Tumor markers: a proteomic approach

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ABSTRACT

This article reviews the recently published data on the diagnosis of cancer with proteomics, including the major proteomics technologies and promising strategies for biomarker discovery and development. Most of the tumor markers are proteins that either numerically increase in response to the alteration of cancer conditions or are produced by cancer cells. However, they are natural compounds ordinarily available in the typical cells to a little extent what are affected by increase of expression due to cancer and its intensity in blood, body fluids or tissues. Tumor markers are substances normally available in body fluids such as serum, urine, blood, and tissues that increase in the desired tissue of cancer patients. Most of tumor markers are proteins that either are produced in response to changes in cancer conditions or are made by the cancer cells. However, most of tumor markers are among the natural compounds of normal cells present in normal conditions in the cell in small amounts and are affected by increase of expression, due to cancer and their levels in the blood, body fluids or tissues.

Key Words: Tumor marker: Proteomic: Cancer: Biomarker

INTRODUCTION

Tumor markers are substances available in body fluids such as blood, urine, serum and tissues that are increased in patients with cancer in different tissues. Most of tumor markers are proteins that either are increased in response to changes in cancer conditions or are made by the cancer cells. However, most of tumor markers are among the natural compounds of normal cells present in normal conditions in the cell in small amounts that are affected by increase of expression due to cancer and their levels in the blood, body fluids or tissues [1]. Tumor marker may be considered as a molecule determining the cancer risk or even provides us with the information about cancer risk and behavior such as metastases, cancer spread, and cancer recurrence and back [1]. Tumor markers are divided into two main classes of "tissue-specific" and "cancer-specific". The first class tumor markers are related to which tracing them indicates presence of tumor in tumor tissue in the body. Some tumor markers like CEA (Carcinoembryonic Antigen), CA125 (Cancer Antigen 125), and CA19-9 (Cancer Antigen 19-9) can also be applied in treatment, follow-up, evaluating the extent of tumor spread and response to therapy. Cancer-specific class is related to the tumor markers which tracing them indicates presence of tumor tissue in a particular tissue where the tumor has spread. These types of tumor markers are likely to increase in non-tumor conditions as well. PSA (Prostate Specific Antigen), GCC (Guanylyl Cyclase C), AFP (Alpha-fetoprotein), and beta-globulin are in this class [2].

Generally, there are five main types of tumor markers [3]:

1.Enzymes: Alkaline phosphatase, phosphatides, PSA, and etc.

2.Tissue receptors: GCC, ER (Estrogen Receptor), PR (Progestrone Receptor), and etc.
3.Antigens: CA125, CA19-9, CEA, and etc.
4.Oncogenes: Ras, Myc, and etc.
5.Hormones: HCG (Human Chorionic Gonadotropin), calcitonin, and etc.

History of tumor markers

The first modern tumor marker used to check out cancer was HCG that was used for pregnancy test. High concentrations of this marker in the blood may be a sign of cancers related to the placenta so-called Gestational Trophoblastic Disease (GTD). Some types of uterine and testicular cancers produce this marker where germ cells are affected [4].

The purpose of researches in the field of tumor markers is to reach to a point that any type of cancer could be diagnosed by a blood (or urine) test. In addition to detecting the cancer at early phases, this simple blood test can prevent millions of deaths each year. The first success in finding a blood test for detect a cancer was achieved in 1965 when Carcinoembryonic Antigen (CEA) was detected in the blood of patients with colon cancer. By late 1970s, several other blood tests for various types of cancer were discovered including marker CA19.9 for colon and pancreatic cancer, CA15-3 for breast cancer and CA125 for uterine cancer. After them, some markers were identified that were not investigated further, because they had no benefit in compare with recent markers [5]. However, due to the following reasons, none of these markers met the main goal in cancer diagnosis at early phases:

1.Most people have a small amount of this marker in their blood; therefore, this it is difficult to diagnosis the cancer in early phases.
2.The amount of these markers significantly goes higher than normal when too much time has passed since the onset of the cancer.
3.The elevated level of these markers in the blood of some patients is not observed though they are affected by cancer.
4.High level of these markers in the blood may not be only due to cancer; for example, CA125 also rises in women with gynecologic problems.

The most important and only marker used for screening is Prostate Specific Antigen (PSA) that has been used since the early 1990s as a screen marker for early phases of prostate cancer; it is now used because of two reasons: firstly, this marker is made only by prostatic cells and therefore, diagnosis of this marker is an indicator of prostatic problems; secondly, this marker is increased at the onset of cancer that can help to diagnose the cancer at the early phases [6].

