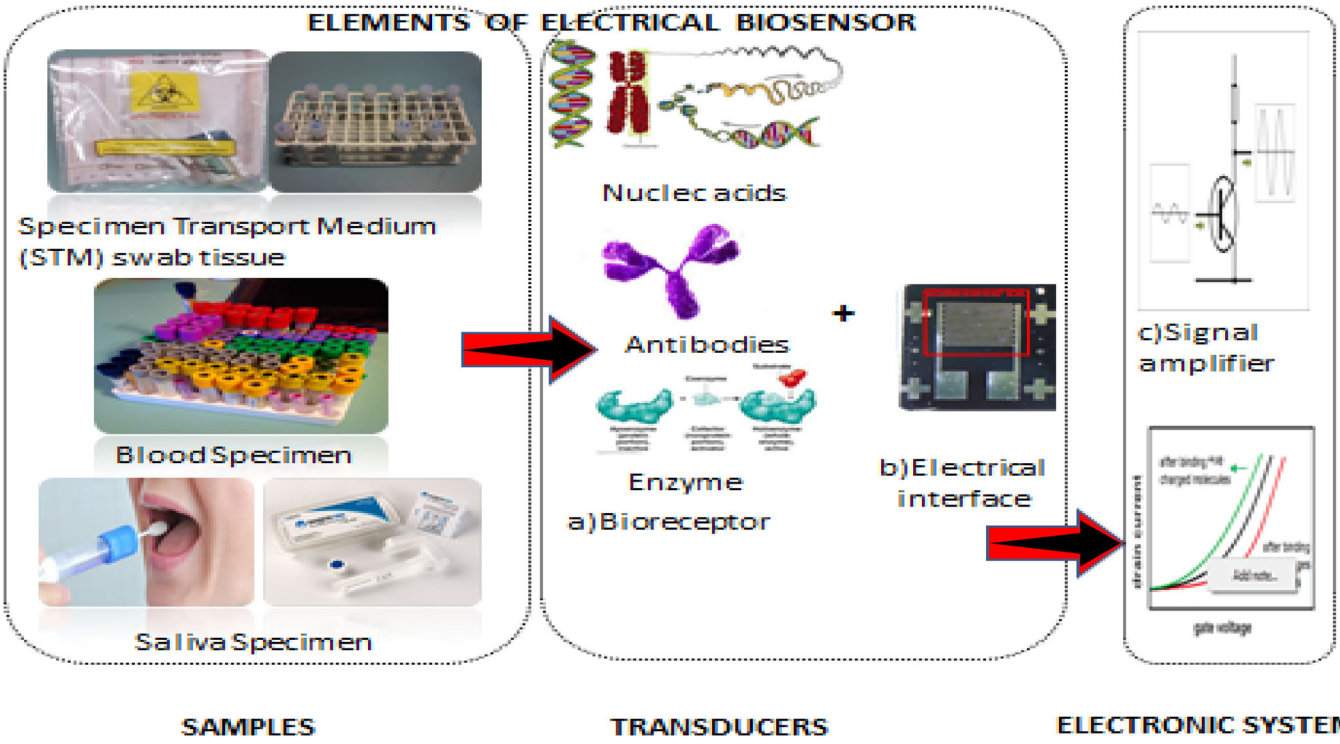

Figure 3. Components and selected constituent of a distinctive electrical biosensor. (a) Bioreceptor that particularly binds to the analyte, (b) a port architecture where a peculiar biological occurrence takes place and produce a signal, which is discovered by the transducer element, and (c) the transducer signal is changed into an electronic signal and amplified the signal to the useful parameter by observing the current change (conductance or resistance).

Northern blotting still required ethidium bromide, formaldehyde and radioactive probes, sample degradation by using RNases. It also had low sensitivity compared to other techniques, time consuming, and involves a lot of labor work. Northern Blotting required a higher concentration of the sample (5-25 mg total RNA for each sample) and not practical for the detection of more than hundreds of miRNAs.

