Chapter 1
Recent Advances in Molecular Diagnosis of Gastric Cancer

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Abstract

Gastric cancer (GC) is one of the most common cancers worldwide. Despite recent advances in early detection, most cancerous patients are detected in advanced stages and so, 5-year survival rate is very low. So, there is an urge need for the discovery of sensitive early detection biomarkers for GC patients. Several serum tumor markers have been used in clinic for several years such as α-fetoprotein (AFP), carcino embryonic antigen (CEA) and so on. Due to low sensitivity and specificity of these traditional biomarkers, researchers seek for novel non-invasive and specific biomarkers. For this purpose, micro-RNAs and long non-coding RNAs have been reported as novel cancer biomarkers. Studies indicate that these RNAs have important roles in tumorgenesis and so they could potentially use as early detection biomarkers. This review summarizes major advances in our current knowledge about molecular diagnosis of GC that have been reported in recent decades.

Gastric Cancer

Gastric cancer (GC) is one of the most common cancers worldwide and the second cause of cancer mortality in developing countries [1]. Therefore, gastric malignancies have a higher rate of incidence in Africa and East Asia compared to Europe and North America. Also, the prevalence of GC in men is higher than women [1,2].

The main type of GC is adenocarcinoma, originating from gastric planar layer glands or mucosa. However,
there are other types of GC, originated from the stomach, including lymphoma and leiomyosarcoma which belong to lymphoid tissue of the stomach and surrounding muscles of the mucosa, respectively [2]. Like other cancers, GC is a multi-factorial disease and several environmental, genetic and epigenetic factors are involved in its etiology. Some environmental risk factors include age, sex, smoking, Helicobacter pylori infection and obesity [2].

Although recent radical surgery and chemotherapy could be medicinal for early-stages of GC, the five-year survival rate is only 20-30% [3]. Also, Most GCs are diagnosed at advanced stages that could not be helpful in defining a therapeutic decision. While, Endoscopic biopsy is the best way to find GC before clinical symptoms, few patients would like to undergo endoscopy due to potential offensive side effects, including aspiration, pneumonia, bleeding, and perforation. Hence, it is necessary to find non-invasive biomarkers for early detection of GC patients. Beside conventional detection methods, specific molecular markers could be employed as diagnostic and prognostic means [4].

**Genetic Biomarkers**

Genetic biomarkers are considered as genes or any related products which facilitate the prognosis and diagnosis of diseases. These biological molecules have specific characteristics such as their facile availability to detect as well as different levels of activity in healthy and patient individuals. Numerous genes and proteins could be considered as molecular biomarkers. Therefore, they are an objective to evaluate normal or pathogenic biological processes. For this aim, it exploits diagnostic tests, imaging technologies and other objective measures for a person's health status. Genetic biomarkers are therefore valuable tools for cancer diagnosis, prognosis and treatment. These can also be used to determine tumor's stage, subtype and response to therapy. Cancer cells represent a broad spectrum of alterations in genes including gene rearrangements, point mutations and gene amplifications which lead to disturbances in the molecular pathways such as cell growth, survival and metastasis. When such specific changes are detected in majority of patients with a tumor malignancy, these variations could be used as signals for detection and better decision for treatment [5].

Diagnostic and prognostic genetic biomarkers are quantifiable parameters that could help oncologists to manage suspected patients. These particularly aid in (i) identifying who is at risk, (ii) diagnosis at an early stage, (iii) selecting the best treatment and (iv) monitoring response to treatment [5]. Different forms of these biomarkers were reported; traditional biomarkers include those that can be assessed by measurement of circulating levels of tumor specific antigens with radiological techniques, mammograms and etc. With the availability of complete human genome sequence and developing recent new technologies such as next generation sequencing (NGS), microarrays, and mass spectrometry, a lot of potentially
informative cancer biomarkers have expanded to include the sequence and expression levels of DNA, RNA, and protein as well as metabolites [6].

