(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 2 430 193 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
09.03.2016 Bulletin 2016/10

(21) Application number: **10775152.1**

(22) Date of filing: **14.05.2010**

(51) Int Cl.:
C12Q 1/68 *(2006.01)* ***G01N 33/53*** *(2006.01)*
G01N 33/50 *(2006.01)* ***G01N 33/68*** *(2006.01)*

(86) International application number:
PCT/NZ2010/000089

(87) International publication number:
WO 2010/131984 (18.11.2010 Gazette 2010/46)

(54) **MARKERS FOR DETECTION OF GASTRIC CANCER**

MARKER FÜR DEN NACHWEIS VON MAGENKREBS

MARQUEURS DE DÉTECTION DE CANCERS GASTRIQUES

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO SE SI SK SM TR

(30) Priority: **15.05.2009 NZ 57701209**

(43) Date of publication of application:
21.03.2012 Bulletin 2012/12

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Description

FIELD OF THE INVENTION

[0001] This invention relates to detection of cancer. Specifically, this invention relates to the use of a protein marker for detection of gastric cancer.

BACKGROUND

[0002] Survival of cancer patients is greatly enhanced when the cancer is detected and treated early. In the case of gastric cancer, patients diagnosed with early stage disease have 5-year survival rates of 90%, compared to approximately 10% for patients diagnosed with advanced disease. However, the vast majority of gastric cancer patients currently present with advanced disease. Therefore, developments that lead to early diagnosis of gastric cancer can lead to an improved prognosis for the patients.

[0003] Identification of specific cancer-associated markers in biological samples, including body fluids, for example, blood, urine, peritoneal washes and stool extracts can provide a valuable approach for the early diagnosis of cancer, leading to early treatment and improved prognosis. Specific cancer markers also can provide a means for monitoring disease progression, enabling the efficacy of surgical, radiotherapeutic and chemotherapeutic treatments to be tracked. However, for a number of major cancers, the available markers suffer from insufficient sensitivity and specificity. For example, the most frequently used markers for gastric cancer, ca19-9, ca72-4 and carcino-embryonic antigen (CEA) detect only about 15-50% of gastric tumors of any stage, declining to approximately 2-11% for early stage disease. Thus, there is a very high frequency of false negative tests that can lead patients and health care practitioners to believe that no disease exists, whereas in fact, the patient may have severe cancer that needs immediate attention. Moreover, these markers can give false positive signals in up to 1/3 of individuals affected by benign gastric disease.

[0004] WO 2005/010213 describes markers for detection of gastric cancer. WO 02/100336 describes tissue-specific endothelial membrane proteins. Liang et al (Proceedings of the American Associate for Cancer Research Annual Meeting, Vol. 46 April 2005, p452) describes loss of ZG16 in colorectal cancer.

[0005] The Affymetrix Gene Chip Human Genome U133 set (http://www.helmholtz-hzi.de/fileadmin/user upload/research/Research/Programme/Technological-platforms/Gene Expression Analysis/Service/humangenomeu133) describes oligonucleotide sequences for an Affymetrix Gene Chip.

SUMMARY OF THE INVENTION

[0006] Described herein are methods, compositions and devices that can provide for detection of early stage cancer, and decrease the frequency of false positives and false negative test results.

[0007] Molecular analyses can be used to identify genes that are highly expressed in gastric tumor tissue, but not necessarily over-expressed compared to non-malignant gastric tissue. Such analyses include microarray and quantitative polymerase chain reaction (qPCR) methods. Cancer genes, RNAs and proteins encoded by those genes are herein termed gastric tumor markers (GTM). It is to be understood that the term GTM does not require that the marker be specific only for gastric tumors. Rather, expression of GTM can be increased in other types of tumors, including malignant or non-malignant tumors, including gastric, bladder, colorectal, pancreatic, ovarian, skin (e.g., melanomas), liver, esophageal, endometrial and brain cancers, among others. It should be understood, however that the term GTM does not include the prior art markers, such as CA19-9, CA72-4, pepsinogen and CEA, or any other markers that have been previously identified as being indicative of gastric tumors. Some GTM are secreted or escape from tumors at sufficient levels to be diagnostic of gastric cancer with a high degree of reliability, and in other cases, measurement of two or more GTM can provide reliable diagnosis of gastric cancer.

[0008] Proteins that are secreted by or cleaved from the cell, either alone or in combination with each other, have utility as serum or body fluid markers for the diagnosis of gastric cancer or as markers for monitoring the progression of established disease. Detection of protein markers can be carried out using methods known in the art, and include the use of monoclonal antibodies, polyclonal antisera and the like.

[0009] Described herein is a method for detecting gastric cancer, comprising:

(i) providing a biological sample; and
(ii) detecting the levels of human zymogen granule protein 16 ("ZG16") in said sample.

The present invention provides a method for detecting gastric cancer, comprising: (i) providing a blood, plasma or serum sample; (ii) detecting the protein levels of human zymogen granule protein 16 ("ZG16") in said sample; and (iii) comparing the amount of ZG16 in said sample with a value obtained from one or more control samples not having gastric cancer,

wherein the overexpression of ZG16 in the biological sample is indicative of gastric cancer. The present invention further provides use of an antibody specific for human zymogen granule protein 16 ("ZG16") for detecting gastric cancer in a blood, plasma or serum sample.

[0010] In one aspect, and over expression of ZG16 in a patient is indicative of the patient having gastric cancer.

[0011] A further GTM family member may be selected from the group consisting of mucin 5AC ("MUC5AC"), or mucin 17 ("MUC17"). The method may involve the detection of ZG16 and MUC5AC, ZG16 and MUC17, or ZG16 and MUC5AC and MUC17.

[0012] The further GTM family member may also comprise one or more further GTM family member, for example anyone of MUC5AC, MUC17, ZG16, carboxypeptidase N, polypeptide 2, 83 kDa chain (CPN2), matrix metalloproteinase 12 (MMP12), inhibin ("INHBA"), insulin-like growth factor 7 ("IGFBP7"), gamma-glutamyl hydrolase ("GGH"), leucine proline enriched proteoglycan ("LEPRE1"), cystatin S ("CST4"), secreted frizzled-related protein 4 ("SFRP4"), asporin ("ASPN"), cell growth regulator with EF hand domain 1 ("CGREF1"), kallikrein 10 (KLK10), tissue inhibitor of metalloproteinase 1 ("TIMP1"), secreted acidic cysteine-rich protein ("SPARC"), transforming growth factor, 13-induced ("TGFBI"), EGF-containing fibulin-like extracellular matrix protein 2 ("EFEMP2"), lumican ("LUM"), stannin ("SNN"), secreted phosphoprotein 1 ("SPP1"), chondroitin sulfate proteoglycan 2 ("CSPG2"), N-acylsphingosine amidohydrolase ("ASAH1"), serine protease 11 ("PRSS11"), secreted frizzled-related protein 2 ("SFRP2"), phospholipase A2, group XIIB ("PLA2G12B"), spondin 2, extracellular matrix protein ("SPON2"), olfactomedin 1 ("OLFM1"), thrombospondin repeat containing 1 ("TSRC1"), thrombospondin 2 ("THBS2"), adlican, cystatin SA ("CST2"), cystatin SN ("CST1"), lysyl oxidase-like enzyme 2 ("LOXL2"), thyroglobulin ("TG"), transforming growth factor beta1 ("TGFB1"), serine or cysteine proteinase inhibitor Clade H, member 1 ("SERPINH1"), serine or cysteine proteinase inhibitor Clade B, member 5 ("SERPINB5"), matrix metalloproteinase 2 ("MMP2"), proprotein convertase subtilisin/kexin type 5 ("PCSK5"), hyaluronan glycoprotein link protein 4 ("HAPLN4"), CA19-9, CA72-4, pepsinogen, CEA, MUC5AC and MUC17.

[0013] One example of a combination of GTM markers that may be used according to the present invention is MUC5AC, MUC17, ZG16, cystatin SN, serpinH1 and serpinB5

[0014] Any suitable method for detecting the level of the GTM is described herein, and may include detecting the levels of a GTM mRNA, GTM cDNA, using an oligonucleotide complementary to at least a portion of said GTM cDNA., using qRT-PCR method using a forward primer and a reverse primer, detecting the levels of a GTM protein, detecting the levels of a GTM peptide, for example using an antibody directed against said GTM. Any suitable antibody can be used, and may be a monoclonal antibody or a polyclonal antiserum. The method may be carried out using a sandwich-type immunoassay method, or using an antibody chip.

[0015] Also described herein is a device for detecting a GTM, comprising: a substrate having a GTM capture reagent thereon; and a detector associated with said substrate, said detector capable of detecting a GTM associated with said capture reagent.

[0016] The GTM capture reagent may be an oligonucleotide or an antibody specific for either a GTM oligonucleotide, a GTM protein or a GTM peptide.

[0017] Additionally described herein is a kit for detecting cancer, comprising:

a substrate having a GTM capture reagent thereon;
a means for visualizing a complex of said GTM capture agent and a GTM; reagents; and
instructions for use, wherein said GTM comprises human zymogen granule protein 16 ("ZG16").

[0018] The GTM capture reagent is a GTM-specific oligonucleotide or a GTM-specific antibody selective for a GTM oligonucleotide, a GTM protein or a GTM peptide.

[0019] Further described herein is a method for detecting gastric cancer, comprising the steps of:

providing a test sample from a patient at risk of having gastric cancer; measuring the presence of a GTM protein in said test sample; and
comparing the amount of GTM present in said test sample with a value obtained from a control sample from a subject not having gastric cancer, wherein said GTM comprises human zymogen granule protein 16 ("ZG16").

[0020] Additionally described herein is a method for screening for gastric cancer, comprising the steps of: providing a test sample from a test subject;
measuring the presence of a GTM in said test sample; and
comparing the amount of GTM present in said test sample with a value obtained from a control sample from a subject not having gastric cancer, wherein said GTM comprises human zymogen granule protein 16 ("ZG16").

[0021] The GTM may be a GTM protein or peptide, or an oligonucleotide specific for a GTM. The oligonucleotide may be DNA or RNA.

[0022] According the method, the step of measuring may use an ELISA assay.

[0023] The test sample may be obtained from plasma, tissue, urine, gastric fluid, serum and stool.

BRIEF DESCRIPTION OF THE FIGURES

[0024] This invention is described with reference to specific embodiments thereof and with reference to the figures, in which:

Figure 1 depicts a table of microarray analysis showing genes with high relative expression in tumor tissue. Signal intensity for each gene in both tumor tissue and non-malignant tissue was ranked. The table shows GTMs with a higher ranking than the existing gastric cancer marker CEA (encoded by the gene CEACAM5).
Figure 2 depicts a table showing the characteristics of serum samples used in antibody array analysis.
Figure 3 depicts histograms showing the distribution of tumor and nonmalignant samples according to their level of expression of (a) ZG16 and (b) MUC17. The level of expression of the two genes was obtained using RT-qPCR.
Figure 4 depicts boxplots showing the detection of (a) MUC17 and (b) ZG16 in the serum of gastric cancer patients and controls using antibody arrays and RCA detection.

DETAILED DESCRIPTION

Definitions

[0025] Before describing embodiments of the invention in detail, it will be useful to provide some definitions of terms as used herein.

[0026] The term "GTM" or "gastric tumor marker" or "GTM family member" means a gene, gene fragment, RNA, RNA fragment, protein or protein fragment related or other identifying molecule associated with gastric cancer. The GTMs disclosed herein do not include molecules that are known in the prior art to be associated with gastric cancer, e.g. CA19-9, CA72-4., pepsinogen and CEA. However, the markers disclosed herein can be used in novel and inventive combinations with previously disclosed GTMs.