Quantitative real-time polymerase chain reaction (qRT-PCR) has been widely used for quantifying the expression of the genes in sensitive and accurate technique. Rosenwald et al.[18] have been profiling 104 miRNAs for the identification of tumor tissue by using qRT-PCR for the clinical use. The requirement of individual miRNA-specific fluorescent probes makes qRT-PCR costly and not suitable for the high-throughput analysis of a large number of miRNAs.

Microarray analysis became the most widely preferred techniques for miRNA detection when it comes to high-throughput identification of a range of miRNAs. The method is based on the solid-phase hybridization of fluorescent labeling miRNAs with oligonucleotide probes that were immobilized on the platform. The fluorescent signal emit shows the hybridization for the quantitative information on miRNA in each spot. The critical steps in microarray are designing of capturing probes and fluorescent labeling of miRNAs. It had lower specificity compared to qRT-PCR but it can identify the expression level of miRNA.

The differentiation about mRNA and miRNA

Specific roles of miRNA and the relation between their mRNA targets during differentiation were not still well defined.[19] MiRNA repression of mRNA was important mechanism to regulate the expression during apoptosis, metabolism, and cell fate specification.[20] Comprehensive miRNA and mRNA expression was being profiled to identify the expression pattern. The importance of precise control of target mRNA expression by miRNA to make sure that lineage specification during identification and differentiation process.

MiRNA was small non-coding RNA while mRNA was classified as coding RNA. MiRNA have different function compare with mRNA (Table 2).[21] MiRNA was allowed to silence the gene regulation while mRNA was allowed for coding of genetic information. Due to stability, miRNA can be stored at 4 °C[22] while mRNA needs to be stored in colder storage, −70 °C. Platelet was known to contain a lot of functional miRNA. miRNA can be expressed outside from the cells.

Why the miRNAs?

miRNAs are carcinogenesis included in the biological processes. MiRNAs were found in *Caenorhabditis elegans,* which begans in 1993.[27] MiRNAs contained 19-24 nucleotides, and no protein expression was encoded in this sequence.[28] MiRNAs interact with the 3′ untranslated target mRNA region, resulting in target mRNA degradation and translation inhibition. MiRNAs have appeared in tumors, including ovarian carcinoma.[29] Mutations in cancer-related miRNAs in ovarian carcinoma are very minimal.[30] MiRNA makes them attractive to gene expression regulators for viruses. As they are small in amount, the small size genomes can easily

Table 5. Therapeutic miRNA for detection by using biosensor.

No	miRNA type	Mechanism	Nanoparticle	Detection range	LOD	Reference
1.	21	Gold electrodes/Amperometry	AuNP	10.0fM-5.0pM	3.0 fM	[76]
2.	21	Glassy carbon electrodes/square wave voltammetry	AuNP	100.00 aM-1.0 nM	30.0 aM	[77]
3.	21	Gold electrodes/differential pulse voltammetry	AuNP, DNA Nanostructure	100.00 pM-1.0 mM	10.0 pM	[78]
4.	21	Glassy carbon electrodes/square wave voltammetry	Graphene oxide/Titanium	1.0 aM-10.0pM	0.76 aM	[75]
5.	24	Gold electrodes/differential pulse voltammetry	Multiwall carbon nanotubes	1.0pM-1.0 nM	1.0 nM	[79]
6.	24	Gold electrodes/differential pulse voltammetry	Multiwall carbon nanotubes	1.0fM-10.0pM	0.1 fM	[80]
7.	26	Gold electrodes/ Electrochemical Impedance Spectroscopy	AuNP	30.0 aM-10.0fM	15.0 aM	[81]
8.	34	Pencil graphite electrode/ Electrochemical Impedance Spectroscopy	Graphene oxide	20.5 pM-10.0pM	2.5 pM	[82]
9.	34	Screen printed carbon electrodes/ differential pulse voltammetry	Carbon	1.0fM-10.0pM	0.5 fM	[83]
10.	126	Gold electrodes/differential pulse voltammetry	Graphene/Au-Ag	1.0fM-10.0 nM	0.79 fM	[84]
11.	155	Gold electrodes/differential pulse voltammetry	Graphene oxide/AuNP	10.0fM-1.0 nM	3.3 fM	[85]
12.	155	Gold electrodes/ square wave voltammetry	Quantum dots	50.0fM-○0pM	12.0 fM	[86]