Establishment of powerful cancer biomarkers requires a comprehensive knowledge about the cellular and molecular mechanisms by which cancers initiate and progress, especially those that are consequences of small changes in only a few master regulators. A major challenge in cancer diagnosis is to establish an exact relationship between cancer biomarkers and clinical pathology to provide the possibility of detecting tumors at an early stage in non-invasive manner. Since, alterations in the DNA content and gene expression occur in many types of cancers, the genetic-based approaches are helpful in diagnosis and prognosis. Analysis of global gene expression profiles provided by microarray and NGS technology has improved the genome-based approaches for studying biomarkers in cancer researches. These techniques are quite successful in clearly discriminating subtle changes between different stages of tumors as well as resolving tumor types which are apparently similar, but different at the level of molecular aspect [5].

Biomarkers for Early Diagnosis of Gastric Cancer

Classical Biomarkers

Until now, several serum tumor markers such as carcino-embryonic antigen (CEA), carbohydrate antigens 19-9, 27-4 and 50 (CA 19-9, CA 27-4 and CA 50), α-fetoprotein (AFP), metalloproteinases and Serum Glycan have been used in clinic. Here, we aim to discuss briefly about these biomarkers.

CEA

CEA was initially identified in 1965 and was first applied for the early detection of GC in 1980. This protein marker is currently used as the most valuable serum protein marker for early diagnosis of patients with risk of developing GC. Nevertheless, serum level of CEA can be altered in patients with other types of carcinomas, thus it exhibits low specificity and sensitivity [7].

CA 19-9, CA 72-4 and CA-50

CA 19-9 has previously been a commonly used marker in gastrointestinal cancers such as particular pancreatic carcinoma and GC. The CA 19-9 test in combination with the CEA test is a useful adjunct for monitoring carcinoma of the stomach. However, the sensitivity of these assays in GC is similar to the CEA test alone [7].
The combined assay of preoperative serum levels of CEA, CA 19-9, and CA 72-4 has provided additional prognostic information for patients with resection of gastric cancer. In fact, patients with preoperative positivity for one of these markers have a high risk of recurrence, even in the early stages of gastric cancer [8].

It was also found that elevation of CA 19-9 and CA-50 levels after chemotherapy is correlated with increased incidence of relapse, but not with the disease-free interval. The estimation of CA 19-9 and CA-50 levels could be useful for early detection of GC recurrence after surgery or adjuvant chemotherapy [9].

**AFP**

Many cases of AFP-producing GCs, characterized by increased AFP serum levels and AFP+ gastric tissues, have been reported. AFP-producing GCs have been associated with a poor prognosis due to high proliferative activity, weak apoptosis rate and rich neovascularization, compared to that of AFP-types. These biological characteristics of AFP-producing GCs reflect the aggressive behavior of the tumor and the poor prognosis of patients with this type of cancer [8].

**Metalloproteinases**

Levels of serum and plasma metalloproteinases, especially type IV collagenases, are important factors in metastasis and invasion of various human cancers. Serum protein proMMP-9 levels and plasma protein proMMP-9 levels may serve as tumor biomarkers, independent from the known factors such as CEA [10].

**Serum Glycan**

Serum glycan profiles may provide biomarker to discriminate GC cases from controls who just suffered from non-atrophic gastritis. Further studies need to be done to convert these findings to biomarkers and elucidate the role of protein glycosylation in GC pathology [11].

**Novel Biomarkers**

**Assessment**

Due to low sensitivity and specificity of traditional biomarkers, early detection of primary tumors has been controversial. However, tumor biomarkers can be used as monitoring factor in tumor recurrence and/or prognostic factor particularly when their higher levels are observed in advanced stages of disease. Therefore, identification of novel applicable biomarkers for early diagnosis of GC is becoming attractive worldwide. Hereunder, we briefly discuss the latest research in this field.

