

Reprimo as a Potential Biomarker for Gastric Cancer Diagnosis and Early Detection

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Abstract:

Searching effective biomarkers for use in gastric cancer diagnosis, prognosis and early detection is an imperative challenge. Some biomarkers have been used in diagnosis of gastric cancer clinically, however, no effective biomarkers have been currently used for early detection. Here, we recapitulate the current biomarkers used for gastric cancer diagnosis, describe aberrant Reprimo gene methylation and a detection method, and discuss its potential use for gastric cancer diagnosis, prognosis and early detection.

Keywords:

Reprimo (RPRM), DNA Methylation, Methylation-Sensitive Melt Curve Analysis, Tumor Biomarkers

1. Introduction

Gastric cancer is one of the major causes of cancer-related death in the world [1]. Early treatments including surgery provide the probability of better survival for gastric cancer patients. However, most cases of gastric cancer are diagnosed at the advanced stages, which loses the best intervention timing during the early cancer development. The conventional methods used for gastric cancer examination include the endoscopy and barium swallow. However, these techniques have many shortcomings, such as the requirement of complex operation, irradiation exposure or potential invasive harms to the patients. Other methods, such as the fluorescence imaging, the utilization of which in screening was reported to reduce the mortality rate of gastric cancer by a half [2], are not applicable for a large-scale screening in asymptomatic populations due to their high costs and complex procedures. In this paper, we will focus on describing the potential of the molecular, genetic and epigenetic factors used as biomarkers for the gastric cancer diagnosis and early detection.

2. Molecular Biomarkers for Gastric Cancer Detection

Tumor biomarkers mainly refer to the molecules secreted or shed into the body fluids or tissues by tumor cells, or the substances produced and secreted by host due to the immune response against a neogenic object. These molecules or substances can provide hints for the existence of a tumor, help to determine the tumor characteristics, such as the malignancy, metastasis or differentiation degrees, and provide information for prognosis and treatment. Among these, about 24 are approved by the US Food and Drug Administration and commonly used clinically [3]. But as for gastric cancer, the biomarkers are quite scarce, and lack of high sensitivity and specificity. Several gastric cancer biomarkers are currently clinically used in serological examination, such as 2-MG, CEA, CA199, and Fer, etc. However, these biomarkers are lack of consistent detection outcomes. Thus, the possibility for detecting the early-stage gastric cancer is usually less than 5%, which constraints their application for early diagnosis.

One major approach for finding the early detection biomarkers is in search of the regulatory molecules or factors involved in the gastric cancer development. In general, transformation of normal cells to tumor requires multiple stages of cellular changes. Accumulation of mutations, dysfunction, and genetic defects among a variety of oncogenes, tumor suppressor genes, cell cycle regulating genes or other related ones will eventually cause immortality and malignancy of the tumor cells. Currently, a variety of genes have been found to be associated with gastric cancer development, including oncogenes such as *c-met*, *c-myc* and *k-sam*, et.al; tumor suppressor genes such as *p53*, *DCC*, *APC* and *MCC*; apoptosis related genes such as *bcl-2*; metastasis related genes such as *nm23* and *CD44*; and other genes such as *EGFR*, *HER-2/neu*, *TGF α* , *MMPs* and *TIMP*. Some of these genes are mutated in a variety of human tumors, while some others are silenced or over-expressed in early or advanced gastric cancer patients. However, these changes are often in correspondence to particular pathological subtypes or specific pathogenic pathways so that they are not universal phenotypic features for gastric cancer [4]. Besides, the expression of some of these genes is not directly associated with the early development of gastric cancer, impeding their applications in the early diagnosis.

So far, the only available early test is measuring the ratio of pepsinogens (PGs)IandII concentrations in serum. PG belongs to the family of aspartic proteases, and is the precursor of pepsin which specifically localizes in gastric mucosal to exert the protease function. PG can be divided into two subgroups, PGIand PGII, according to their immunogenicity. A change of the ratio of PGItoII can be used to indicate the potential gastric atrophy, which is a kind of gastric precancerous lesions. And PGIand PGII were significantly increased in patients with gastric cancer, and decreased after surgery [5]. Thus, the PGIand PGI/II ratio may serve as “serological biopsy” to monitor the pathological changes at the gastric gland mucosa, and may be used in the early diagnosis of gastric cancer. However, this method is only somewhat useful for predicting intestinal-type gastric cancer but is not good for the large-scale detection.