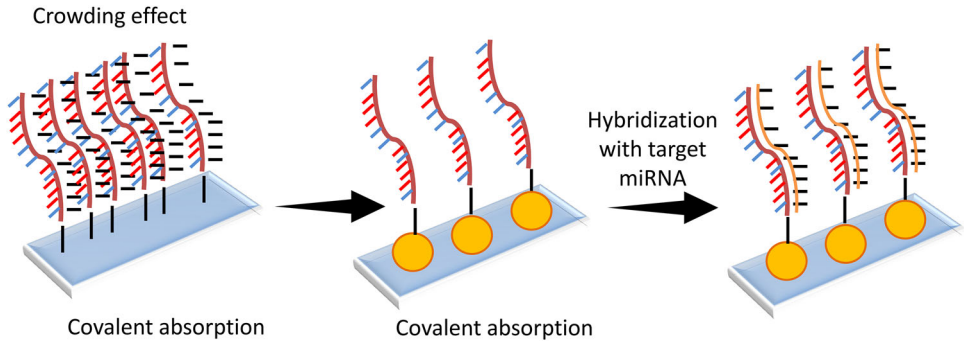

Figure 6. Crowding effect on the DNA-miRNA hybridization due to the high immobilization density.

be transferred. In addition, miRNAs are non-immunogenic and use the host regulatory mechanism.[31]

Across a number of solid and hematopoietic tumors, miRNAs were found to be expressed differently in the tumor versus normal tissues. Separate signatures of miRNA can reliably discern tumors from normal tissues in certain cases and are associated with the outcome of the disease. MiRNA was more effective in classifying human cancer compared with cDNA.[32] As diagnostic and prognostic biomarkers, miRNA contains around 21 nucleotides with a great potential.

Various cases of epithelial ovarian cancer (EOC) had specific characteristics, such as tumor level, subtype, and resistance to prognosis and therapy. EOC has been diagnosed with pelvic organs and abdomen since cancer has progressed to the advanced stages. EOC has become very dangerous and in 5 years they have a survival rate of 30% for the patients who identified at this point. At stage 1, 20% is diagnosed where cancer only narrows at the ovaries and does not pass to the pelvic and abdomen.

Nowadays, reliability is required using serum biomarkers to detect early ovarian cancer and to increase the patient's survival rate to 80%. Pelvic test, TVS, and CA125 serum were conducted as diagnostic procedures for the detection of ovarian cancer, but they cannot diagnose the disease at an early stage and minimize death by 80%. Medical doctor recommended platinum and taxane-based chemotherapy for the cancer treatment but sadly, high clinical response, relapses, and repeated treatment established a tolerance to these types of therapies through the use of cytotoxic chemotherapy.

Current Blood Isolation methods for miRNA are ineffective. Nguyen et al.[33] developed a reverse-transcription quantitative real-time PCR (RT-qPCR) test for the direct detection of miRNA circulating in breast cancer serum detection. Serum concentration in miRNA 21, used for the detection of breast tumors has a high correlation with the presence of breast cancer.

MiRNAs as an expression marker

Several studies have examined the role of miRNAs in ovarian carcinoma. MiRNAs do not need complete supplementation of target sites and can identify short supplement sites at 5′ terminals containing 2-8 nucleotides instead of complete miRNAs. Table 4 showed therapeutic miRNA for detection of ovarian cancer. Overexpress miRNAs acted as oncogenes via the negative regulation of oncogenes by the downregulated tumor suppressor genes. The functions of miRNAs have been highlighted in cancer biology and showed that miRNAs dysregulation in malignant differences like ovarian cancer. Naturally, miRNAs are small non-coding RNA molecules containing 18-24 nucleotides that interact with their target coding mRNAs to inhibit translation by promoting mRNA degradation or blocking translation by binding to complementary sequences in the 3′ untranslated regions (3′ UTR) of mRNA.[34] Current studies have shown that miRNA reported is more stable in blood than mRNA found in serum or plasma.[35]