**Oncogenes/Tumor Suppressors**

**Oncogenes**

To date, numerous GC-related oncogenes have been reported. Oncogenes are overexpressed genes in
cancers which promote cell growth and cell cycle progression. They also inhibit apoptosis by repressing apoptosis-related genes. When a molecule has low expression in gastric or non-gastric part of healthy individuals, detection of aberrantly activated oncogenes can be of great diagnostic value. As shown in Table 1, recently, several human genes were reported as GC-related oncogenes whose expression was increased in different tissues [12,13].

Table 1: Genes up-regulated in gastric cancer.

Pt: Number of patients enrolled in expression analysis; IHC: Immunohistochemistry; QPCR: Quantitative realtime reverse transcription-polymerase chain reaction; OS: Overall survival; RFS: Recurrence free survival; N: Lymph node metastasis. (Reprinted from: Mitsuro Kanda, Yasuhiro Kodera. “Recent advances in the molecular diagnostics of gastric cancer.” World J Gastroenterol, 2015; 21(34): 9838-9852. Copyright 2015 by Baishideng Publishing Group Inc.)

MicroRNAs (miRNAs)

miRNAs are small non-coding RNAs of about 17-25 nucleotides in length that control vast variety of biological processes. Since the first discovery of two miRNAs (lin-4 and let-7) in 1990s, an increasing number of miRNAs have...
been identified in various organisms. miRNA biogenesis begins with the transcription of the miRNA encoding gene as a long primary transcript known as pri-miRNA. A Hairpin-shaped segment which is embedded in pri-miRNAs, undergoes more processing steps including cleavage of pri-miRNA by the RNase III family enzyme, Drosha and its cofactor DGCR8 that takes place in nucleus to generate pre-miRNA. The pre-miRNA is transported to the cytoplasm and cleaved by another RNase III enzyme, Dicer to generate miRNA/miRNA* duplex. The RNA duplex is loaded into Argonaute protein (Ago) complex, which preferentially degrades the miRNA strand and sustains the mature miRNA. Ago complex associates with GW182/TNRC6 family to form a complex named RNA-induced silencing complex (RISC). MiRNA/Ago complexes recognize target mRNAs by pairing ~7 nucleotides of 5’-end of mature miRNA (mostly nucleotides 2-8, that is called “seed region”). Although miRNAs often recognize and target the 3’-UTR of mRNAs, they have been shown to target mRNAs at their coding region and 5’-UTR as well as promoter region [14,15].

**Role of miRNAs in GC**

As illustrated in figure 1, regulation of miRNAs' expression plays a crucial role in the pathogenesis of GC such as proliferation, invasion, metastasis, apoptosis, Helicobacter Pylori infections and so on. Moreover, a number of miRNAs have been shown to be associated with tumor type, tumor stage, and patient’s survival. Understanding novel biological functions of miRNAs have provided insights into the use of miRNAs as novel biomarkers of GC [16,17].

**Figure 1:** Role of miRNAs in GC. (Reprinted from: Han-Shao Liu, Hua-Sheng Xiao. “MicroRNAs as potential biomarkers for gastric cancer.” World J Gastroenterol. 2014; 20(34): 12007-12017. Copyright 2014 by Baishideng Publishing Group Inc.)

**MicroRNAs as Diagnostic Biomarkers**

Analyzing the expression profile and next-generation sequencing of miRNAs revealed that the expression level of cancerous tissues is obviously different from non-tumoral...
ones. As a result, observed miRNA differential expression in tumor tissues could be used for tissue specificity and classification of cancer types. As shown in figure 2, studies have revealed dozens of miRNAs that play roles in gastric tumorigenesis and influence gastric cancer growth, cell cycle progression and metastasis processes, thus might be used as potential biomarkers [18].