[0027] The term "marker" refers to a molecule that is associated quantitatively or qualitatively with the presence of a biological phenomenon. Examples of "markers" include a polynucleotide, such as a gene or gene fragment, RNA or RNA fragment; or a gene product, including a polypeptide such as a peptide, oligopeptide, protein, or protein fragment; or any related metabolites, by products, or any other identifying molecules, such as antibodies or antibody fragments, whether related directly or indirectly to a mechanism underlying the phenomenon. The markers disclosed herein include the nucleotide sequences (e.g., GenBank sequences) as disclosed herein, in particular, the full-length sequences, any coding sequences, any fragments, or any complements thereof, and any measurable marker thereof as defined above.

[0028] As used herein "antibodies" and like terms refer to immunoglobulin molecules and immunologically active portions of immunoglobulin (Ig) molecules, i.e., molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen. These include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, Fc, Fab, Fab', and Fab_2 fragments, and a Fab expression library. Antibody molecules relate to any of the classes IgG, IgM, IgA, IgE, and IgD, which differ from one another by the nature of heavy chain present in the molecule. These include subclasses as well, such as IgGI, IgG2, and others. The light chain may be a kappa chain or a lambda chain. Reference herein to antibodies includes a reference to all classes, subclasses, and types. Also included are chimeric antibodies, for example, monoclonal antibodies or fragments thereof that are specific to more than one source, e.g., a mouse or human sequence. Further included are camelid antibodies, shark antibodies or nanobodies.

[0029] The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by abnormal or unregulated cell growth. Cancer and cancer pathology can be associated, for example, with metastasis, interference with the normal functioning of neighbouring cells, release of cytokines or other secretory products at abnormal levels, suppression or aggravation of inflammatory or immunological response, neoplasia, pre-malignancy, malignancy, invasion of surrounding or distant tissues or organs, such as lymph nodes, etc. Specifically included are melanomas.

[0030] The term "tumour" refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues.

[0031] The term "gastric cancer" refers to a tumor originating in the stomach. These tumors are able to metastasize to any organ.

[0032] The terms "differentially expressed," "differential expression," and like phrases, refer to a gene marker whose expression is activated to a higher or lower level in a subject (e.g., test sample) having a condition, specifically cancer, such as melanoma, relative to its expression in a control subject (e.g., reference sample). The terms also include markers whose expression is activated to a higher or lower level at different stages of the same condition; in diseases with a good or poor prognosis; or in cells with higher or lower levels of proliferation. A differentially expressed marker may be

either activated or inhibited at the polynucleotide level or polypeptide level, or may be subject to alternative splicing to result in a different polypeptide product. Such differences may be evidenced by a change in mRNA levels, surface expression, secretion or other partitioning of a polypeptide, for example.

[0033] Differential expression may include a comparison of expression between two or more markers (e.g., genes or their gene products); or a comparison of the ratios of the expression between two or more markers (e.g., genes or their gene products); or a comparison of two differently processed products (e.g., transcripts or polypeptides) of the same marker, which differ between normal subjects and diseased subjects; or between various stages of the same disease; or between diseases having a good or poor prognosis; or between cells with higher and lower levels of proliferation; or between normal tissue and diseased tissue, specifically cancer, or melanoma. Differential expression includes both quantitative, as well as qualitative, differences in the temporal or cellular expression pattern in a gene or its expression products among, for example, normal and diseased cells, or among cells which have undergone different disease events or disease stages, or cells with different levels of proliferation.

[0034] The term "expression" includes production of polynucleotides and polypeptides, in particular, the production of RNA (e.g., mRNA) from a gene or portion of a gene, and includes the production of a polypeptide encoded by an RNA or gene or portion of a gene, and the appearance of a detectable material associated with expression. For example, the formation of a complex, for example, from a polypeptide-polypeptide interaction, polypeptide-nucleotide interaction, or the like, is included within the scope of the term "expression". Another example is the binding of a binding ligand, such as a hybridization probe or antibody, to a gene or other polynucleotide or oligonucleotide, a polypeptide or a protein fragment, and the visualization of the binding ligand. Thus, the intensity of a spot on a microarray, on a hybridization blot such as a Northern blot, or on an immunoblot such as a Western blot, or on a bead array, or by PCR analysis, is included within the term "expression" of the underlying biological molecule.

[0035] The terms "expression threshold," and "defined expression threshold" are used interchangeably and refer to the level of a marker in question outside which the polynucleotide or polypeptide serves as a predictive marker for patient survival. The threshold will be dependent on the predictive model established are derived experimentally from clinical studies such as those described in the Examples below. Depending on the prediction model used, the expression threshold may be set to achieve maximum sensitivity, or for maximum specificity, or for minimum error (maximum classification rate). For example a higher threshold may be set to achieve minimum errors, but this may result in a lower sensitivity. Therefore, for any given predictive model, clinical studies will be used to set an expression threshold that generally achieves the highest sensitivity while having a minimal error rate. The determination of the expression threshold for any situation is well within the knowledge of those skilled in the art.

[0036] The term "sensitivity" means the proportion of individuals with the disease who test (by the model) positive. Thus, increased sensitivity means fewer false negative test results.

[0037] The term "specificity" means the proportion of individuals without the disease who test (by the model) negative. Thus, increased specificity means fewer false positive test results.

[0038] The term "microarray" refers to an ordered or unordered arrangement of capture agents, preferably polynucleotides (e.g., probes) or polypeptides on .a substrate. See, e.g., Microarray Analysis, M. Schena, John Wiley & Sons, 2002; Microarray Biochip Technology, M. Schena, ed., Eaton Publishing, 2000; Guide to Analysis of DNA Microarray Data, S. Knudsen, John Wiley & Sons, 2004; and Protein Microarray Technology, D. Kambhampati, ed., John Wiley & Sons, 2004.

[0039] The term "oligonucleotide" refers to a polynucleotide, typically a probe or primer, including, without limitation, single-stranded deoxyribonucleotides, single- or double-stranded ribonucleotides, RNA: DNA hybrids, and double-stranded DNAs. Oligonucleotides, such as single-stranded DNA probe oligonucleotides, are often synthesized by chemical methods, for example using automated oligonucleotide synthesizers that are commercially available, or by a variety of other methods, including *in vitro* expression systems, recombinant techniques, and expression in cells and organisms.

[0040] The term "overexpression" or "overexpressed" refers to an expression level of a gene or marker in a patient that is above that seen in normal tissue. Expression may be considered to be overexpressed if it is 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, or greater then 2 times the expression in normal tissue.

[0041] The term "polynucleotide," when used in the singular or plural, generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. This includes, without limitation, single- and double-stranded DNA, DNA including single- and double- stranded regions, single-and double-stranded RNA, and RNA including single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or include single- and double-stranded regions. Also included are triple-stranded regions comprising RNA or DNA or both RNA and DNA. Specifically included are mRNAs, cDNAs, and genomic DNAs, and any fragments thereof. The term includes DNAs and RNAs that contain one or more modified bases, such as tritiated bases, or unusual bases, such as inosine. The polynucleotides of the invention can encompass coding or non-coding sequences, or sense or antisense sequences. It will be understood that each reference to a "polynucleotide" or like term, herein, will include the full-length sequences as well as any fragments, derivatives, or variants thereof.

[0042] "Polypeptide," as used herein, refers to an oligopeptide, peptide, or protein sequence, or fragment thereof, and

to naturally occurring, recombinant, synthetic, or semi-synthetic molecules. Where "polypeptide" is recited herein to refer to an amino acid sequence of a naturally occurring protein molecule, "polypeptide" and like terms, are not meant to limit the amino acid sequence to the complete, native amino acid sequence for the full-length molecule. It will be understood that each reference to a "polypeptide" or like term, herein, will include the full-length sequence, as well as any fragments, derivatives, or variants thereof.

[0043] The term "qPCR" or "QPCR" refers to quantative polymerase chain reaction as described, for example, in PCR Technique: Quantitative PCR, J.W. Larrick, ed., Eaton Publishing, 1997, and A-Z of Quantitative PCR, S. Bustin, ed., IUL Press, 2004.

[0044] The term RCA is an abbreviation for rolling circle amplification. RCA is a technique which involves the repeated copying of a circular template to amplify a signal in a linear manner.

[0045] "Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization generally depends on the ability of denatured DNA to reanneal when complementary strands are present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridisable sequence, the higher the relative temperature which can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. Additional details and explanation of stringency of hybridization reactions, are found e.g., in Ausubel et al., Current Protocols in Molecular Biology, Wiley Interscience Publishers, (1995).

[0046] "Stringent conditions" or "high stringency conditions", as defined herein, typically: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ a denaturing agent during hybridization, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Fico11/0.1% polyvinylpyrrolidone/50 mM sodium phosphate, buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5X SSC (0.75 M NaC1, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5X, Denhardt's solution, sonicated salmon sperm DNA (50 ug/m1), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2X SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash comprising 0.1X SSC containing EDTA at 55°C.

[0047] "Moderately stringent conditions" may be identified as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, New York: Cold Spring Harbor Press, 1989, and include the use of washing solution and hybridization conditions (e. g., temperature, ionic strength, and % SDS) less stringent that those described above. An example of moderately stringent conditions is overnight incubation at 37°C in a solution comprising: 20% formamide, 5X SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5X Denhardt's solution, 10% dextran sulfate, and 20 mg/ml denatured sheared salmon sperm DNA, followed by washing the filters in 1X SSC at about 37-50°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

[0048] The term "MUC5AC" means mucin 5AC (Seq ID Nos 1 and 4), and includes the marker MUC5AC, including a polynucleotide, such as a gene or gene fragment, RNA or RNA fragment; or a gene product, including a polypeptide such as a peptide, oligopeptide, protein, or protein fragment; or any related metabolites, by products, or any other identifying molecules, such as antibodies or antibody fragments

[0049] The term "MUC17" means human mucin 17, cell surface associated (Seq ID Nos 2 and 5), and includes the marker MUC17, including a polynucleotide, such as a gene or gene fragment, RNA or RNA fragment; or a gene product, including a polypeptide such as a peptide, oligopeptide, protein, or protein fragment; or any related metabolites, by products, or any other identifying molecules, such as antibodies or antibody fragments,.

[0050] The term "ZG16" means human zymogen granule protein 16 (Seq ID Nos 3 and 6), and includes the marker ZG16, including a polynucleotide, such as a gene or gene fragment, RNA or RNA fragment; or a gene product, including a polypeptide such as a peptide, oligopeptide, protein, or protein fragment; or any related metabolites, by products, or any other identifying molecules, such as antibodies or antibody fragments.

[0051] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, and biochemistry, which are within the skill of the art. Such techniques are explained fully in the literature, such as, Molecular Cloning: A Laboratory Manual, 2nd edition, Sambrook et al., 1989; Oligonucleotide Synthesis, MJ Gait, ed., 1984; Animal Cell Culture, R.I. Freshney, ed., 1987; Methods in Enzymology, Academic Press, Inc.; Handbook of Experimental Immunology, 4th edition, D .M. Weir & CC. Blackwell, eds., Blackwell Science Inc., 1987; Gene Transfer Vectors for Mammalian Cells, J.M. Miller & M.P. Calos, eds., 1987; Current Protocols in Molecular Biology, F.M. Ausubel et al., eds., 1987; and PCR: The Polymerase Chain Reaction, Mullis et al., eds., 1994.