Applications of Tumor Markers

Most tumor markers can be potentially used in any of the items listed below for diagnosis, prevention and in some cases even for treatment. Here are some applications of tumor markers:

1.Screening of malignant tumors in the initial phase:

Despite the limitations and lack of specificity for tumor markers in the initial phases of formation, several tumor markers currently have the potential to be used in the screening tests, including Vanillylmandelic Acid (VMA) and Homovanellic Acid in the screening of infants suffering from neuroblastoma [7], AFP for cancers of hepatic cells, CA125 for women with uterine cancer and PSA marker for prostate cancer. Nevertheless, study on none of these markers could reduce mortality in patients with malignant tumors [8, 9, and 10].

2.Help to cancer diagnosis:

One of the important problems in cancer diagnosis is concerning identification the primary tissue in a metastatic tumor, because identification of primary tissue is essential in many cancers for management the patient and treatment. In some cases, despite the analysis of pathologic results, medical examinations, routine blood and serum analysis, and X-ray examinations they are still unable to identify the primary tumor tissue. Fortunately, in such cases, several tumor markers have been identified which their determination in the blood and serum may help to identification of primary tissue [11]. The production rate of HCG marker increases in the case of gestational choriocarcinoma, AFP and HCG markers in germinal tumors, PSA in prostate cancer, and the amount of CA15-3 marker in breast cancer [12]. According to the statement of National Cancer Center Network (NCCN) and European Society of Medical Oncology (ESMO), the level of AFP,

HCG and PSA markers should be measured in the men who have cancer with unknown origin and primary tissue; and according to the same statement, the level of AFP, HCG, CA125, estrogen receptor, progesterone receptor and HER2 (Human Epidermal Growth Factor Receptor 2) should be measured in women [13].

3.Prognosis prediction:

Prognostic markers are the factors may predict the outcome of a cancer or a patient when he/she is not treated e.g. adjuvant therapy. Predictive markers are the factors indicating the response or sensitivity of a cancer to the therapeutic agents during the treatment [14]. The common prognosis factors for malignant tumors include tumor size, tumor grade, and number of involved metastasized lymph nodes; furthermore, tumor markers can be considered as a prognosis factor for malignant tumors if they possess the following characteristics [15]:

- Being independent of the previous common prognosis factors or able to provide sufficient information in addition to them;
- The information provided by tumor markers would be more accurate and more precise than the ones obtained from previous factor; and
- Tumor markers would be able to provide prognosis information in the cases that common clinical factors are unable to provide information. For example, in the patients with breast cancer of the negative lymph node and the patient with colon cancer of stage II, prognosis markers may distinguish between the patients who must receive chemotherapy and those who do not need to receive this therapy.

The prognosis markers can be measured both in serum level and tumor tissue. The serum markers have this ability to identify the spread of tumor bulk or hidden metastases by examining their concentration. The most widely used tissue markers in this field are probably the molecules able to indicate the tumor spread through increased cell proliferation or metastatic spread in the tissue [16].

The most popular and best prognosis tumor markers used in clinics are AFP, HCG, and LDH. Another common marker is CEA in the diagnosis of colorectal cancer that is able to distinguish between the patients of stage II who are eligible to receive chemotherapy and those who do not need to receive this therapy. Two popular prognosis markers in the people with breast cancer of negative lymph node are Urokinase Plasminogen Activator (UPA) and Plasminogen Activator Inhibitor-1 (PAI-1) [17].

4.Post surgical care:

One of the main applications of tumor markers is currently to monitor the patients' situation after surgery. This is important since the timely diagnosis of metastatic and returning the disease after surgery improves the chance of recovery or treatment results [7]. Thyroglobin (TG) is one of the markers in patients with advanced thyroid carcinoma. Normally, the concentration of this marker in the people serum is not measurable and its increase may be sign of metastatic or return of thyroid cancer in the patients when it is detectable and is observed in the patients' serum [18]. Another most widely used markers in the review of recovery process after surgery of patients with colorectal cancer is CEA. The recent investigations has shown that serial evaluation of this marker in patients' serum can indicate the return of cancer and its metastasis in patient after surgery with a sensitivity and specificity of about %80 and %70, respectively. CEA is currently one of the most widely used markers in the review of colorectal cancer and also is the most applicable test to determine the potential of therapeutic in the people with returning the cancer [19].