Circulating miRNAs

Since application of tissue-expressed biomarkers needs to use of invasive methods such as endoscopic biopsy and aspiration pneumonia, few patients would like to be subject of such medical services. Therefore, there is a demand to find non-invasive diagnostic procedures. Nowadays, researchers paid their attention to find circulating tumor markers which are secreted from the tumor tissues to the body fluids, such as serum. As a potential non-invasive biomarker that could be applied in clinical use, miRNAs are easy to detect from body fluids, such as blood plasma or serum [4]. Cellular miRNAs can be packaged into exosomes or freely exported from the cells to blood [19]. The presence of miRNAs in blood circulation was first reported by Chim et al. [20] and Lawrie et al. [21] in 2008. Immediately after these initial findings, three other groups found and characterized a list of miRNAs present in sera and other body fluids [22]. For the first time, Tsujiura et al. in 2010 reported alterations in level of circulating miRNAs in patients with GC [23]. These miRNAs were further followed by robust studies that have demonstrated differential levels of these circulating miRNAs in various type and stages of GC. Nowadays, researchers have devoted to identify sensitive miRNA biomarkers in the blood of GC patients. These novel tumor markers have several advantages such as long time stability even after incubation at room temperature or multiple times of freeze-thawing. Their stability and easily testable
length (~ 22nt) make miRNAs suitable for being used as cancer biomarkers [4]. Table 3 reviewed several circulating miRNAs have been identified, which are significantly modulated in their expression in GC patients compared to that of normal population [19].

**Table 3:** Dysregulated circulating miRNAs reported in gastric cancer patients.

<table>
<thead>
<tr>
<th>Dysregulated miRNAs (miR)</th>
<th>Down-regulated miRNAs (miR)</th>
<th>miRNA detecting methods</th>
<th>Samples &amp; sample sizes (gastric cancer patients/healthy controls)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17, 19, 19a</td>
<td>17, 19, 19a</td>
<td>qPCR</td>
<td>Plasma (69/30)</td>
</tr>
<tr>
<td>1, 10.4, 10.9, 10.5</td>
<td>1, 10.4, 10.9, 10.5</td>
<td>qPCR</td>
<td>Plasma (90/27)</td>
</tr>
<tr>
<td>48, 406</td>
<td>48, 406</td>
<td>qPCR</td>
<td>Plasma (50/30)</td>
</tr>
<tr>
<td>21, 225</td>
<td>21, 225</td>
<td>qPCR</td>
<td>Plasma (70/70)</td>
</tr>
<tr>
<td>87-89, 87-90, 87h</td>
<td>87-89, 87-90, 87h</td>
<td>qPCR</td>
<td>Plasma (50/30)</td>
</tr>
<tr>
<td>27a, 27b, 19a, 21a, 22b, 27a, 27b, 19a, 21a, 22b</td>
<td>27a, 27b, 19a, 21a, 22b, 27a, 27b, 19a, 21a, 22b</td>
<td>qPCR</td>
<td>Plasma (50/30)</td>
</tr>
<tr>
<td>37a, 37b</td>
<td>37a, 37b</td>
<td>qPCR</td>
<td>Plasma (50/30)</td>
</tr>
<tr>
<td>11a</td>
<td>11a</td>
<td>qPCR</td>
<td>Plasma (50/30)</td>
</tr>
<tr>
<td>421</td>
<td>421</td>
<td>qPCR</td>
<td>Plasma (60/40)</td>
</tr>
<tr>
<td>50a, 50b, 50c</td>
<td>50a, 50b, 50c</td>
<td>qPCR</td>
<td>Plasma (60/40)</td>
</tr>
<tr>
<td>105, 50</td>
<td>105, 50</td>
<td>qPCR</td>
<td>Plasma (50/30)</td>
</tr>
<tr>
<td>37a, 37b</td>
<td>37a, 37b</td>
<td>qPCR</td>
<td>Plasma (50/30)</td>
</tr>
</tbody>
</table>