[0052] It is to be understood that the above terms may refer to protein, DNA sequence and/or RNA sequence. It is also to be understood that the above terms also refer to non-human proteins, DNA and/or RNA having homologous

sequences as depicted herein.

DESCRIPTION OF EMBODIMENTS OF THE INVENTION

[0053] Typically, tumor markers are differentially expressed between tumor tissue and corresponding non-malignant tissue. This provides a means to distinguish between patients with and without cancer. However, it is probable that the anatomical structure and physiological characteristics of tumor tissues will lead to differences in the accumulation of markers in serum and other biological fluids even when those markers aren't over-expressed in tumor tissue. In particular, the abnormal polarity of tumor cells, the leaky vasculature and the high interstitial pressure of tumor tissue would be predicted to favour the efflux of specific markers out of tumor tissue compared to non-malignant tissue. Consequently, it is hypothesized that secreted proteins that are expressed at very high levels in gastric tumour tissue, but not necessarily over-expressed compared to non-malignant gastric tissue, would constitute useful gastric cancer markers.

[0054] Using a combination of microarray analysis and quantitative polymerase chain reaction (qPCR), novel markers for the detection of gastric cancer have been identified. This novel gastric tumor marker (GTM), provide further tools in the early detection of gastric cancer. Specifically, the invention comprises the novel GTMs: MUC5AC (Seq ID Nos 1 and 4), MUC17 (Seq ID Nos 2 and 5), and ZG16 (Seq ID Nos 3 and 6).

[0055] The novel GTMs can be used in isolation, or alternatively they can be combined together as signature (comprising two or more GTMs). A signature described herein includes at least one of MUC5AC, MUC 17, and ZG16, and at least one further GTM, which can either be a GTM according to the present invention, or any other GTM, including known GTMs.

[0056] Known GTMs suitable for use in combination with the presently disclosed GTMs include carboxypeptidase N, polypeptide 2, 83 kDa chain (CPN2), matrix metalloproteinase 12 (MMP12), inhibin ("INHBA"), insulin-like growth factor 7 ("IGFBP7"), gamma-glutamyl hydrolase ("GGH"), leucine proline-enriched proteoglycan ("LEPREI"), cystatin S ("CST4"), secreted frizzled-related protein 4 ("SFRP4"), asporin ("ASPN"), cell growth regulator with EF hand domain 1 ("CGREF1"), kallikrein 10 (KLK10), tissue inhibitor of metalloproteinase 1 ("TIMP1"), secreted acidic cysteine-rich protein ("SPARC"), transforming growth factor, 13-induced ("TGFBI"), EGF-containing fibulin-like extracellular matrix protein 2 ("EFEMP2"), lumican ("LUM"), stannin ("SNN"), secreted phosphoprotein 1 ("SPP1"), chondroitin sulfate proteoglycan 2 ("CSPG2"), N-acylsphingosine amidohydrolase ("ASAH1"), serine protease 11 ("PRSS11"), secreted frizzled-related protein 2 ("SFRP2"), phospholipase A2, group XIIB ("PLA2G12B"), spondin 2, extracellular matrix protein ("SPON2"), olfactomedin 1 ("OLFM1"), thrombospondin repeat containing 1 ("TSRC1"), thrombospondin 2 ("THBS2"), adlican, cystatin SA ("CST2"), cystatin SN ("CSTI"), lysyl oxidase-like enzyme 2 ("LOXL2"), thyroglobulin ("TG"), transforming growth factor beta I ("TGFB1"), serine or cysteine proteinase inhibitor Clade H, member 1 ("SERPINH1 "), serine or cysteine proteinase inhibitor Clade B, member 5 ("SERPINB5"), matrix metalloproteinase 2 ("MMP2"), proprotein convertase subtilisin/kexin type 5 ("PCSK5"), hyaluronan glycoprotein link protein 4 ("HAPLN4"), CA19-9, CA72-4, pepsinogen and CEA, or any other markers that have been previously identified as being indicative of gastric tumors.

[0057] By the term "reliability" we include the low incidence of false positives and/or false negatives. Thus, with higher reliability of a marker, fewer false positives and/or false negatives are associated with diagnoses made using that marker. Therefore, in certain embodiments, markers are provided that permit detection of gastric cancer with reliability greater than the reliability of prior art markers of about 50%. In other embodiments, markers are provided that have reliability greater than about 70%; in other embodiments, greater than about 73%, in still other embodiments, greater than about 80%, in yet further embodiments, greater than about 90%, in still others, greater than about 95%, in yet further embodiments greater than about 98%, and in certain embodiments, about 100% reliability.

General Approaches to Cancer Detection

[0058] General methodologies for determining expression levels are outlined below, although it will be appreciated that any method for determining expression levels would be suitable.

Quantitative PCR (qPCR)

[0059] Quantitative PCR (qPCR) can be carried out on tumour samples, on serum and plasma using GTM specific primers and probes. In controlled reactions, the amount of product formed in a PCR reaction (Sambrook, J., E Fritsch, E. and T Maniatis, Molecular Cloning: A Laboratory Manual 3rd. Cold Spring Harbor Laboratory Press: Cold Spring Harbor (2001)) correlates with the amount of starting template. Quantification of the PCR product can be carried out by stopping the PCR reaction when it is in log phase, before reagents become limiting. The PCR products are then electrophoresed in agarose or polyacrylamide gels, stained with ethidium bromide or a comparable DNA stain, and the intensity of staining measured by densitometry. Alternatively, the progression of a PCR reaction can be measured using PCR machines such as the Applied Biosystems' Prism 7000 or the Roche LightCycler which measure product accumulation in real-time. Real-time PCR measures either the fluorescence of DNA intercalating dyes such as Sybr Green into

the synthesized PCR product, or the fluorescence released by a reporter molecule when cleaved from a quencher molecule; the reporter and quencher molecules are incorporated into an oligonucleotide probe which hybridizes to the target DNA molecule following DNA strand extension from the primer oligonucleotides. The oligonucleotide probe is displaced and degraded by the enzymatic action of the Taq polymerase in the next PCR cycle, releasing the reporter from the quencher molecule. In one variation, known as Scorpion®, the probe is covalently linked to the primer.

Reverse Transcription PCR (RT-PCR)

[0060] RT-PCR can be used to compare RNA levels in different sample populations, in normal and tumour tissues, with or without drug treatment, to characterize patterns of expression, to discriminate between closely related RNAs, and to analyze RNA structure.

[0061] For RT-PCR, the first step is the isolation of RNA from a target sample. The starting material is typically total RNA isolated from human tumours or tumour cell lines, and corresponding normal tissues or cell lines, respectively. RNA can be isolated from a variety of samples, such as tumour samples from breast, lung, colon (e.g., large bowel or small bowel), colorectal, gastric, esophageal, anal, rectal, prostate, brain, liver, kidney, pancreas, spleen, thymus, testis, ovary, uterus, bladder etc., tissues, from primary tumours, or tumour cell lines, and from pooled samples from healthy donors. If the source of RNA is a tumour, RNA can be extracted, for example, from frozen or archived paraffin-embedded and fixed (e.g., formalin-fixed) tissue samples.

[0062] The first step in gene expression profiling by RT-PCR is the reverse transcription of the RNA template into cDNA, followed by its exponential amplification in a PCR reaction. The two most commonly used reverse transcriptases are avian myeloblastosis virus reverse transcriptase (AMV-RT) and Moloney murine leukaemia virus reverse transcriptase (MMLV-RT). The reverse transcription step is typically primed using specific primers, random hexamers, or oligo-dT primers, depending on the circumstances and the goal of expression profiling. For example, extracted RNA can be reverse-transcribed using a GeneAmp RNA PCR kit (Perkin Elmer, CA, USA), following the manufacturer's instructions. The derived cDNA can then be used as a template in the subsequent PCR reaction.

[0063] Although the PCR step can use a variety of thermostable DNA-dependent DNA polymerases, it typically employs the Taq DNA polymerase, which has a 5'-3' nuclease activity but lacks a 3'-5' proofreading endonuclease activity. Thus, TaqMan qPCR typically utilizes the 5' nuclease activity of Taq or Tth polymerase to hydrolyze a hybridization probe bound to its target amplicon, but any enzyme with equivalent 5' nuclease activity can be used.

[0064] Two oligonucleotide primers are used to generate an amplicon typical of a PCR reaction. A third oligonucleotide, or probe, is designed to detect nucleotide sequence located between the two PCR primers. The probe is non-extendible by Taq DNA polymerase enzyme, and is labeled with a reporter fluorescent dye and a quencher fluorescent dye. Any laser-induced emission from the reporter dye is quenched by the quenching dye when the two dyes are located close together as they are on the probe. During the amplification reaction, the Taq DNA polymerase enzyme cleaves the probe in a template-dependent manner. The resultant probe fragments disassociate in solution, and signal from the released reporter dye is free from the quenching effect of the second fluorophore. One molecule of reporter dye is liberated for each new molecule synthesized, and detection of the unquenched reporter dye provides the basis for quantitative interpretation of the data.

[0065] TaqMan RT-PCR can be performed using commercially available equipment, such as, for example, ABI PRISM 7700 Sequence Detection System (Perkin-Elmer-Applied Biosystems, Foster City, CA, USA), or Lightcycler (Roche Molecular Biochemicals, Mannheim, Germany). In a preferred embodiment, the 5' nuclease procedure is run on a real-time quantitative PCR device such as the ABI PRISM 7700 Sequence Detection System. The system consists of a thermocycler, laser, charge-coupled device (CCD), camera, and computer. The system amplifies samples in a 96-well format on a thermocycler. During amplification, laser-induced fluorescent signal is collected in real-time through fibre optics cables for all 96 wells, and detected at the CCD. The system includes software for running the instrument and for analyzing the data.

[0066] 5' nuclease assay data are initially expressed as Ct, or the threshold cycle. As discussed above, fluorescence values are recorded during every cycle and represent the amount of product amplified to that point in the amplification reaction. The point when the fluorescent signal is first recorded as statistically significant is the threshold cycle.

Real-time Quantitative PCR (qRT-PCR)

[0067] A more recent variation of the RT-PCR technique is the real time quantitative PCR, which measures PCR product accumulation through a dual-labeled fluorigenic probe (i.e., TaqMan probe). Real time PCR is compatible both with quantitative competitive PCR and with quantitative comparative PCR. The former uses an internal competitor for each target sequence for normalization, while the latter uses a normalization gene contained within the sample, or a housekeeping gene for RT-PCR. Further details are provided, e.g., by Held et al., Genome Research 6: 986-994 (1996).

[0068] Expression levels can be determined using fixed, paraffin-embedded tissues as the RNA source. According to

one aspect of the present invention, PCR primers are designed to flank intron sequences present in the gene to be amplified. In this embodiment, the first step in the primer/probe design is the delineation of intron sequences within the genes. This can be done by publicly available software, such as the DNA BLAT software developed by Kent, W. J., Genome Res. 12 (4): 656-64 (2002), or by the BLAST software including its variations. Subsequent steps follow well established methods of PCR primer and probe design.

[0069] In order to avoid non-specific signals, it is useful to mask repetitive sequences within the introns when designing the primers and probes. This can be easily accomplished by using the Repeat Masker program available on-line through the Baylor College of Medicine, which screens DNA sequences against a library of repetitive elements and returns a query sequence in which the repetitive elements are masked. The masked sequences can then be used to design primer and probe sequences using any commercially or otherwise publicly available primer/probe design packages, such as Primer Express (Applied Biosystems); MGB assay-by-design (Applied Biosystems); Primer3 (Steve Rozen and Helen J. Skaletsky (2000) Primer3 on the VIMNV for general users and for biologist programmers in: Krawetz S, Misener S (eds) Bioinformatics Methods and Protocols: Methods in Molecular Biology. Humana Press, Totowa, NJ, pp 365-386).