Predict the response to therapy: The predictive markers are very important in oncology, because the cancers are different in terms of response to therapy. Only small number of cancer patients benefits from the specific systemic methods; therefore, we will be probably able to avoid the unnecessary therapeutics and apply most beneficial therapy for patients if we could properly select the patients in terms of response to therapy. Having the accurate predictive markers in this field can help us to get reliable and accurate results. Nevertheless, only few of oncologic predictive markers are clinically useable [7]. The first of these markers in oncology is ER that can indicate the probability of response to hormone therapy in patients with breast cancer [20]. The new marker been introduced for patients with breast cancer is HER-2 that is used to check the patients who are treated by Trastuzumab (Herceptin, Roch). Trastuzumab is a monoclonal antibody synthesized against HER-2 [21]. Epidermal growth factor receptor (EGFR) is another predictive marker that its mutation or increase of expression was seen in various types of cancer; two therapeutic agents of monoclonal antibodies and tyrosine kinase inhibitors (TKIs) have been synthesized against this marker [22].

Modern techniques for measuring the tumor markers

The common methods for measuring the tumor markers are ELISA technique for serum markers and Immunohistochemical technique for tissue markers; usage of these two techniques, we are now able to track one or a few markers simultaneously [7]. But two new techniques of microarray gene expression and proteomic are capable to track hundreds or thousands of markers simultaneously. According to recent investigations, it is claimed that use of microarray helps us to identification of tumors with unknown primary site that are similar in terms of morphology; it also provides beneficial and predictive information to evaluate the response or resistance of tumors to special therapy. While this method can measure thousands of genes at RNA level, proteomic method measures the markers at protein level [23]. There are many new proteomic techniques including gel electrophoresis, tissue microarray, antibody microarray and mass spectrometry (SELDI-TF), that the recent technique can detect different kinds of cancer with more remarkable specificity and sensitivity than existing markers [24].

The matter in making these techniques applicable is that standardization and simplification should be done at first and also their cost must be reduced to become available and usable in laboratory centers easily [25].

Clinical tumor markers

Currently, there are over 60 kinds of analytes as tumor marker that have been approved by Association of Food and Drug (AFD). Some of them are only for research goals, but they are mostly for diagnostic, screening or cancer evaluation purposes [26]. Here, we explain some more common clinical tumor markers that are used in laboratories as diagnostic tests.

Alpha-fetoprotein (AFP):

AFP is a glycoprotein, which is produced by developed fetuses but its amount declines in the blood after the birth. AFP test is primarily used to detect some abnormalities that occur during embryo development. However, in adults the higher level of 1000 ng/l of this marker in the blood is often associated with cancer. AFP test has been confirmed for the detection and evaluation of testis cancer. Also, the level of this marker rises in 80% of patients with hepatic cell carcinoma and liver cancer. The biopsy for evaluation of liver cell carcinoma should be performed when AFP levels as high as 500 ng/l (or higher) would be observed [27].

CA125:

This marker is as kind of glycoprotein that is expressed by epithelial cells during embryo development [5]. The high amount of this marker is often associated with uterine cancer, so that the level of this marker rises in serum in 75% of the patients with uterine cancer. Also, the level of this marker increases in 50% of the patients as stage I and 90% of the patients with stage II and higher [26]. The level of marker CA125 is associated with the amount of tumor mass; therefore, this test is used to check out the return of tumor followed by chemotherapy [26]. In this test, the level of this marker is checked in patients with uterine cancer

every 3 months for 2 years after the therapy and elevated level of this marker during this assessment represents the return of disease in most cases [28]. In addition to uterine cancer, this marker is also used to evaluate some other malignant cancers such as liver cancer, lung cancer, breast cancer and colon cancer [4].

Carcinoma embryonic Antigen (CEA):

This marker is a kind of glycoprotein that is expressed by normal mucosal cells. Increased expression of CEA is seen in adenocarcinomas particularly colorectal cancer [29]. The amount of this marker rises in 50% of colorectal cancers that have spread to lymph nodes and 75% of patients with metastasis. The highest amount of CEA is 100 ng/ml that has been observed in patients with metastasis [30]. CEA marker is not suitable for screening test of patients with colorectal cancer [31]. The main role of this marker is investigating return of disease after receiving a suitable therapy in patients with colorectal cancer [30]. American Society of Clinical Oncology has proposed to assess the amount of CEA in patients with colon cancer stage II and III and those who are surgical candidates every 2 to 3 months for at least 2 years. If we observe elevation of this marker, a metastasis or return may be happened [32]. The investigations represent that the amount of this marker may be mild elevated in the smokers and 5% of healthy people [26].