**Exosomal miRNAs**

Exosomes are secretary vesicles that are derived from most of human cell types. The reported diameter of exosomes is between 30 and 100 nm. These cellular vesicles are present in many and perhaps all biological fluids, including blood, urine, etc. Most of the tumor cells secrete exosomes and give the possibility to detect them in different fluids of the human body, including plasma, serum, urine, saliva, milk and amniotic fluid. Although previous studies considered exosomes as “garbage bag” that deplete wasteful metabolic products of the cells, preceding researchers have discovered multiple different functions for these micro-vessels. Nowadays, several biological functions are attributed to exosomes: they commence cell-cell communications through packaging and transferring the bio-molecules such as proteins, mRNAs and miRNAs throughout the body [24]. The miRNA recruitment to the exosomes depends on the attachment of RNA-induced silencing complexes (RISCs) to the ESCRT components. However, the release of exosomal miRNAs is under control of ceramide-dependent machinery, as reported by Kosaka et al [25]. EVs confer several potential benefits over current clinical biomarkers. EVs may carry either previously known biomarkers or novel ones to be used as surrogate factors. Moreover, the advantage of using these types of biomarkers is due to their protection by the exosomic membranes against destructive conditions of bloodstream or other extracellular environments. Evidence of miRNA existence in exosomes that was firstly reported by Valadi et al. showed that these vesicles contain many types of RNAs including miRNAs, which can be transferred to another cell, where they can be functional.
Since then, the number of studies in order to identify other exosomal miRNAs in cancer has been over-growing in the literatures. The principle of using exosome as cancer biomarker is that the miRNA content of an exosome, derived from a tumor cell, may be different from that of normal cells. Using this principle, the exosomal miRNA profile from fluid samples of patients with GC has also been assessed. The data reported the presence of let-7 family (a, b, c, d, e, f, g, i) in exosomes which were derived from gastric tumor cells [26].

Long Non-Coding RNAs (lncRNAs)

LncRNAs are endogenous cellular RNA molecules with approximate 200 nucleotides long, which lack the capability of encoding proteins. These RNAs are predominantly transcribed by RNA polymerase II and are subsequently subjected to 5'-end capping, 3'-end polyadenylation and splicing. However, multiple lncRNAs were reported that are transcribed by RNAPolymerase III and have not 3’poly-A tail. LncRNAs have different subcellular localization where they can interact with DNA, RNA, and proteins and act as gene expression regulators at different levels. Like other RNAs, lncRNAs have detected in several body fluids and in comparison with other types of RNAs, they have more specificity to express in a time- and cell-type specific manner [27].

Observed aberrant expression of many lncRNAs across both solid and non-solid malignancies brought these molecules to the forefront of cancer researches and accelerated studies to elucidate their mechanistic roles in cancer. LncRNAs play important roles in many biological processes of normal cells such as proliferation, differentiation and migration and homeostasis. In cancer cells, they are involved in proliferation, invasion, angiogenesis and therapeutic sensitivity. Confirmed functions of some lncRNAs make them as potential novel biomarkers for diagnosis or therapeutic targets. Disregarding to their mechanisms, accumulating in vitro and in vivo studies strengthen their role as oncogenes (e.g., HOTAIR, ANRIL, MALAT1, SRA, HULC, UCA1, PCA3, PCAT-1, PCGEM1, and PRNCR1) or tumorsuppressors (LincRNA-p21, GAS5, MEG3, TERRA, PANDA, and TUG1) [27,28].

Role of lncRNAs in GC

LncRNAs Involved in Angiogenesis

Angiogenesis is an important event for proliferation, metastasis, and drug sensitivity of tumor mass that not only provides nutrients and oxygen for cancer cells and transports tumor metabolites, but also provides favorable conditions for transferring the in situ cells to other part of the body (metastasis) [29]. LncRNAs promote carcinoma metastasis by activating an angiogenic system which contains phosphoglycerate kinase (PGK). PGK is a disulfide
reductase enzyme, conventionally thought to be involved in glycolysis. It also functions as an inhibitor of metastasis through attenuating the angiogenesis processes [30]. Certain lncRNAs were found as modulators of angiogenesis in GC. For example, H19 promotes vasculogenesis by activating tumor necrosis factor-α, which indirectly induces angiogenic factors [31]. Other lncRNAs regulate basal sprouting and migration, endothelial cell proliferation, capillary density by regulation of vascular endothelial growth factor α (VEGFα) and its receptor [32].