[0070] The most important factors considered in PCR primer design include primer length, melting temperature (Tm), and G/C content, specificity, complementary primer sequences, and 3' end sequence. In general, optimal PCR primers are generally 1730 bases in length, and contain about 20-80%, such as, for example, about 50-60% G+C bases. Melting temperatures between 50 and 80°C, e.g., about 50 to 70°C, are typically preferred. For further guidelines for PCR primer and probe design see, e.g., Dieffenbach, C. W. et al., General Concepts for PCR Primer Design in: PCR Primer, A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York, 1995, pp.133-155; Innis and Gelfand, Optimization of PCRs in: PCR Protocols, A Guide to Methods and Applications, CRC Press, London, 1994, pp. 5-11; and Plasterer, T. N. Primerselect: Primer and probe design. Methods Mol. Biol. 70: 520-527 (1997).

Microarray Analysis

[0071] Differential expression can also be identified, or confirmed using the microarray technique. Thus, the expression profile of GTMs can be measured in either fresh or paraffin-embedded tumour tissue, using microarray technology. In this method, polynucleotide sequences of interest (including cDNAs and oligonucleotides) are plated, or arrayed, on a microchip substrate. The arrayed sequences (i.e., capture probes) are then hybridized with specific polynucleotides from cells or tissues of interest (i.e., targets). Just as in the RT-PCR method, the source of RNA typically is total RNA isolated from human tumours or tumour cell lines, and corresponding normal tissues or cell lines. Thus RNA can be isolated from a variety of primary tumours or tumour cell lines. If the source of RNA is a primary tumour, RNA can be extracted, for example, from frozen or archived formalin fixed paraffin-embedded (FFPE) tissue samples and fixed (e.g., formalin-fixed) tissue samples, which are routinely prepared and preserved in everyday clinical practice.

[0072] In a specific embodiment of the microarray technique, PCR amplified inserts of cDNA clones are applied to a substrate. The substrate can include up to 1, 2, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, or 75 nucleotide sequences. In other aspects, the substrate can include at least 10,000 nucleotide sequences. The microarrayed sequences, immobilized on the microchip, are suitable for hybridization under stringent conditions. As other embodiments, the targets for the microarrays can be at least 50, 100, 200, 400, 500, 1000, or 2000 bases in length; or 50-100, 100-200, 100-500, 100-1000, 100-2000, or 500-5000 bases in length. As further embodiments, the capture probes for the microarrays can be at least 10, 15, 20, 25, 50, 75, 80, or 100 bases in length; or 10-15, 10-20, 10-25, 10-50, 10-75, 10-80, or 20-80 bases in length.

[0073] Fluorescently labeled cDNA probes may be generated through incorporation of fluorescent nucleotides by reverse transcription of RNA extracted from tissues of interest. Labeled cDNA probes applied to the chip hybridize with specificity to each spot of DNA on the array. After stringent washing to remove non-specifically bound probes, the chip is scanned by confocal laser microscopy or by another detection method, such as a CCD camera. Quantitation of hybridization of each arrayed element allows for assessment of corresponding mRNA abundance. With dual colour fluorescence, separately labeled cDNA probes generated from two sources of RNA are hybridized pairwise to the array. The relative abundance of the transcripts from the two sources corresponding to each specified gene is thus determined simultaneously.

[0074] The miniaturized scale of the hybridization affords a convenient and rapid evaluation of the expression pattern for large numbers of genes. Such methods have been shown to have the sensitivity required to detect rare transcripts, which are expressed at a few copies per cell, and to reproducibly detect at least approximately two-fold differences in the expression levels (Schena et al., Proc. Natl. Acad. Sci. USA 93 (2): 106-149 (1996)). Microarray analysis can be performed by commercially available equipment, following manufacturer's protocols, such as by using the Affymetrix GenChip technology, Illumina microarray technology or Incyte's microarray technology. The development of microarray methods for large-scale analysis of gene expression makes it possible to search systematically for molecular markers of cancer classification and outcome prediction in a variety of tumour types.

RNA Isolation, Purification, and Amplification

[0075] General methods for mRNA extraction are well known in the art and are disclosed in standard textbooks of molecular biology, including Ausubel et al., Current Protocols of Molecular Biology, John Wiley and Sons (1997). Methods for RNA extraction from paraffin embedded tissues are disclosed, for example, in Rupp and Locker, Lab Invest. 56: A67 (1987), and De Sandres et al., BioTechniques 18: 42044 (1995). In particular, RNA isolation can be performed using purification kit, buffer set, and protease from commercial manufacturers, such as Qiagen, according to the manufacturer's instructions. For example, total RNA from cells in culture can be isolated using Qiagen RNeasy mini-columns. Other commercially available RNA isolation kits include MasterPure Complete DNA and RNA Purification Kit (EPICENTRE (D, Madison, WI), and Paraffin Block RNA Isolation Kit (Ambion, Inc.). Total RNA from tissue samples can be isolated using RNA Stat-60 (Tel-Test). RNA prepared from tumour can be isolated, for example, by cesium chloride density gradient centrifugation.

[0076] The steps of a representative protocol for profiling gene expression using fixed, paraffin-embedded tissues as the RNA source, including mRNA isolation, purification, primer extension and amplification are given in various published journal articles (for example: T. E. Godfrey et al. J. Molec. Diagnostics 2: 84-91 (2000); K. Specht et al., Am. J. Pathol. 158: 419-29 (2001)). Briefly, a representative process starts with cutting about 10 micron thick sections of paraffin-embedded tumour tissue samples. The RNA is then extracted, and protein and DNA are removed. After analysis of the RNA concentration, RNA repair and/or amplification steps may be included, if necessary, and RNA is reverse transcribed using gene specific promoters followed by RT-PCR. Finally, the data are analyzed to identify the best treatment option(s) available to the patient on the basis of the characteristic gene expression pattern identified in the tumour sample examined.

Immunohistochemistry and Proteomics

[0077] Immunohistochemistry methods are also suitable for detecting the expression levels of the proliferation markers described herein. Thus, antibodies or antisera, preferably polyclonal antisera, and most preferably monoclonal antibodies specific for each marker, are used to detect expression. The antibodies can be detected by direct labeling of the antibodies themselves, for example, with radioactive labels, fluorescent labels, hapten labels such as, biotin, or an enzyme such as horseradish peroxidase or alkaline phosphatase. Alternatively, unlabeled primary antibody is used in conjunction with a labeled secondary antibody, comprising antisera, polyclonal antisera or a monoclonal antibody specific for the primary antibody. Immunohistochemistry protocols and kits are well known in the art and are commercially available.

[0078] Proteomics can be used to analyze the polypeptides present in a sample (e.g., tissue, organism, or cell culture) at a certain point of time. In particular, proteomic techniques can be used to assess the global changes of polypeptide expression in a sample (also referred to as expression proteomics). Proteomic analysis typically includes: (1) separation of individual polypeptides in a sample by 2-D gel electrophoresis (2-D PAGE); (2) identification of the individual polypeptides recovered from the gel, e.g., by mass spectrometry or N-terminal sequencing, and (3) analysis of the data using bioinformatics. Proteomics methods are valuable supplements to other methods of gene expression profiling, and can be used, alone or in combination with other methods, to detect the products of the proliferation markers described herein.

Hybridization Methods Using Nucleic Acid Probes Selective for a Marker

[0079] These methods involve binding the nucleic acid probe to a support, and hybridizing under appropriate conditions with RNA or cDNA derived from the test sample (Sambrook, J., E Fritsch, E. and T Maniatis, Molecular Cloning: A Laboratory Manual 3rd. Cold Spring Harbor Laboratory Press: Cold Spring Harbor (2001)). These methods can be applied to GTM derived from a tumour tissue or fluid sample. The RNA or cDNA preparations are typically labeled with a fluorescent or radioactive molecule to enable detection and quantification. In some applications, the hybridizing DNA can be tagged with a branched, fluorescently labeled structure to enhance signal intensity (Nolte, F.S., Branched DNA signal amplification for direct quantitation of nucleic acid sequences in clinical specimens. Adv. Clin. Chem. 33, 201-35 (1998)). Unhybridized label is removed by extensive washing in low salt solutions such as 0.1x SSC, 0.5% SDS before quantifying the amount of hybridization by fluorescence detection or densitometry of gel images. The supports can be solid, such as nylon or nitrocellulose membranes, or consist of microspheres or beads that are hybridized when in liquid suspension. To allow washing and purification, the beads may be magnetic (Haukanes, B-1 and Kvam, C., Application of magnetic beads in bioassays. Bio/Technology 11, 60-63 (1993)) or fluorescently-labeled to enable flow cytometry (see for example: Spiro, A., Lowe, M. and Brown, D., A Bead-Based Method for Multiplexed Identification and Quantitation of DNA Sequences Using Flow Cytometry. Appl. Env. Micro. 66, 4258-4265 (2000)).

[0080] A variation of hybridization technology is the QuantiGene Plexe assay (Genospectra, Fremont) which combines a fluorescent bead support with branched DNA signal amplification. Still another variation on hybridization technology is the Quantikine® mRNA assay (R&D Systems, Minneapolis). Methodology is as described in the manufacturer's instructions. Briefly the assay uses oligonucleotide hybridization probes conjugated to Digoxigenin. Hybridization is

detected using anti-Digoxigenin antibodies coupled to alkaline phosphatase in colorometric assays.

[0081] Additional methods are well known in the art and need not be described further herein.

Enzyme-Linked Immunological Assays (ELISA)

[0082] Briefly, in sandwich ELISA assays, a polyclonal or monoclonal antibody against the GTM is bound to a solid support (Crowther, J.R. The ELISA guidebook. Humana Press: New Jersey (2000); Harlow, E. and Lane, D., Using antibodies: a laboratory manual. Cold Spring Harbor Laboratory Press: Cold Spring Harbor (1999)) or suspension beads. Other methods are known in the art and need not be described herein further. Monoclonal antibodies can be hybridoma-derived or selected from phage antibody libraries (Hust M. and Dubel S., Phage display vectors for the in vitro generation of human antibody fragments. Methods Mol Biol. 295:71-96 (2005)). Nonspecific binding sites are blocked with non-target protein preparations and detergents. The capture antibody is then incubated with a preparation of sample or tissue from the patient containing the GTM antigen. The mixture is washed before the antibody/antigen complex is incubated with a second antibody that detects the target GTM. The second antibody is typically conjugated to a fluorescent molecule or other reporter molecule that can either be detected in an enzymatic reaction or with a third antibody conjugated to a reporter (Crowther, Id.). Alternatively, in direct ELISAs, the preparation containing the GTM can be bound to the support or bead and the target antigen detected directly with an antibody-reporter conjugate (Crowther, Id.).

[0083] Methods for producing monoclonal antibodies and polyclonal antisera are well known in the art and need not be described herein further.

Immunodetection

[0084] The methods can also be used for immunodetection of marker family members in sera or plasma from gastric cancer patients taken before and after surgery to remove the tumour, immunodetection of marker family members in patients with other cancers, including but not limited to, colorectal, pancreatic, ovarian, melanoma, liver, oesophageal, stomach, endometrial, and brain and immunodetection of marker family members in urine and stool from gastric cancer patients.