Prostate Specific Antigen (PSA):

It is a glycoprotein produced by epithelium cells of prostate and its level rises in men suffering from prostate cancer and any other diseases related to prostate [33]. PSA has been approved as a screening test for prostate carcinoma. Normal serum level of this marker is less than 4ng/l. The level between 4 and 10 ng/l means that it is a positive test, because the level of this marker goes higher than 4 and 10 ng/l in 20-30% and 50% of patients with prostate carcinoma, respectively [33]. The presence of metastasis can be predicted by PSA test [34]. In patients that the level of PSA is recently elevated and PSA is less than 20 ng/l, the probability of cancer is very rare and the probability of metastasis is below 2% [34]. After treatment of prostate cancer, PSA level should be controlled every 6 months for 5 years and then every 2 years [35].

Human Chronic Gonadotropin (HCG):

Beta subunit of this glycoprotein marker is normally expressed by cells of the placenta and high level of this marker has been observed in pregnancy and cancers associated with germ cells and gestational trophoblastic disease [36]. HCG along with AFP rises in 85% of patients suffering from tumors related to germ cells, but the level of this marker rises in only 20% of patients with stage I; therefore, this marker is not suitable for screening test since it rises only in small percentage of patents at early stages [36]. In metastatic cases, the high level of AFP and HCG can help to diagnosis of germ cells tumors at the current phase of cancer [37]. The higher levels than 10000 ng/ml of AFP and 50000 u/ml of HCG at diagnosis time suggests poor prognostic outcome, means that the cure rate is low [38]. The level of these two markers is also used to assess the response to the therapy of patients with germ cells tumors. In the patients who their level of these two markers does not decrease appropriately after receiving the therapy, it is necessary to consider another therapy method [38].

Common cancers and tumor markers

Breast cancer

Nowadays, the diagnosis of breast cancer is accomplished in the laboratories by common biological parameters such as lymph node status, tumor size, tumor grade, malignancy rate, age and expression of gene HER-2 [39]. Despite this, it seems that to know these parameters would not be sufficient for precise diagnosis or proper selection of the patients for radiotherapy; also to estimate the rate of disease progression and preventing the return of cancer after therapy, it is necessary to present newer markers with higher sensitivity and specificity [40]. Nowadays, the only marker that is used for evaluation of therapy is estrogen and progesterone receptor (ER/PR) that is applied to select the patients who need to receive an approach for hormone therapy. Also, marker HER-2 is used to select the patients who should receive Trastuzumab (Herceptin) [39]. In the recent years, Tissue Inhibitor of Metalloproteinase 1 (TIMP1) has been proposed as a new prognostic marker and the marker assessing response to the therapy in the patients.

The molecular role of this marker is inhabitation of apoptosis in the tumor cells [39]. According to the investigation conducted in 2009, gene Gli1 has been introduced as another prognostic marker for breast cancer [40]. During this study, the scientists concluded that there is a strong correlation between increase in the expression of this marker and unsuccessful therapy results in the patients suffering from breast cancer, also the increase in the expression of this gene is associated with lymph node status and tumor stage [41].

Uterine cancer

According to the various studies have been conducted on identifying the tumor markers of uterine cancer, the best known and most widely used marker to study and diagnosis of uterine cancer is CA125 that is used as a test to check out the therapy during and after the therapy [42]. However, the use of this marker has been restricted due to this fact that the level of this marker also rises in the serum of patients with heart failure and liver cancer [43]. Hence, the use of this marker, along with other common diagnostic methods is currently being investigated [44]. Therefore, there is an essential need for more sensitive and more specific markers beside CA125 for diagnosis and study of the therapy in patients with uterine cancer [44]. One of these new markers is B7-H4 that is used beside CA125 to detect the cancer at early stages and the combination of these two markers together has the greater specificity than the marker alone. The increase expression of CA125 is observed in cases whom the tumor is malignant or has spread [45]. At an investigation conducted in 2007, Spondin2, which is a member of spondin family, and dcr3, which is a secretory member of TNFR, beside markers CA125 and B7-H2 are presented as a group to improve the diagnosis and detection of uterine cancer [46].