**LncRNAs that Participate in Cell-Cell Junction and Adhesion**

Tight junctions (TJs) join the cells together and regulate the diffusion of ions and specific molecules through the cell environment. They also maintain the integrity of the cell-cell protective barrier. Recent studies have revealed that cell-cell junctions are the main protectors of GC metastasis. The absence or aberrant distribution of TJ proteins on the surface of gastric cells leads to facile disjunction of cancer cells to spread around finally initiates the metastasis [33]. TUC339 is an ultra-conserved lncRNA with 1198-bp long that regulates both gastric cancer cell growth and adhesion [32].

**Degradation of Extracellular Matrix by LncRNAs**

The extracellular matrix plays important protective role against invasion and metastasis of GC cells. Its damage ruins the protective barrier against cancer cell migration and provides a favorable environment for metastasis. Pri-cellular proteases originated from cancer cells can digest matrix proteins and hence facilitate cancer metastasis [34]. Matrix metalloproteinases (MMPs) are a subgroup of such proteases that degrade the extracellular matrix and modulate GC metastasis [35]. As regulators of proteases, lncRNAs have great importance in stabilizing or degrading the extracellular matrix. For example, H19 is down-regulated in prostate cancer and its corresponding microRNA (miR-675), inhibits the expression of extracellular matrix protein, and thereby inhibits prostate cancer metastasis [36]. Park et al. found that expression level of the lncRNA, BM742401 in normal gastric cells is necessary to inhibit migration through repression of MMP9 secretion. In many GCs, BM742401 is down-regulated and promotes the invasion and metastasis [37].

Other lncRNAs that are deregulated in GC include HOTAIR and FENDRR which contribute in metastasis. These lncRNAs control extracellular matrix degradation by modulating the expression of metastasis-related genes such as ICAM-1, MMP1, MMP2, MMP3 and MMP9 [32].
LncRNAs as Cancer Diagnostic Markers

Changes in the expression levels of lncRNAs are increasingly reported in various malignancies suggesting that lncRNAs play roles in tumor initiation and progression. On the other hand, lncRNAs also exist in serum, plasma and other body fluids [27]. Therefore, lncRNAs could be considered as potential diagnostic and/or prognostic biomarkers. Table 4 represents recent important GC-related lncRNAs [12].

Table 4: Gastric cancer-associated long non coding RNAs.

<table>
<thead>
<tr>
<th>Symbol (location)</th>
<th>Materials</th>
<th>Detection methods</th>
<th>Pt</th>
<th>Survival</th>
<th>Relevant clinical factors</th>
<th>Functional analysis</th>
<th>Interacting molecules</th>
<th>In vivo study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early detection</td>
<td>CDDO (19:3:12)</td>
<td>Circulating</td>
<td>QPCR</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>HOTAR1 (12p3.1)</td>
<td>Tissue</td>
<td>QPCR</td>
<td>40</td>
<td>-</td>
<td>Premortal, invasion, stage</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Prediction of survival</td>
<td>BLCAT1 (10p2.1)</td>
<td>Tissue</td>
<td>QPCR</td>
<td>35</td>
<td>Size, N, stage</td>
<td>Prognostic, migration, invasion</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>CCAT1 (8q24.21)</td>
<td>Tissue</td>
<td>QPCR</td>
<td>67</td>
<td>Size, stage</td>
<td>Prognostic, migration, invasion</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>GAPLINC (18)</td>
<td>Tissue</td>
<td>Microarray</td>
<td>90</td>
<td>Size, N</td>
<td>Prognostic, MAPK, migration, invasion</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>M/F5A32 (4p16.3)</td>
<td>Tissue</td>
<td>QPCR</td>
<td>30</td>
<td>Depth, N, stage</td>
<td>Prognostic</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Pt: Number of patients enrolled in expression analysis; QPCR: Quantitative real-time reverse transcription-polymerase chain reaction; OS: Overall survival; N: Lymph node metastasis