[0085] GTMs can also be detected in tissues or samples using other standard immunodetection techniques such as immunoblotting or immunoprecipitation (Harlow, E. and Lane, D., Using antibodies: a laboratory manual. Cold Spring Harbor Laboratory Press: Cold Spring Harbor (1999)). In immunoblotting, protein preparations from tissue or fluid containing the GTM are electrophoresed through polyacrylamide gels under denaturing or non-denaturing conditions. The proteins are then transferred to a membrane support such as nylon. The GTM is then reacted directly or indirectly with monoclonal or polyclonal antibodies as described for immunohistochemistry. Alternatively, in some preparations, the proteins can be spotted directly onto membranes without prior electrophoretic separation. Signal can be quantified by densitometry.

[0086] In immunoprecipitation, a soluble preparation containing the GTM is incubated with a monoclonal or polyclonal antibody against the GTM. The reaction is then incubated with inert beads made of agarose or polyacrylamide with covalently attached protein A or protein G. The protein A or G beads specifically interact with the antibodies forming an immobilized complex of antibody-GTM-antigen bound to the bead. Following washing the bound GTM can be detected and quantified by immunoblotting or ELISA.

Threshold Determination

[0087] For tests using GTM, thresholds will be derived that will enable a sample to be called either positive or negative for gastric cancer. These thresholds will be determined by the analysis of cohorts of patients who are being investigated for the presence of gastric cancer. Thresholds may vary for different test applications; for example, thresholds for use of the test in population screening will be determined using cohorts of patients who are largely free of urological symptoms, and these thresholds may be different to those used in tests for patients who are under surveillance for gastric cancer recurrence. A threshold could be selected to provide a practical level of test specificity in the required clinical setting; that is, a specificity that allows reasonable sensitivity without excessive numbers of patients receiving false positive results. This specificity may be within the range of 80-90%. An alternative method to obtain a test threshold is to plot sensitivity against specificity for different test thresholds (ROC curves) then select the point of inflexion of the curve.

[0088] As an alternative to single thresholds, the test may use test intervals which provide different degrees of likelihood of presence of disease and which have different clinical consequences associated with them. For example, a test may have three intervals; one associated with a high (e.g. 90%) risk of the presence of gastric cancer, a second associated with a low risk of gastric cancer and a third regarded as being suspicious of disease. The "suspicious" interval could be associated with a recommendation for a repeat test in a defined period of time.

Antibodies to Gastric Cancer Markers

[0089] Also described herein is manufacture of antibodies against GTMs. Using methods described herein, novel GTMs can be identified using microarray and/or qRT-PCR methods. Once a putative marker is identified, it can be produced in sufficient amount to be suitable for eliciting an immunological response. In some cases, a full-length GTM can be used, and in others, a peptide fragment of a GTM may be sufficient as an immunogen. The immunogen can be injected into a suitable host (e.g., mouse, rabbit, etc) and if desired, an adjuvant, such as Freund's complete adjuvant or Freund's incomplete adjuvant can be injected to increase the immune response. It can be appreciated that making antibodies is routine in the immunological arts and need not be described herein- further. As a result, one can produce antibodies, including monoclonal or phage-display antibodies, against GTMs identified using methods described herein.

[0090] In yet further embodiments, antibodies can be made against the protein or the protein core of the tumour markers identified herein or against an oligonucleotide sequence unique to a GTM. Although certain proteins can be glycosylated, variations in the pattern of glycosylation can, in certain circumstances, lead to mis-detection of forms of GTMs that lack usual glycosylation patterns. Thus, GTM immunogens can include deglycosylated GTM or deglycosylated GTM fragments. Deglycosylation can be accomplished using one or more glycosidases known in the art. Alternatively, GTM cDNA can be expressed in glycosylation-deficient cell lines, such as prokaryotic cell lines, including E. coli and the like.

[0091] Vectors can be made having GTM -encoding oligonucleotides therein. Many such vectors can be based on standard vectors known in the art. Vectors can be used to transfect a variety of cell lines to produce GTM -producing cell lines, which can be used to produce desired quantities of GTM for development of specific antibodies or other reagents for detection of GTMs or for standardizing developed assays for GTMs.

Kits

[0092] Based on the discoveries described herein, several types of test kits can be envisioned and produced. First, kits can be made that have a detection device pre-loaded with a detection molecule (or "capture reagent"). In embodiments for detection of GTM mRNA, such devices can comprise a substrate (e.g., glass, silicon, quartz, metal, etc) on which oligonucleotides as capture reagents that hybridize with the mRNA to be detected is bound. In some embodiments, direct detection of mRNA can be accomplished by hybridizing mRNA (labeled with cy3, cy5, radiolabel or other label) to the oligonucleotides on the substrate. In other embodiments, detection of mRNA can be accomplished by first making complementary DNA (cDNA) to the desired mRNA. Then, labeled cDNA can be hybridized to the oligonucleotides on the substrate and detected.

[0093] Antibodies can also be used in kits as capture reagents. In some embodiments, a substrate (e.g., a multiwell plate) can have a specific GTM capture reagent attached thereto. In some embodiments, a kit can have a blocking reagent included. Blocking reagents can be used to reduce non-specific binding. For example, non-specific oligonucleotide binding can be reduced using excess DNA from any convenient source that does not contain GTM oligonucleotides, such as salmon sperm DNA. Non-specific antibody binding can be reduced using an excess of a blocking protein such as serum albumin. It can be appreciated that numerous methods for detecting oligonucleotides and proteins are known in the art, and any strategy that can specifically detect GTM associated molecules can be used.

[0094] Antibodies can also be used when bound to a solid support, for example using an antibody chip, which would allow for the detection of multiple markers with a single chip.

[0095] In addition to a substrate, a test kit can comprise capture reagents (such as probes), washing solutions (e.g., SSC, other salts, buffers, detergents and the like), as well as detection moieties (e.g., cy3, cy5, radiolabels, and the like). Kits can also include instructions for use and a package.

[0096] Cancer markers can be detected in a sample using any suitable technique, and can include, but are not limited to, oligonucleotide probes, qPCR or antibodies raised against cancer markers.

[0097] It will be appreciated that the sample to be tested is not restricted to a sample of the tissue suspected of being a tumour. The marker may be secreted into the serum or other body fluid. Therefore, a sample can include any bodily sample, and includes biopsies, blood, serum, peritoneal washes, cerebrospinal fluid, urine and stool samples.

[0098] It will also be appreciate that the present disclosure is not restricted to the detection of cancer in humans, but is suitable for the detection of cancer in any animal, including, but not limited to dogs, cats, horses, cattle, sheep, deer, pigs and any other animal known to get cancer.

Tests for Gastric Cancer Markers in Body Fluids

[0099] In several embodiments, assays for GTM can be desirably carried out on samples obtained from blood, plasma, serum, peritoneal fluid obtained for example using peritoneal washes, or other body fluids, such as urine, lymph, cerebrospinal fluid, gastric fluid or stool samples.

[0100] In general, methods for assaying for oligonucleotides, proteins and peptides in these fluids are known in the art. Detection of oligonucleotides can be carried out using hybridization methods such as Northern blots, Southern blots or microarray methods, or qPCR. Methods for detecting proteins include such as enzyme linked immunosorbent assays (ELISA), protein chips having antibodies, suspension beads radioimmunoassay (RIA), Western blotting and lectin binding. However, for purposes of illustration, fluid levels of a GTM can be quantified using a sandwich-type enzyme-linked immunosorbent assay (ELISA). For plasma assays, a 5 uL aliquot of a properly diluted sample or serially diluted standard GTM and 75 uL of peroxidaseconjugated anti-human GTM antibody are added to wells of a microtiter plate. After a 30 minute incubation period at 30°C, the wells are washed with 0.05% Tween 20 in phosphate-buffered saline {PBS) to remove unbound antibody. Bound complexes of GTM and anti-GTM antibody are then incubated with o-phenylendiamine containing H_2O_2 for 15 minutes at 30°C. The reaction is stopped by adding 1 M H_2SO_4, and the absorbance at 492 nm is measured with a microtiter plate reader.

[0101] It can be appreciated that anti-GTM antibodies can be monoclonal antibodies or polyclonal antisera. It can also be appreciated that any other body fluid can be suitably studied.

[0102] It is not necessary for a marker to be secreted, in a physiological sense, to be useful. Rather, any mechanism by which a marker protein or gene enters the serum can be effective in producing a detectable, quantifiable level of the marker. Thus, normal secretion of soluble proteins from cells, sloughing of membrane proteins from plasma membranes, secretion of alternatively spliced forms of mRNA or proteins expressed therefrom, cell death (either apoptotic) can produce sufficient levels of the marker to be useful.

[0103] There is increasing support for the use of serum markers as tools to diagnose and/or evaluate efficacy of therapy for a variety of cancer types.

Yoshikawa et al., (Cancer Letters, 151: 81-86 (2000) describes tissue inhibitor of matrix metalloproteinase-1 in plasma of patients with gastric cancer.

Rudland et al., (Cancer Research 62: 3417-3427 (2002) describes osteopontin as a metastasis associated protein in human breast cancer.

Buckhaults et al., (Cancer Research 61:6996-7001 (2002) describes certain secreted and cell surface genes expressed in colorectal tumors.

Kim et al., (JAMA 287(13):1671-1679 (2002) describes osteopontin as a potential diagnostic biomarker for ovarian cancer.

Hotte et al., (AJ. American Cancer Society 95(3):507-512 (2002) describes plasma osteopontin as a protein detectable in human body fluids and is associated with certain malignancies.

Martin et al., (Prostate Cancer Prostatic Dis. March 9, 2004 (PMID: 15007379) (Abstract) described use of human kallikrein 2, prostate-specific antigen (PSA) and free PSA as markers for detection of prostate cancer.

Hall et al (Laryngoscope 113(1):77-81 (2003) (PMID: 12679418) (Abstract) described predictive value of serum thyroglobulin in thyroid cancer.

Mazzaferri et al., (J. Clin. Endocrinol. Metab. 88(4):1433-1441 (2003) (Abstract) describes thyroglobulin as a potential monitoring method for patients with thyroid carcinoma.

Whitley et al, (Dim Lab. Med. 24(1):29-47 (2004) (Abstract) describes thyroglobulin as a serum marker for thyroid carcinoma.

Kuo et al (Clin. Chim. Acta. 294(1-2):157-168 (2000) (Abstract) describes serum matrix metalloproteinase-2 and -9 in HCF- and HBV-infected patients.

Koopman et al., (Cancer Epidemiol. Biomarkers Prey 13(3):487-491 (2004) (Abstract) describes osteopontin as a biomarker for pancreatic adenocarcinoma.

Pellegrini et al., (Cancer Immunol. Immunother. 49(7):388-394 (2000) (Abstract) describes measurement of soluble carcinoembryonic antigen and TIMP 1 as markers for pre-invasive colorectal cancer.

Melle et al., (Clin. Chem. 53(4), 629-635 (2007) (Abstract) describes HSP27 as a serum marker for pancreatic adenocarcinoma.

Leman et al., (Urology, 69(4) 714-20 (2007) (Abstract) describes EPCA-2 as a serum marker for prostate cancer.

Tsigkou et al., (I Clin Endocrinol Metab, 92(7) 2526-31 (2007) (Abstract) describes total inhibin as a potential serum marker for ovarian cancer.

Marchi et al., (Cancer 112, 1313-1324 (2008) (Abstract) describes ProApolipoprotein AI as a serum marker of brain metastases in lung cancer patients.

Methods

[0104] The following general methods were used to evaluate the suitability of various approaches to molecular identification of markers associated with gastric tumors.

Tumor Collection

[0105] Gastric tumor samples and non-malignant gastric tissues were collected from surgical specimens resected at Seoul National University Hospital. Diagnosis of gastric cancer was made on the basis of symptoms, physical findings and histological examination of tissues.