Efficient serum or plasma extraction of circulating nucleic acids was difficult when too small in mer with the amount of nucleic acids is reduced, especially from the blood. To overcome this difficulty, the direct serum after centrifugation from the blood sample was used to detect miRNA.[33] The reverse transcriptase quantitative PCR assay showed that the significant between breast cancer patients with healthy female results. Unfortunately, this kind of method detection cannot differentiate the different stages of cancer.

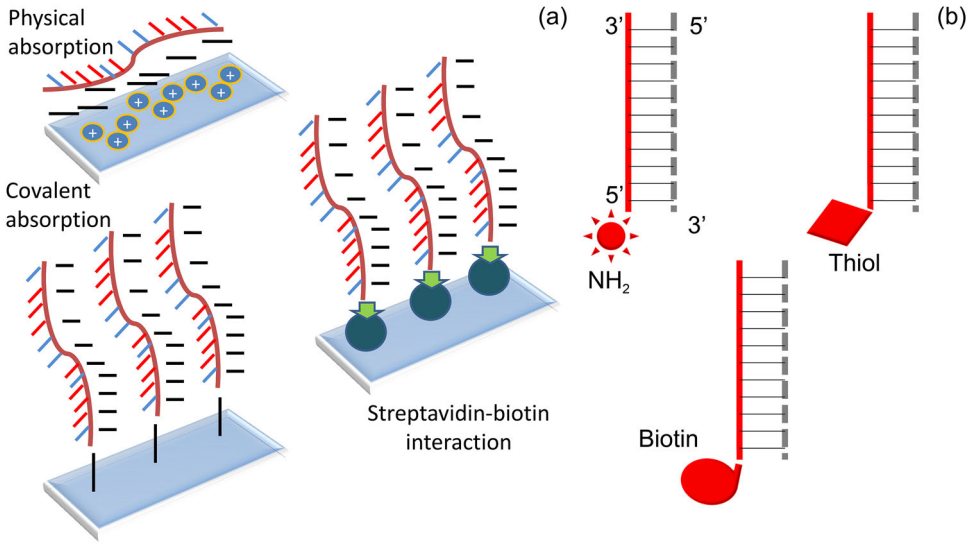

Figure 4. Immobilization techniques for fabrication of DNA biosensor for detection of miRNA.

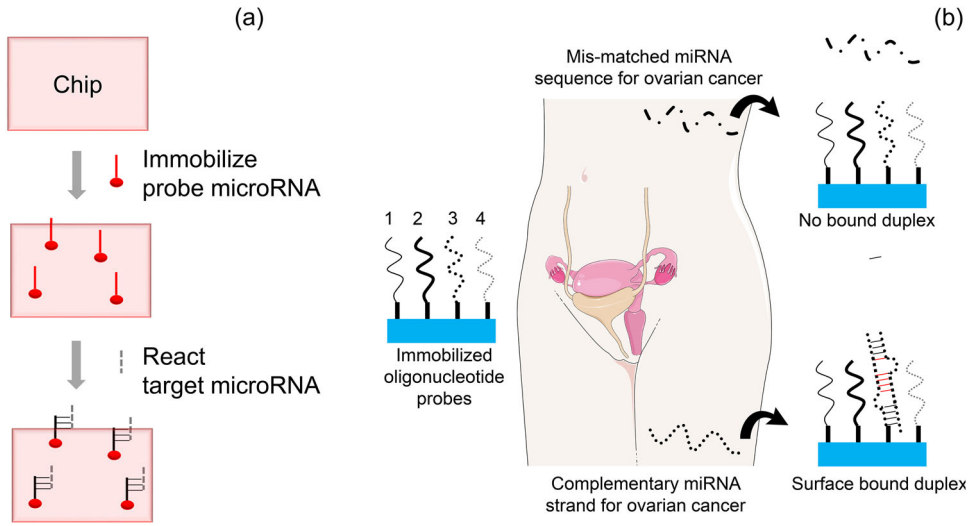

Figure 5. Complementation of probe and target miRNAs. (a) Singleplex; (b) multiplex. Shown for the ovarian cancer detection.