Conclusion

Gastric cancer, also known as stomach cancer, is one of the most common cancers worldwide and it is of great importance to understand its clinical pathology and also its molecular basis. This malignancy is originated from the altered phenotype of lining cells of the stomach. In the study of cancers, two main trends are being followed: 1) cancer diagnosis and 2) cancer treatment. Like other cancers, uncovering the molecular basis of gastric cancer could provide facilities to both gastric cancer diagnosis and treatment. In the past years, gastric cancer cases have mostly been detected through apparent phenotypes such as indigestion, feeling bloated after eating meal, heartburn, slight nausea, loss of appetite and so on. But thereafter, as the medical technologies emerged, many researchers around the world have paid attention to discover and use of cancer biomarkers. Above all, there are many reports about the genes, which their expression alterations were validated in many types of human cancers [38]. Based on their function in tumor behavior, scientists have classified these genes as two main groups: oncogenes (ONCs) and tumor suppressors (TSGs) [39,40]. Although these genes have different pattern of expression between tumor and normal tissues, they must have other criteria to exploit them as useful cancer biomarkers [40]. These criteria include remarkable and differential change in expression in cancer cells, availability of clinical samples without invasive methods and high detection precision [41]. In this chapter we compiled a set of well-documented genes, involving in gastric cancer.

Firstly, traditional genetic biomarkers were collected and categorized as ONCs and TSGs. Thereafter, new class
of biomarkers including miRNAs and long non-coding RNAs were evaluated. Nevertheless, in a comprehensive vision, we would not discard the use of traditional biomarkers such as many protein-coding genes. A medical diagnostic procedure for gastric cancer should consider both protein-coding genes in combination with non-coding RNA genes. That is why both are under the regulation of each other by diverse set of complicated mechanisms [42]. Disregarding to the mechanisms, a genetic biomarker must be clinically feasible to detect. Mostly, genetics biomarkers are extra-cellular particles which finally could be traced in body fluids, bloodstream, urine and stool. Old procedures, such as biopsy, which exploited tissue-expressed genes as cancer biomarkers, were offensive for patients. In many cases, the biopsy was reported as causal factor in tumorigenesis for healthy individuals who referred to clinic be screened. In this study we represented lists of gastric cancers biomarkers. They are diverse in their functional role and expression level. However, the usefulness of appropriate biomarkers mostly depends on the frequency of observed expression change of the genes in patients, the detection method(s) and the type of molecular marker [43,44].

Rather than protein biomarkers as well as long non-coding RNAs, recent studies are going to use miRNAs as gastric biomarkers. Of these, the use of GC-related miRNAs which are in fact, due to their small size (19-25 nt long), they are protected from natural ribonucleases which are abundantly present in the body fluids finally exported to the cell outside (secretory miRNAs) is the main area of cancer researches [45]. Moreover, some miRNAs are encompassed in extracellular vesicles (EVs) which are successfully protected and exported through the plasma membrane and they could be used as cancer therapy. In this method, EVs which carry tumor suppressor miRNAs can be exposed to ONC-expressing cancer cells [24].

Like other cancers, administration of gastric cancer contain simultaneous use of ONC- and TSGs. Disregarding to the detailed molecular function of genes, scientists must exploited both protein-coding and non-coding RNA genes for diagnosis and treatment of gastric cancer. Invasive and expensive methods must be improved by using stable, accessible and detectable biomarkers (such as miRNAs). However, the power of biomarkers must be tested in a general population to acquire the “diagnosis precision”. Designing inhibitors against intracellular biomarkers could be also used as a therapeutic tool.

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