RNA Extraction

[0106] In some embodiments, expression of genes associated with gastric tumors was analyzed by determining the levels of RNA in samples taken from tumors. Frozen surgical specimens were embedded in OCT medium. 60 micron sections were sliced from the tissue blocks using a microtome, homogenized in a TriReagent: water (3:1) mix, then chloroform extracted. Total RNA was then purified from the aqueous phase using the RNeasy™ procedure (Qiagen). In total, RNA from 58 gastric tumors and 58 non-malignant ("normal") gastric tissue samples were extracted and used in the microarray analysis described below. RNA was also extracted from 16 cancer cell lines and pooled to serve as a reference RNA.

Microarray Slide Preparation

[0107] Epoxy coated glass slides were obtained from MWG Biotech AG, Ebersberg, Germany) and were printed with -30,000 50mer oligonucleotides using a Gene Machines microarraying robot, according to the manufacturer's protocol.

RNA labeling and Hybridization

[0108] cDNA was transcribed from 10ug total RNA using Superscript II reverse transcriptase (Invitrogen) in reactions containing 5-(3 -aminoallyl)- 2' deoxyuridine - 5'-triphosphate. The reaction was then de-ionized in a Microcon column before being incubated with Cy3 or Cy5 in bicarbonate buffer for 1 hour at room temperature. Unincorporated dyes were removed using a Qiaquick column (Qiagen) and the sample concentrated to 15ul in a SpeedVac. Cy3 and Cy5 labeled cDNAs were then mixed with Ambion ULTRAhyb buffer, denatured at 100°C for 2 minutes and hybridized to the microarray slides in hybridization chambers at 42°C for 16 hours. The slides were then washed and scanned twice in an Axon 4000A scanner at two power settings to yield primary fluorescence data on gene expression.

Normalization Procedure

[0109] To measure the expression of cancer genes in tumors and non-cancerous tissues, median fluorescence intensities detected by Genepix^{Tm} software were corrected by subtraction of the local background fluorescence intensities. Spots with a background corrected intensity of less than zero were excluded. To facilitate normalization, intensity ratios and overall spot intensities were log-transformed. Log-transformed intensity ratios were corrected for dye and spatial bias using local regression implemented in the LOCFIT^{Tm} package. Log-transformed intensity ratios were regressed simultaneously with respect to overall spot intensity and location. The residuals of the local regression provided the corrected log-fold changes. For quality control, ratios of each normalized microarray were plotted with respect to spot intensity and localization. The plots were subsequently visually inspected for possible remaining artifacts. Additionally, an analysis of variance (ANOVA) model was applied for the detection of pin-tip bias. All results and parameters of the normalization were inserted into a Postgres-database for statistical analysis.

Marker Selection

[0110] Microarray gene expression data for each of 29,718 genes was ranked according to the relative intensity of signal for each gene in both tumor and non-malignant tissue. Further analysis was limited to (i) genes encoding secreted proteins (ii) genes with an intensity rank in tumor tissue higher than that observed for the gene (CEACAM5) encoding the existing tumor marker CEA and (iii) genes with no significant expression in blood or vascular tissue, as determined by EST counts in the Unigene database (Wheeler DL et al 2003). Secreted proteins were predicted by identifying transcripts expected to contain an N-terminal signal peptide. Proteins with predicted transmembrane helices that were not in the first 20 N-terminal amino acids [Krogh A. et al 2001] were discarded. Further subcellular localization was predicted using TARGETP [Emanuelsson 0 et al 2000].

[0111] Reference numbers (MWG oligo #) for relevant oligonucleotides, and the NCBI mRNA and protein reference sequences of selected GTMs are shown in Figure 1. Figure 1 also shows the rank intensity of the selected GTMs in both tumor and nonmalignant tissue. Full DNA sequences of the GTM of this invention are shown herein below.

Quantitative Real-Time PCR

[0112] In other embodiments, real-time, or quantitative PCR (qPCR) can be used for absolute or relative quantitation of PCR template copy number. The primer set for MUC17 (Fwd: GAGGTGGTCAGCAGCATTGAC; Rev: CCTGGGAAGAGTGGTTTTTTAGC) was designed using Primer Express V 2.0TM (Applied Biosystems) and amplified product detected using SYBR green labelling. ZG16 was represented by the Assay-on-Demand™ expression assay Hs.00380609_ml (Applied Biosystems). Amplification was carried out on an ABI Prism™ 7000 sequence detection system under standard cycling conditions.

[0113] Assays were performed over two 96 well plates with each RNA sample represented by a single cDNA. Up to 45 RNA samples from both gastric tumours and non-malignant gastric tissue was analysed. Each plate contained a reference cDNA standard curve, over a 625-fold concentration range, in duplicate. Analysis consisted of calculating the ΔCT (target gene CT - mean reference cDNA CT). ΔCT is directly proportional to the negative log2 fold change. Log2 fold changes relative to the median non-malignant log2 fold change were then calculated (log2 fold change -median normal log2 fold change). These fold changes were then clustered into frequency classes and graphed.

Protein expression and antibody generation

[0114] To validate ZG16 at the protein level it was necessary to generate new antibodies against the recombinant protein. The coding region 17-167 of ZG16 was PCR amplified from human cell line cDNA using the forward primer CACCAATGCCATTCAGGCCAGGT and the reverse primer TCAGCATCTGCTGCAGCTA. The PCR product was gel purified and cloned into the "Gateway" entry vector "pENTR/dTOPO" from Invitrogen before being sequence to verify correct insert. Using the "Gateway" system ZG16 was then cloned from pENTR/dTOPO into the Invitrogen expression vector pDEST17 containing an N terminal 6xHIS tag. Expression of ZG16 was carried out in BL21-AI *E.coli* cells (Invitrogen), cells were grown at 37°C on a shaker until they were in mid log phase ($0D_{600}$ = 0.5) whereby they were induced at a final concentration of 0.2% arabinose and grown for a further 3hours at 37°C on a shaker. Cells were harvested by centrifuging at 6000xg for 15 minutes and supernatant discarded. The cells were resuspended in PBS (pH7.0) and lysed by sonication using a Sonics Vibra cell at 60% power. Lysed cells were cleared by centrifuging at 12000xg for 10 minutes and the supernatant was discarded. Cell pellet was washed three times in PBS (pH7.0) buffer containing 0.5% Triton X-100 followed by one wash with PBS (pH7.0). Then, pellet was further washed once using 8M urea in PBS (pH7.0). Each wash step was clarified by centrifuging at 12000xg and supernatant was discarded. The pellet was then solubilised in solubilisation buffer containing 10mM TRIS (pH8.0), 8M urea, 100mM NaC1 overnight at room temperature. Solubilisation buffer was further centrifuged at 12000xg, filtered through a 0.45nm membrane and loaded onto a NiSepharose colum pre-washed with washing buffer containing PBS (pH7.0), 8M Urea and 20mM Imidazole. After loading, column was washed with 10 column volumes of washing buffer and solubilised proteins were eluted in washing buffer, supplemented with 500mM Imidazole. Eluted proteins were desalted into PBS (pH7.0) and 8M urea buffer and then refolded by drop-wise dilution in refolding buffer containing 50mM Sodium Acetate (pH 4.5), 0.1M NDSB-201, 10% Glycerol, ImM/0.1mM GSH/GSSH. Refolding buffer was clarified by centrifugation at 12000xg and refolded protein was concentrated using Centriprep filters with nominal molecular cut-off of 10KDa (Millipore). Refolded proteins were buffer exchanged into a buffer containing 100mM sodium acetate (pH 5.0) supplemented with 10% glycerol using a G25 desalt column and aliquots were stored at -80°C. Coomassie stained 10% SDS PAGE gel and Western blot analysis collectively indicated the presence of a His-tagged protein of 18KDa at up to 95% purity. The 18KDa Coomassie stained band was excised and identified by MALDI-TOF/TOF MS/MS to contain ZG16.

[0115] Antibodies against ZG16 were obtained by panning a phage display antibody library with the purified ZG16 protein (Antibodies by Design; a division of Morphosys AG, Germany. www.morphosys.com).

Antibody arrays

[0116] Antibody arrays were used to validate the candidate markers. Serum samples were obtained from patients with gastric cancer, colorectal cancer (before and after surgery) and from surgical patients with non-malignant disease. Samples were made available by Dunedin Public Hospital, New. Zealand, and the Christchurch Cancer Society tissue bank, Christchurch, New Zealand. Antibodies against ZG16 and MUC17 that were obtained from either commercial sources or selected from phage libraries (Morphosys) were printed onto glass slides (Schott Nexterion Slide H) using the GeneMachines OmniGrid 100 array robot. Each array was circumscribed with a hydrophobic pen. Slides were then washed in 3X PBS-0.5% Tween 20 (3X PBS-T) before blocking with 50mM ethanolamine in 50mM sodium borate buffer, pH8.0 followed by caseinate blocking buffer (3X PBS-T, 1% sodium caseinate). Biotin-labelled serum samples were then added to the slides before incubation overnight at 4°C. Slides were then washed in 3X PBS-T before being air-dried. Bound antibody was then detected using rolling circle amplification (RCA), largely as previously described (Haab BB, Lizardi PM. RCA-enhanced protein detection arrays. Methods Mol Biol. 2006;328:15-29). Briefly, the slides were

incubated with anti-biotin antibodies that had been conjugated with an oligonucleotide primer (5' - CCT GGT GCT CAA ATT TCA GTT CTG C - 3'). A circular DNA template was then hybridised to the slides at 37°C for 30mins in a humidified sealed chamber, before the slides were washed in decreasing concentrations of PBS-T (3X PBS-0.05% Tween 20, 1X PBS-0.05% Tween 20 and 0.1X PBS-0.05% Tween 20) and dried. The template was then extended using phi29 at 30°C for 3hrs before the slides were washed and dried by centrifugation. The amplified template was then detected using homologous fluorescently labeled probes. Slides were scanned with an Axon 4000A scanner and signal measured with the GenePix Pro 6.1.0.4 software.

[0117] Cy5 fluorescence intensity was adjusted using quantile normalization, using the normalizeBetweenArrays function from the limma (Smith, 2005) package for R (the R package for statistical computing (R Development Core). Quantile normalization adjusts the values of the intensities so that the distribution of intensities is the same for each block (each block corresponding to a separate sample), by setting the quantiles of the intensities from different blocks to the same value. The rank of each intensity value does not change during this procedure, only the relative magnitude of the intensities. The assumption is that the underlying probability distribution function describing the range of antigen concentrations is the same for all samples. This procedure improved the average correlation of signals between blocks across all samples and also when considering reference-only blocks, which indicates an improvement in the quality of the data. Genepix-flagged spots were removed before taking the median across replicates to obtain normalized intensities for each antibody.

[0118] Thus, we have identified three genes and/or proteins that are useful for developing reagents, devices and kits for detecting and evaluating gastric cancer. One or more markers of gastric cancer can be used, either singly or in combination to provide a reliable molecular test for gastric cancer.

EXAMPLES

[0119] The examples described herein are for purposes of illustrating embodiments of the invention. Other embodiments, methods and types of analyses are within the scope of persons of ordinary skill in the molecular diagnostic arts and need not be described in detail hereon.