3. Gastric Cancer-Related Gene Methylation Biomarkers

Epigenetic regulation manipulates the cells' normal development or disease progress by controlling the genome stability, gene expression and cell development through multiple modifications of the human genome[6], and provides an essential source for finding tumor biomarkers. Aberrant DNA methylation frequently occurs in

tumors, including a reduction in the overall methylation level in the genome and the hypermethylation in certain gene promoter regions, particularly in tumor suppressor genes and mismatch repair genes. This modification will alter the gene expression or accelerate the gene's mutation, favoring for tumorigenesis. Different from genetic mutations or other genetic changes, aberrant gene methylation is a reversible process [7].

Within the genome of a cancer cell, there might be a variety of genes abnormally methylated at the same time. These hypermethylated genes may play roles in cell cycle regulation, DNA repair, drug resistance, differentiation, apoptosis, tumor metastasis or angiogenesis. However, different types of tumor may have their own gene hypermethylation patterns. Thus, DNA methylation alterations may serve as another type of biomarkers for tumor diagnosis [7]. The establishment of gene methylation patterns of different cancer types has become an important mission in the related fields [8,9,10]. So far, aberrant gene methylation has been found in lung cancer, gastric cancer, prostate cancers, leukemia, lymphomas, and gallbladder carcinomas, *etc* [11]. Hypermethylated genes in these cancers include *APC*, *E-cadherin*, *ER*, *Reprimo*, *14-3-3Sigma*, and *3OST2*, *etc*. However, effects caused by methylation of these genes might be inversed in different cancer types. Methylation of these genes might be correlated with the cancer progression. For example, in the non-small cell lung cancer tissues, *Reprimo* and *14-3-3Sigma* promoters were frequently hypermethylated, but no significant difference of methylation frequency was observed among tumor specimens at the different clinical stages, and neither was it associated with lymphnode metastasis, suggesting that the promoter methylation of both genes might be an early event in tumor development.

The epigenetic variation in gastric cancer was first observed in the DNA mismatch repair gene (*hMSH2* and *hMLH1*) promoter [12]. Afterwards, a number of aberrant methylated genes were found, including tumor suppressor genes *p16*, *pS2*, cell cycle regulation gene *APC*, cell adhesion molecule *CDC1*, tumor metastasis inhibitor *DCC*, *P14INK4a/ARF (CDKN2A)*, calcium-dependent adhesion molecule *E-cadherin*, multifunctional transmembrane hyaluronic acid receptor *CD44*, cyclooxygenase *COX-2*, tyrosinephosphatase *SHP1* and *SEMA3B*, *etc* [4,13]. However, the results of methylation studies may be inconsistent due to the influences of race, region or detection methodologies [13,14]. So far, a comprehensive DNA methylation profile for gastric cancer has not yet been laid out.

4. Roles of Reprimo Gene and Its Methylation In Gastric Cancer

Reprimo is a glycosylated protein located in the cytoplasm. It was first identified as a p53 tumor suppressor-inducible expression gene by differential display PCR after X-ray irradiation of wild-type and p53/IRF-1 deficient mouse embryonic fibroblasts [15,16]. The *Reprimo* gene localizes on the chromosome 2q23. Allelic gene defects at this site have been associated with lung cancer, breast cancer, and colon cancer [17,18]. P53 can induce *Reprimo* mRNA expression [15], and *Reprimo* may exert its anti-tumor function by inhibiting the activity of Cdc2 and the nuclear translocation of the Cdc2/cyclinB1 complex, causing G2 cell cycle arrest in cells [15]. It has been shown that aberrant methylation in the *Reprimo* gene promoter can interfere with its transcription, causing a G2/M checkpoint regulation disorder [15,16]; and this abnormal methylation is not correlated with p53 mutations [19,20]. Therefore, inactivation of these checkpoint molecules may lead to or accelerate the genetic instability.