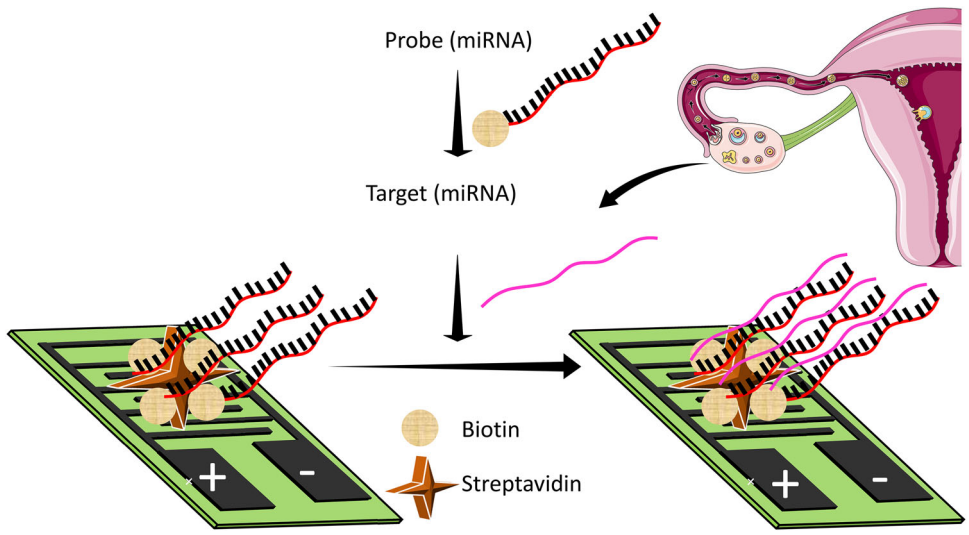

Figure 7. miRNA detection on interdigitated electrode. Surface is modified by streptavidin-biotin conjugation tetravalent strategy.

In a total of 489 HGSOCs, the Cancer Genome Atlas (TCGA) project analyzed mRNA expression, miRNA expression, methylation promoter, and copy number of DNA, showed that ovarian cancers could be classified into three subtypes of miRNA.[36,37] Poor survival was associated with a robust integrated mesenchymal subtype, and eight key

miRNAs (miR-25, miR-29c, miR-101, miR-128, miR-141, miR-182, miR-200a, and miR-506) were identified. Kinose et al. have shown that miR-199a-3p is de-regulated in high-grade serous ovarian carcinomas and that the loss of miR-199a-3p is correlated with ovarian carcinogenesis and peritoneal dispersion by causing upregulation of c-Met, the hep- atocyte growth factor receptor.[38] Considering the high levels of endogenous nucleases in body fluids, it was expected that the rapid degradation of freely circulating RNAs would occur. Recent evidence has shown that miRNAs are secreted from cells into circulation where they are stable and can sometimes be functionally transferred to the recipient cells.[39]

MiRNAs circulating have been exceptional as a biomarker because of their blood stability. Circulating miRNA as a stable blood-based biomarker for several cancer detections and becomes an interesting area in research because miRNA is protected from degradation in the extracellular environment.[40] This characteristic led to the option of miRNA as a suitable biomarker in the biosensor. Cell-free miRNA can be detected in eleventh different normal body fluids rather than blood and urine. miRNA in serum have been identified as biomarkers for the identification of cancer and other diseases.[41] The advantages of circular miRNA are resistant to RNAse digestion compared to mRNA. miRNA showed high resistance to the enzymatic cleavage by RNAse A that is most present in our hand. The stability of miRNA had been studied using serum from various sources including treatment with harsh conditions such as boiling, low or high pH, extended storage, and freeze-thaw cycles.