Example 1: Identification of Markers for Gastric Malignancy

[0120] Markers were selected using the gene expression data obtained from gastric tumors and non-malignant samples. The following criteria was used for marker selection: (i) the presence of a signal sequence characteristic of a secreted protein (ii) the microarray signal intensity ranking in tumor tissue and (iii) the levels of corresponding ESTs in blood or vascular tissues. The use of these criteria enabled the identification of secreted markers that are abundantly expressed in tumor tissue but likely to have a low background in serum, blood or plasma. Figure 1 depicts a table that shows the three markers for gastric malignancy selected using the above criteria, MUC5AC, MUCI7 and ZG16. Figure 1 includes the symbol for the gene ("symbol"), the MWG oligo number, the NCBI mRNA reference sequence number, the protein reference sequence number, the rank intensity of the gene on the arrays derived using tumor tissue, and the rank intensity of the gene on the arrays derived using nonmalignant tissue. All three GTMs had a higher expression (intensity) rank than CEACAM5, the gene that encodes the existing gastric cancer marker CEA. The lowest expressing rank possible was 29,718. Examination of the ranking also shows that the expression of these GTMs in tumor tissue was comparable to non-malignant tissue, indicating that the genes had not been strongly down-regulated during carcinogenesis. Unigene EST counts (Wheeler et al, 2003) for the three GTMs in blood and vascular tissue were all zero.

Example 2: qRT-PCR Analysis

[0121] The abundance and identity of the GTMs ZG16 and MUC17 was confirmed in tumor tissue using the more sensitive and accurate gene expression quantification technique, qPCR. Up to 45 gastric tumor samples and an equal number of nonmalignant gastric tissue samples from the same patients were analysed by RT-qPCR using the primers and probes described in the methods section. Expression of these genes was quantified using the number of PCR cycles required to reach a threshold level of product amplification (Ct).

[0122] qPCR analysis confirmed the array data: both markers were readily detected in tumor tissue by qPCR and there was no evidence for a significant decrease in expression in tumor tissue compared to non-malignant tissue. The abundance of these RNAs in tumor tissue compared to non-malignant tissue is illustrated by the histograms in Figure 2a-b.

Example 3: Detection of Gastric Tumor Marker Proteins in Serum

[0123] In certain embodiments, detection of GTM proteins can be accomplished using antibodies directed against either the entire protein, a fragment of the protein (peptide) or the protein core. Methods for detecting and quantifying

expression of proteins and peptides are known in the art and can include methods relying on specific antibodies raised against the protein or peptide. Monoclonal antibodies and polyclonal antisera can be made using methods that are well known in the art and need not be described herein further.

[0124] To detect the GTMs in serum, antibodies against the GTMs were printed onto glass slides using Gene Machine OmniGrid™ robotics. Each antibody was repeated 8 times on the array. Serum samples from 33 gastric cancer patients and 41 controls were then labeled with biotin before being incubated with the antibody slides. Bound proteins were detected with anti-biotin antibodies and the signal amplified using rolling circle amplification (RCA) and fluorescent labeling. The amount of bound protein was quantified using an Axon 4000a scanner and the Genepix 6.1.0.4 software. The characteristics of the patients are shown in Figure 2.

[0125] The fluorescent signal from each antibody on the array was normalized and the median signal for the 8 replicates expressed in arbitrary fluorescent units. Box plots illustrating the data spread are shown in Figure 3. The median signal for MUC 17 was 18,836AU for gastric cancer patients and 16,130 for the control group. These medians were significantly different (p=0.007). Significant differences between the medians were observed for two phage display ZG16 antibodies (5902 and 5905) obtained from MorphoSys. The median signal for ZG16_5902 in gastric cancer patient samples was 2139AU compared to 1837AU for controls; the median ZG16_5905 signal in patients was 3063AU compared to 1675AU for controls. The median signal between patients and controls for both ZG16_5902 and ZG16_5905 were significantly different (p=0.05 and p=0.005, respectively).

[0126] This data demonstrates that MUC17 and ZG16 are present at significantly higher levels in the serum of gastric cancer patients than controls. Further differentiation between patient and control groups will be achieved by refinement of the immunological testing procedure, the identification of antibodies with greater specificity for the target antigens and the use of combinations of markers.

Example 4: Cells Transfected with GTM-Containing Vectors

[0127] In still further embodiments, cells are provided that can express GTMs, GTM fragments or peptide markers. Both prokaryotic and eukaryotic cells can be so used. For example, E. coli (a prokaryotic cell) can be use to produce large quantities of GTMs lacking in mature glycosylation (if the particular GTM normally is glycosylated). COS cells, 293 cells and a variety of other eukaryotic cells can be used to produce GTMs that are glycosylated, or have proper folding and therefore, three-dimensional structure of the native form of the GTM protein. Methods for transfecting such cells are known in the art and need not be described further herein.

Example 5: Kits

[0128] Based on the discoveries described herein, several types of test kits can be produced. First, kits can be made that have a detection device pre-loaded with a detection molecule (or "capture reagent"). In embodiments for detection of GTM mRNA, such devices can comprise a substrate (e.g., glass, silicon, quartz, metal, etc) on which oligonucleotides as capture reagents that hybridize with the mRNA to be detected. In some embodiments, direct detection of mRNA can be accomplished by hybridizing mRNA (labeled with cy3, cy5, radiolabel or other label) to the oligonucleotides on the substrate. In other embodiments, detection of mRNA can be accomplished by first making complementary DNA (cDNA) to the desired mRNA. Then, labeled cDNA can be hybridized to the oligonucleotides on the substrate and detected.

[0129] Regardless of the detection method employed, comparison of test GTM expression with a standard measure of expression is desirable. For example, RNA expression can be standardized to total cellular DNA, to expression of constitutively expressed RNAs (for example, ribosomal RNA) or to other relatively constant markers.

[0130] Antibodies can also be used in kits as capture reagents. In some embodiments, a substrate (e.g., a multiwell plate) can have a specific GTM capture reagent attached thereto. In some embodiments, a kit can have a blocking reagent included. Blocking reagents can be used to reduce non-specific binding. For example, non-specific oligonucleotide binding can be reduced using excess DNA from any convenient source that does not contain GTM oligonucleotides, such as salmon sperm DNA. Non-specific antibody binding can be reduced using an excess of a blocking protein such as serum albumin. It can be appreciated that numerous methods for detecting oligonucleotides and proteins are known in the art, and any strategy that can specifically detect GTM associated molecules can be used.

[0131] In embodiments relying upon antibody detection, GTM proteins or peptides can be expressed on a per cell basis, or on the basis of total cellular, tissue, or fluid protein, fluid volume, tissue mass (weight). Additionally, GTM in serum can be expressed on the basis of a relatively high-abundance serum protein such as albumin.

[0132] In addition to a substrate, a test kit can comprise capture reagents (such as probes), washing solutions (e.g., SSC, other salts, buffers, detergents and the like), as well as detection moieties (e.g., cy3, cy5, radiolabels, and the like). Kits can also include instructions for use and a package.

[0133] Although this invention is described with reference to specific embodiments thereof, it can be appreciated that other embodiments involving the use of the disclosed markers can be used.

INDUSTRIAL APPLICABILITY

[0134] Methods for detecting GTM family members include detection of nucleic acids using microarray and/or real time PCR methods and detection of proteins and peptides. The compositions and methods described herein are useful in the manufacture of diagnostic devices and kits, diagnosis of disease, evaluating efficacy of therapy, and for producing reagents suitable for measuring expression of GTM family members in biological samples.

SEQUENCE LISTING

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Glu Ser Ser Tyr Lys His His Pro Ala Leu Ser Pro Ile Ala Arg Gly
        35                  40                  45

Pro Ser Gly Val Pro Leu Arg Gly Ala Thr Val Phe Pro Ser Leu Arg
    50                  55                  60

Thr Ile Pro Val Val Arg Ala Ser Asn Pro Ala His Asn Gly Arg Val
65                  70                  75                  80

Cys Ser Thr Trp Gly Ser Phe His Tyr Lys Thr Phe Asp Gly Asp Val
                85                  90                  95

Phe Arg Phe Pro Gly Leu Cys Asn Tyr Val Phe Ser Glu His Cys Gly
            100                 105                 110

Ala Ala Tyr Glu Asp Phe Asn Ile Gln Leu Arg Arg Ser Gln Glu Ser
        115                 120                 125

Ala Ala Pro Thr Leu Ser Arg Val Leu Met Lys Val Asp Gly Val Val
    130                 135                 140

Ile Gln Leu Thr Lys Gly Ser Val Leu Val Asn Gly His Pro Val Leu
145                 150                 155                 160

Leu Pro Phe Ser Gln Ser Gly Val Leu Ile Gln Gln Ser Ser Ser Tyr
                165                 170                 175

Thr Lys Val Glu Ala Arg Leu Gly Leu Val Leu Met Trp Asn His Asp
            180                 185                 190

Asp Ser Leu Leu Leu Glu Leu Asp Thr Lys Tyr Ala Asn Lys Thr Cys
        195                 200                 205

Gly Leu Cys Gly Asp Phe Asn Gly Met Pro Val Val Ser Glu Leu Leu
    210                 215                 220

Ser His Asn Thr Lys Leu Thr Pro Met Glu Phe Gly Asn Leu Gln Lys
225                 230                 235                 240

Met Asp Asp Pro Thr Glu Gln Cys Gln Asp Pro Val Pro Glu Pro Pro
                245                 250                 255

Arg Asn Cys Ser Thr Gly Phe Gly Ile Cys Glu Glu Leu Leu His Gly
            260                 265                 270

Gln Leu Phe Ser Gly Cys Val Ala Leu Val Asp Val Gly Ser Tyr Leu
        275                 280                 285

Glu Ala Cys Arg Gln Asp Leu Cys Phe Cys Glu Asp Thr Asp Leu Leu
    290                 295                 300
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Ser Cys Val Cys His Thr Leu Ala Glu Tyr Ser Arg Gln Cys Thr His
305                 310                 315                 320

Ala Gly Gly Leu Pro Gln Asp Trp Arg Gly Pro Asp Phe Cys Pro Gln
                325                 330                 335

Lys Cys Pro Asn Asn Met Gln Tyr His Glu Cys Arg Ser Pro Cys Ala
            340                 345                 350

Asp Thr Cys Ser Asn Gln Glu His Ser Arg Ala Cys Glu Asp His Cys
        355                 360                 365

Val Ala Gly Cys Phe Cys Pro Glu Gly Thr Val Leu Asp Asp Ile Gly
    370                 375                 380

Gln Thr Gly Cys Val Pro Val Ser Lys Cys Ala Cys Val Tyr Asn Gly
385                 390                 395                 400

Ala Ala Tyr Ala Pro Gly Ala Thr Tyr Ser Thr Asp Cys Thr Asn Cys
                405                 410                 415

Thr Cys Ser Gly Gly Arg Trp Ser Cys Gln Glu Val Pro Cys Pro Gly
            420                 425                 430

Thr Cys Ser Val Leu Gly Gly Ala His Phe Ser Thr Phe Asp Gly Lys
        435                 440                 445

Gln Tyr Thr Val His Gly Asp Cys Ser Tyr Val Leu Thr Lys Pro Cys
    450                 455                 460

Asp Ser Ser Ala Phe Thr Val Leu Ala Glu Leu Arg Arg Cys Gly Leu
465                 470                 475                 480

Thr Asp Ser Glu Thr Cys Leu Lys Ser Val Thr Leu Ser Leu Asp Gly
                485                 490                 495

Ala Gln Thr Val Val Val Ile Lys Ala Ser Gly Glu Val Phe Leu Asn
            500                 505                 510

Gln Ile Tyr Thr Gln Leu Pro Ile Ser Ala Ala Asn Val Thr Ile Phe
        515                 520                 525

Arg Pro Ser Thr Phe Phe Ile Ile Ala Gln Thr Ser Leu Gly Leu Gln
    530                 535                 540