Aberrant methylation of *Reprimo* gene is shown to be closely related to the occurrence and development of gastric cancer [15,21], although no preferably methylated locus or session has been observed [22]. Previous studies have also suggested that the methylation degree is varied in the different tissue samples, but not associated with the clinicopathological factors, including aging and stage of tumor [23]. Abnormal methylation of the *Reprimo* gene was also found in many other cancer types [19, 20]; while the methylation occurrence was significantly lower in brain cancer, ovarian cancer, cervical cancer and bladder cancer [24], indicating a tissue-specific regulation pattern of the *Reprimo* gene in tumorigenesis. In many studies, the methylation is thought to be the dominant way to inhibit the *Reprimo* gene expression [25,26,27]. Because the changes of *Reprimo* expression is not associated with the gene mutation [25,26,27], a more complex mechanism may be involved in the regulation of *Reprimo* gene expression.

5. Can *Reprimo* Gene Methylation Be Used for Early Gastric Cancer Detection?

In the early-stage gastric cancer plasma samples, the *Reprimo* gene was found to be frequently methylated (the frequency is over 70%), while was rarely seen in the plasma samples of normal individuals [13]. The *Reprimo* hypermethylation has been reported in premalignant conditions such as Barrett's esophagus and non-malignant gastric epithelia, as well as associated with *cagA* and *vacA s1m1* alleles in the early gastric cancer progression [25,27]. A role for *Reprimo* in gastric cancer development has been postulated because the loss of *Reprimo* expression was significantly associated with the progression from stage I to stages II-IV of gastric cancer [28]. Therefore, the *Reprimo* methylation may be a useful biomarker for early detection and prognosis of gastric cancer. We have recently developed a semi-quantitative method based on methylation-sensitive melt curve analysis (MS-MCA) for detecting *Reprimo* DNA methylation which may provide a good tool for examining the *Reprimo* gene methylation [21]. In this method, a promoter region of *Reprimo* gene is selected containing 30 cytosines which are potential targets for methylation. Two plasmids representing unmethylated and complete methylated DNA are used as controls. DNA of test serum or tissue samples are bisulfite converted and used for polymerase chain reaction. The melt curves of these reactions derived from test samples are analyzed and compared to the controls. Therefore, the methylation degrees can be determined.

6. Conclusions and Perspectives

Early detection and diagnosis remains a major challenge for effective gastric cancer intervention. Recent advances provide the essential evidence for *Reprimo* gene methylation to be developed as a new biomarker for gastric cancer diagnosis, prognosis and early detection. However, its value in the clinical practice needs to be further verified based on a large scale of sample test. The clinical trial for detecting the *Reprimo* gene methylation is approved by the US FDA and is currently undergoing, and hopefully will be performed in the other countries, particularly in China, which accounts for 40% of the total gastric cancer cases of the world [29]. Recently, a MS-MCA method was developed to detect the *Reprimo* gene methylation, providing a promising method for early gastric cancer detectio [21]. However, more methods need to be developed to facilitate the course of clinical tests. In addition, although *Reprimo* methylation can be detected in the circulating DNA from gastric cancer patients' serum, the source of it still remains uncertified. The role and

molecular mechanism of Reprimo in the gastric cancer development are also not clear and need to be further explored. In our unpublished data, we find that three DNA methyltransferases (DNMTs) including DNMT1, DNMT3A and DNMT3B, which are the key enzymes involved in DNA methylation, are involved in the *Reprimo* promoter methylation. Further study still requires to solve the mystery on when *Reprimo* is methylated by DNMTs and how the methylation is connected to the occurrence of gastric cancer. We believe that the above issues will become unraveled and the *Reprimo* gene methylation test will soon be used clinically to benefit human health.

Conflicts of Interest

The author declares that there is no conflict of interest regarding the publication of this article.

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