Gastric cancer

Gastric cancer is one of the most common cancers in the world and there are limited biomarkers to check out or predict the progression of this cancer [47]. Molecular markers are one kind of these markers. CEA has been presented as the most widely used tumor marker for gastric cancer [48]. CA19-9 and CA72-4 are also among the antigen markers that have been presented to detect and check out the patients suffering from gastric cancer [49]. CEA and CA19-9 are most common tumor markers to detect and check the patients suffering from gastric cancer after surgery and the combination of these two markers causes the increase of sensitivity of these markers. But none of these two markers are sensitive enough to diagnose gastric cancer. At a study, these two markers have been reported to be more reliable and more important than other tumor markers for gastric cancer [50]. On the other hand, in an investigation done in 2007, M2-Pyruvate Kinase marker has been presented as a more accurate and more sensitive tumor marker than CEA, CA19-9 and even CA72-4 to detect the esphagogastric patients [51].

Evaluation of TIMP1 gene in the serum of patients with gastric cancer indicated the sensitivity and specificity of %17 and %98 as a marker, respectively. Because the high sensitivity and specificity is required for a diagnostic marker, this marker is not suitable as a prognostic marker in the cases are at high and advanced stages. It has been observed that the increased expression of this marker has a strong association with progression of the cancer. According to the investigations and researches conducted to identify and introduce the tumor marker with high sensitivity and specificity, also the limited markers in this area been identified [52].

Colorectal cancer (CRC)

The rapid increase of signs and molecular symptoms of colorectal cancer caused the identification of the molecular markers could help the therapy and diagnosis of patients. But despite of the assessments and identifications of different markers, the experts have not yet succeeded to use these markers as a common and routine test. Apart from MSI, most identified molecular markers have not yet been able to recognize the stage of CRC patients clearly and successfully [53].

Serum markers

In recent years, various markers for the detection of CRC have been targeted that they could be measured in either serum, or blood, or stool and the most common of them is serum marker CEA [54].

CEA

The oldest and still most widely used marker in CRC patients is CEA. This marker is used in these patients to study the improvement of patients after resection of the primary tumor. Another major advantage of this marker is the early detection of recurrent and metastasis, particularly liver metastasis [55]. Although the best method for improvement and survival increasing of these patients is surgery, the main therapy of patients with metastasis is chemotherapy. In these patients, the CEA level should be measured every 2-3 months for 3 years [56].

Markers based on stool

The most widely used test for CRC screening is the diagnostic test checking the presence of blood in stool. This test is based on the peroxidase-like activity of hemoglobin and immunochemical which evaluates the shape and position of the globin chain of hemoglobin [57]. Another test in this field is checking out the abnormal DNA mutants that since there is no significant and known gene which would be altered in CRC patients, a number of genes are like mutants of K-Ras, APC, P53, BAT26, adenine tract26 and long DNA [58].

Tissue markers:

The serum markers are preliminarily used for improvement and survival increasing after the surgery, while stool markers are mostly used for screening; on the other hand, the tissue markers are valuable in the diagnosis, therapy, and prediction of disease [54].

Thymidylate synthesis (TS)

Thymidylate synthesis (TS) is a rare enzyme causes converting deoxyuridine monophosphate (Dump) to deoxythymidine monophosphate that is a necessary reaction for DNA synthesis. TS is widely used as a tissue marker for diagnosis and prediction of therapy [59]. A review of clinical studies has shown a correlation between high expression of TS in CRC patients and resistance to 5-FU, which is a agent for chemotherapy in the patients. The higher TS in the patients associated with the poor results of chemotherapy [60].

Microsatellite instability (MSI)

One of the most applicable and acceptable molecular markers is MSI tissue marker. Approximately %15 of CRC patients, who are potential candidates for study of MSI, indicate the deactivation of mismatch repair factors that causes increase/decrease in repeating of a repetitive sequence of about 5-10 nucleotides on DNA [61]. Based on an investigation carried out on CRC patients, the patients with MSI have shown the better results for approximately %15 in comparison with the patients without MSI. Moreover, the correlation between MSI and desired diagnosis of CRC patients, according to this study, might be because of activated lymphocytes [62].