Circulating miRNA is highly tissue-specific and can be used as a noninvasive biomarker for cancer detection. miRNA can be an ideal choice blood-based biomarker for ovarian cancer detection because their expression was frequently dysregulated in cancer, the expression pattern of miRNA appears to be tissue-specific, highly stable in formalin-fixed tissue. Circulating miRNAs were first observed in serum and plasma in 2008, and subsequently identified in a number of body fluids such as saliva, urine, breast milk, and so on.[33–58] Endogenous salivary miRNAs have been degraded much slowly compared to exogenous miRNA.

MiRNAs circulating under harsh conditions such as extreme pH values, high temperatures, and long-term storage are known to be remarkably stable. Current studies have suggested that miRNAs are released into circulation from cells through the use of several packaging and transport systems to avoid degradation. Serum miRNA was identified as resistant to multiple freezes thaw cycles compared to large molecular weight RNAs. Serum miRNA was stable even treated for 3 hours in low pH (pH = 1) and high pH (pH = 13).

MiRNA and biosensors

Biosensors

Biosensors are rapid, sensitive, low-cost analytic device, and selective.[61–68] It contains two elements; a biological recognition element to capture target miRNAs and the transducer that is able to convert the signal of recognition into a measurable signal. Biosensors have superior features in sample preparation, cost, assay time, and portability compared to conventional methods. Figure 3 showed the components and selected constituent of a distinctive electrical biosensor including a bioreceptor that particularly binds to the analyte, a port architecture where a peculiar biological occurrence takes place and produce a signal, which is discovered by the transducer element, eventually the transducer signal is changed into an electronic signal and amplified the signal to the useful parameter by observing the current changes (conductance or resistance)

The target microRNA-155 sequence was identified by complementing an interdigitated dual-electrode surface capture-sample sequence-immobilized for early detection of breast cancer that caused by the mutation.[69] Special attention has been paid to breast cancer research by introducing a nanotechnological approach, in particular with biodegradable polymeric nanoparticles, which has resulted in many changes. A biotin-streptavidin complex was used to immobilize the capture probe on the interdigitated electrode surface and the target microRNA-155 was determined. Figure 6(a) showed immobilization techniques for the fabrication of DNA biosensor for the detection of miRNA including physical absorption, covalent absorption, and streptavidin-biotin absorption. Chemical functionalizations have been used widely by modifying the end(s) of the nucleic acid probe with appropriate chemistry (Figure 6(b)).

MiRNAs as biosensor target

Design of miRNA biosensor to become one of the critical points in the biosensor by using miRNA as the target detection component.[70, 71] MiRNA can be used as a potential candidate for the early detection of cancer. MiRNA had two different characteristics as oncogenic miRNA and tumor suppressors. Table 5 showed recent findings associated with certain circulating miRNA together with various kinds of diseases. Depending on miRNA's important roles in several diseases and focusing on cancer detection, the function and identification of expression levels of miRNA were considered as crucial noninvasive biomarkers and raised great to the academician and industry.

There have a lot of relevances of miRNA in development of biosensor to detect ovarian cancer. For enhanced diagnostic, prognostic, and streamlined therapeutic applications, miRNAs have emerged as promising noninvasive or minimally invasive cancer biomarkers. The creation of relatively simple, fast, and inexpensive miRNA biosensor strategies has received considerable attention. The electrochemical sensors showed a because of their intrinsic benefits, such as their great promise of point-of-care diagnostics, simplicity, sensitivity, accessibility to high multiplexing levels and low cost.

Electrochemical biosensor for miRNA analysis

The electrochemical biosensors have widely been used in the medical, environmental, agricultural, and food analysis

fields. The electrochemical nanosensor showed a great promising for fast, point of care analysis, and simple method detection. The transduction element can be in different materials such as gold, glassy carbon, graphite, and modification of these materials with various nanoparticles or nanotubes (Table 5). The principles in the electrochemical detection of miRNAs by measuring the changes in the electrode properties such as conductivity and resistance or in the electroactive compound redox signal that upon hybridization between the complementary probe and target miRNA sequences, can be applied for both singleplex and multiplex analyses with ovarian cancer and other disease diagnoses (Figure 4(a,b)).

Electrochemical biosensor for miRNA detection based on voltammetric measurement produced the changes in the current signal upon the hybridization of target miRNA. The complementation of probe and target miRNAs for singleplex and multiplex was shown in Figure 5(a,b) for the ovarian cancer detection. The voltammetric biosensor can be label-free detection based on their usage.[68, 72, 73] Label-free detection of miRNA was a simple method and additional steps of labeling were not required, more practical, and less time consuming compared to the label-based methods. Label-free miRNA sensing strategy depends on the electroactive of the nucleic acid signal before and after hybridization.[74] Figure 6 showed the crowding effect on the DNA-miRNA hybridization due to the high immobilization density and create label-free detection by using voltammetric sensing. As an example of label-free detection, Cheng et al.[75] designed a voltammetric biosensor for detection of miRNA 21 with a 0.76 aM limit of detection.

The analytical performance of the biosensor has been studied (Table 5). Based on the therapeutic miRNA for detection by using biosensor, the developed biosensor by using miRNA 21, glassy carbon electrodes using square wave voltammetry, and integrated with graphene oxide-titanium has an outstanding electrochemical function and lowest limit of detection, 0.76 aM compared to another sensing element of analytical performance of the miRNA biosensor.[75] This method achieved a wide dynamic linear range from 1.0 aM to 10.0 pM with an ultra low limit detection of 0.76 aM, exerting a major sensitivity enhancement. Additionally, the proposed biosensor was sufficiently selective and could be used to distinguish between the target miRNAs and homologous miRNAs. Its usage may be made for quick and direct study of human serum miRNAs. This strategy therefore offers a fresh and ultrasensitive platform in biomedical science, clinical diagnosis, and miRNA expression profiling.

Interdigitated electrodes (IDEs)

Gopinath et al.[87] focus on identifying the microRNA-155 target sequence by using a capture probe on an interdigitated electrode sensor. The target microRNA-155 sequence was identified by complementation on a capture-probe sequence-immobilized interdigitated dual-electrode surface and the sensitivity was found to be 1 fM, and this strategy can be implemented for miRNA-mediated ovarian cancer detection (Figure 7).

MiRNA analysis by using noninvasive methods for cancer detection

Recent studies have revealed that miRNA expression was similar and differentially enriched in serum, plasma, or other forms of diseased tissue body fluids that show their ability to be minimally ideal and useful biomarker invasive.[88] Understanding the function and detection of the miRNA expression level is considered an important noninvasive method and increase a great attention to the academia and industry. Based on miRNA properties, they are difficult to be amplified because of their short length and being lost in conventional RNA isolation methods. miRNA is also difficult to be amplified by using Polymerase Chain Reaction (PCR) due to their short length. Recent studies showed that miRNAs are not only present in serum[89] or plasma[90, 91] but also in different extracellular bio-fluids including saliva, tears, urine, breast milk, colostrum, peritoneal fluid, cerebrospinal fluid, bronchial lavage and seminal fluid,[92] and follicular fluid.[93-95]

Conclusion and future perspectives

A significant amount of research has been conducted on the detection of miRNA due to their specific signatures and the importance in the clinical part for early detection of cancer. In the present time, molecular biology, biosensor, and combining more than two strategies make them successful with miRNA detection. This review suggested that miRNA sensor needs to be developed with the emphasis of electrochemical detection and focusing on the early detection of ovarian cancer. In the experimental value, all the techniques describe in this review are a complement to each other like screening multi miRNAs, nanomaterial that has been considered for the best method to follow. The best analysis method was verified by using another technique for validation. The field of miRNA sensing for early cancer detection focused on circulating miRNA due to its noninvasive method, stability, and protected from degradation due to in the circular form.

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