Guanylyl Cyclase C (GCC)

GCC is from the receptor family of guanylyl cyclase and six groups of this family have been identified in the mammals so far. These receptors are involved in secretion and the regulation of body fluids and electrolytes [63]. GCC is expressed in mucosal cells of intestine from duodenum to rectum and is not expressed in the outer intestinal tissues [64]. Expression of this receptor increases in the cases that colon tissue undergoes the transformation and metastasis and this increased expression has not been observed in the metastasis of other outer intestinal tumors [65, 66]. Various articles have introduced this receptor as an unique and specific marker to study the metastatic state and the improvement of CRC patients after the surgery [67, 68, and 69]. The spread of tumor cells into the peripheral blood is one of the metastatic cases. In an investigation carried out on the blood of CRC patients, this marker was observed in their peripheral blood and was further known as a specific marker to study the metastatic tumors of colorectal cancer as well as tracking the free tumor cells in the blood [70].

During his researches and investigations in 2008, Dr. Scott Waldma applied this marker to develop a diagnostic kit for cancer patients following by confirming that as a diagnostic marker for CRC [71].

Methylated genes as the new tumor markers

During the cancer process, the first defect takes place in genomic DNA, including molecular alteration of mutation, copy, move, heterozygous, MSI and aberrant methylated of the specific genes. The genes which undergo this change are proposed to be capable of being used as the tumor marker [72]. The aberrant hyper methylation of some specific genes at promoter region is an important

fact toward the formation and progression of the cancer. For instance, in some situations this aberrant methylation happens at the early stages of malignity of the tumor. Many investigations have claimed that measurement of methylation of promoter region of specific genes is likely to be beneficial in early detection of cancer, prognostic determination and prediction of therapeutic response [73]. One of the reasons to consider DNA as a tumor marker is that DNA is more resistance than RNA and also can stay unchanged longer; consequently, it might be a useful tool for laboratory studies. Furthermore, DNA could be copied by PCR easily; therefore, less amount of sample is required than mRNA and protein [74].

The methylation takes place at CpG region on the DNA. The CpG supposed to be metylated has the size of 0.5 to 5 kilos of the pairs. About %50 of mammalian genes contain CpG located in promoter region or first exon that are typically often inactive and just get methylated and activated in cancer conditions. The methylation of CpG in promoter genes is usually associated with genes shutdown due to the prevention and interruption of transcription of genes [75]. Assessment of 98 various primary tumors indicated an average of about 600 aberrant methylated genes in each tumor. The genes methylated at early phase of tumorgenesis are likely to be useful to detect the people at risk of tumor progression or malignancy. However, the genes methylated in the middle of tumorgenesis or in the malignant stages may be used as prognostic marker. In addition, the measurement of the amount of genes methylation in cases that drug resistance or susceptibility is observed, could lead to improvement of the information concerning the prediction of the appropriate therapy [76]. The genes undergo aberrant methylated are suitable to be used as tumor marker due to three reasons. Firstly, since the amount of protein markers increases slightly at early stages of tumorgenesis and cancer, the evaluation of these markers in blood is difficult. Then, they hold diagnosis potential at early stages or in screening; however, the methylation of promoter of several genes currently exist both in early and advanced stages of cancer and freely releasing of a piece of these genes into the blood might be useful as a marker investigating the cancer at early stages or early diagnosis [77]. One of the other characteristics of tumor markers is their tissue specificity, particularly when they are used for diagnosis. Due to some unknown reasons, some genes have tissue specificity and get methylated in certain cancer tissues. Take for instance, Hmlh1 gene gets methylated in colorectal and gastric cancer whilst is rarely and slightly methylted in other types of cancer [78]. BRCA1 gene is methylated in breast and ovarian cancer while PV3 and p15 are methylated in most of the hematologic malignancies. This sort of methylation also helps the identification of metastatic tumors with unknown primary origin [79]. In addition, the promoter of some genes such as RASSF1, 1nk4a and p16 are methylated in several types of tumors [74]. Third characteristic that makes methylated genes the suitable markers is that the location on the gene, which gets methylated, is determined and therefore, designing a pair of primers, the specific gene caused methylation, could be easily detected [80]. In general, according to the various studies conducted so far, it may be claimed that methylated genes can be used for detection of people at risk of cancer spread. Nowadays, the scientists and researchers have considered these genes as appropriate tumor markers since they are measureable in the samples in which only few percentage of tissues have been damaged and also at early stages of the disease, which is much applicable for timely detection of cancer [73].

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