Leu Asn Leu Gln Leu Val Pro Thr Met Gln Leu Phe Met Gln Leu Ala
545                 550                 555                 560

Pro Lys Leu Arg Gly Gln Thr Cys Gly Leu Cys Gly Asn Phe Asn Ser
                565                 570                 575
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Ile Gln Ala Asp Asp Phe Arg Thr Leu Ser Gly Val Val Glu Ala Thr
580 585 590

Ala Ala Ala Phe Phe Asn Thr Phe Lys Thr Gln Ala Ala Cys Pro Asn
595 600 605

Ile Arg Asn Ser Phe Glu Asp Pro Cys Ser Leu Ser Val Glu Asn Ala
610 615 620

Pro Arg Met Gly Ala Pro Ser Ala Cys Arg Thr Leu Val Leu Ala Leu
625 630 635 640

Ala Ala Met Leu Val Val Pro Gln Ala Glu Thr Gln Gly Pro Val Glu
645 650 655

Pro Ser Trp Glu Asn Ala Gly His Thr Met Asp Gly Gly Ala Pro Thr
660 665 670

Ser Ser Pro Thr Arg Arg Val Ser Phe Val Pro Pro Val Thr Val Phe
675 680 685

Pro Ser Leu Ser Arg Lys Gln Met Leu Pro Leu Pro Ala Gly Lys Gly
690 695 700

Val Phe Ala Ser Pro Lys Gly Gly Gly Pro Asp Leu Gly Val Gln Leu
705 710 715 720

Pro Pro Ala Leu Asn Pro Ala His Asn Gly Arg Val Cys Ser Thr Trp
725 730 735

Gly Asp Phe His Tyr Lys Thr Phe Asp Gly Asp Val Phe Arg Phe Pro
740 745 750

Gly Leu Cys Asn Tyr Val Phe Ser Glu His Cys Arg Ala Ala Tyr Glu
755 760 765

Asp Phe Asn Val Gln Leu Arg Arg Gly Leu Val Gly Ser Arg Pro Val
770 775 780

Val Thr Arg Val Val Ile Lys Ala Gln Gly Leu Val Leu Glu Ala Ser
785 790 795 800

Asn Gly Ser Val Leu Ile Asn Gly Gln Arg Glu Glu Leu Pro Tyr Ser
805 810 815

Arg Thr Gly Leu Leu Val Glu Gln Ser Gly Asp Tyr Ile Lys Val Ser
820 825 830

Ile Arg Leu Val Leu Thr Phe Leu Trp Asn Gly Glu Asp Ser Ala Leu
835 840 845

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Leu Glu Leu Asp Pro Lys Tyr Ala Asn Gln Thr Cys Gly Leu Cys Gly
    850                 855                 860

Asp Phe Asn Gly Leu Pro Ala Phe Asn Glu Phe Tyr Ala His Asn Ala
865                 870                 875                 880

Arg Leu Thr Pro Leu Gln Phe Gly Asn Leu Gln Lys Leu Asp Gly Pro
                885                 890                 895

Thr Glu Gln Cys Pro Asp Pro Leu Pro Leu Pro Ala Gly Asn Cys Thr
            900                 905                 910

Asp Glu Glu Gly Ile Cys His Arg Thr Leu Leu Gly Pro Ala Phe Ala
        915                 920                 925

Glu Cys His Ala Leu Val Asp Ser Thr Ala Tyr Leu Ala Ala Cys Ala
    930                 935                 940

Gln Asp Leu Cys Arg Cys Pro Thr Cys Pro Cys Ala Thr Phe Val Glu
945                 950                 955                 960

Tyr Ser Arg Gln Cys Ala His Ala Gly Gly Gln Pro Arg Asn Trp Arg
                965                 970                 975

Cys Pro Glu Leu Cys Pro Arg Thr Cys Pro Leu Asn Met Gln His Gln
            980                 985                 990

Glu Cys Gly Ser Pro Cys Thr Asp Thr Cys Ser Asn Pro Gln Arg Ala
        995                 1000                1005

Gln Leu Cys Glu Asp His Cys Val Asp Gly Cys Phe Cys Pro Pro
    1010                1015                1020

Gly Arg Ser Cys Thr Val Leu Asp Asp Ile Thr His Ser Gly Cys
    1025                1030                1035

Leu Pro Leu Gly Gln Cys Pro Cys Thr His Gly Gly Arg Thr Tyr
    1040                1045                1050

Ser Pro Gly Thr Ser Phe Asn Thr Thr Cys Ser Ser Cys Thr Cys
    1055                1060                1065

Ser Gly Gly Leu Trp Gln Cys Gln Asp Leu Pro Cys Pro Gly Thr
    1070                1075                1080

Cys Ser Val Gln Gly Gly Ala His Ile Ser Thr Tyr Asp Glu Lys
    1085                1090                1095

Leu Tyr Asp Leu His Gly Asp Cys Ser Tyr Val Leu Ser Lys Lys
    1100                1105                1110

Cys Ala Asp Ser Ser Phe Thr Val Leu Ala Glu Leu Arg Lys Cys
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1115 1120 1125

Gly Leu Thr Asp Asn Glu Asn Cys Leu Lys Ala Val Thr Leu Ser
1130 1135 1140

Leu Asp Gly Gly Asp Thr Ala Ile Arg Val Gln Ala Asp Gly Gly
1145 1150 1155

Val Phe Leu Asn Ser Ile Tyr Thr Gln Leu Pro Leu Ser Ala Ala
1160 1165 1170

Asn Ile Thr Leu Phe Thr Pro Ser Ser Phe Phe Ile Val Val Gln
1175 1180 1185

Thr Gly Leu Gly Leu Gln Leu Leu Val Gln Leu Val Pro Leu Met
1190 1195 1200

Gln Val Phe Val Arg Leu Asp Pro Ala His Gln Gly Gln Met Cys
1205 1210 1215

Gly Leu Cys Gly Asn Phe Asn Gln Asn Gln Ala Asp Asp Phe Thr
1220 1225 1230

Ala Leu Ser Gly Val Val Glu Ala Thr Gly Ala Ala Phe Ala Asn
1235 1240 1245

Thr Trp Lys Ala Gln Ala Ala Cys Ala Asn Ala Arg Asn Ser Phe
1250 1255 1260

Glu Asp Pro Cys Ser Leu Ser Val Glu Asn Glu Asn Tyr Ala Arg
1265 1270 1275

His Trp Cys Ser Arg Leu Thr Asp Pro Asn Ser Ala Phe Ser Arg
1280 1285 1290

Cys His Ser Ile Ile Asn Pro Lys Pro Phe His Ser Asn Cys Met
1295 1300 1305

Phe Asp Thr Cys Asn Cys Glu Arg Ser Glu Asp Cys Leu Cys Ala
1310 1315 1320

Ala Leu Ser Ser Tyr Val His Ala Cys Ala Ala Lys Gly Val Gln
1325 1330 1335

Leu Ser Asp Trp Arg Asp Gly Val Cys Thr Lys Tyr Met Gln Asn
1340 1345 1350

Cys Pro Lys Ser Gln Arg Tyr Ala Tyr Val Val Asp Ala Cys Gln
1355 1360 1365

Pro Thr Cys Arg Gly Leu Ser Glu Ala Asp Val Thr Cys Ser Val
1370 1375 1380

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Ser Phe Val Pro Val Asp Gly Cys Thr Cys Pro Ala Gly Thr Phe
1385                1390                1395

Leu Asn Asp Ala Gly Ala Cys Val Pro Ala Gln Glu Cys Pro Cys
1400                1405                1410

Tyr Ala His Gly Thr Val Leu Ala Pro Gly Glu Val Val His Asp
1415                1420                1425

Glu Gly Ala Val Cys Ser Cys Thr Gly Gly Lys Leu Ser Cys Leu
1430                1435                1440

Gly Ala Ser Leu Gln Lys Ser Thr Gly Cys Ala Ala Pro Met Val
1445                1450                1455

Tyr Leu Asp Cys Ser Asn Ser Ser Ala Gly Thr Pro Gly Ala Glu
1460                1465                1470

Cys Leu Arg Ser Cys His Thr Leu Asp Val Gly Cys Phe Ser Thr
1475                1480                1485

His Cys Val Ser Gly Cys Val Cys Pro Pro Gly Leu Val Ser Asp
1490                1495                1500

Gly Ser Gly Gly Cys Ile Ala Glu Glu Asp Cys Pro Cys Val His
1505                1510                1515

Asn Glu Ala Thr Tyr Lys Pro Gly Glu Thr Ile Arg Val Asp Cys
1520                1525                1530

Asn Thr Cys Thr Cys Arg Asn Arg Arg Trp Glu Cys Ser His Arg
1535                1540                1545

Leu Cys Leu Gly Thr Cys Val Ala Tyr Gly Asp Gly His Phe Ile
1550                1555                1560

Thr Phe Asp Gly Asp Arg Tyr Ser Phe Glu Gly Ser Cys Glu Tyr
1565                1570                1575

Ile Leu Ala Gln Asp Tyr Cys Gly Asp Asn Thr Thr His Gly Thr
1580                1585                1590

Phe Arg Ile Val Thr Glu Asn Ile Pro Cys Gly Thr Thr Gly Thr
1595                1600                1605

Thr Cys Ser Lys Ala Ile Lys Leu Phe Val Glu Val Arg Thr Ala
1610                1615                1620

Pro Ala Val Ser Thr Pro Asp Pro Ala Ala Asn Glu Pro Ala Pro
1625                1630                1635
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Arg Glu Ala Ser Val Gly Phe Arg Gln Arg Leu Pro Pro Leu Gln
1640 1645 1650

Ser Tyr Glu Leu Ile Leu Gln Glu Gly Thr Phe Lys Ala Val Ala
1655 1660 1665

Arg Gly Pro Gly Gly Asp Pro Pro Tyr Lys Ile Arg Tyr Met Gly
1670 1675 1680

Ile Phe Leu Val Ile Glu Thr His Gly Met Ala Val Ser Trp Asp
1685 1690 1695

Arg Lys Thr Ser Val Phe Ile Arg Leu His Gln Asp Tyr Lys Gly
1700 1705 1710

Arg Val Cys Gly Leu Cys Gly Asn Phe Asp Asp Asn Ala Ile Asn
1715 1720 1725

Asp Phe Ala Thr Arg Ser Arg Ser Val Val Gly Asp Ala Leu Glu
1730 1735 1740

Phe Gly Asn Ser Trp Lys Leu Ser Pro Ser Cys Pro Asp Ala Leu
1745 1750 1755

Ala Pro Lys Asp Pro Cys Thr Ala Asn Pro Phe Arg Lys Ser Trp
1760 1765 1770

Ala Gln Lys Gln Cys Ser Ile Leu His Gly Pro Thr Phe Ala Ala
1775 1780 1785

Cys Arg Ser Gln Val Asp Ser Thr Lys Tyr Tyr Glu Ala Cys Val
1790 1795 1800

Asn Asp Ala Cys Ala Cys Asp Ser Gly Gly Asp Cys Glu Cys Phe
1805 1810 1815

Cys Thr Ala Val Ala Ala Tyr Ala Gln Ala Cys His Asp Ala Gly
1820 1825 1830

Leu Cys Val Ser Trp Arg Thr Pro Asp Thr Cys Pro Leu Phe Cys
1835 1840 1845

Asp Phe Tyr Asn Pro His Gly Gly Cys Glu Trp His Tyr Gln Pro
1850 1855 1860

Cys Gly Ala Pro Cys Leu Lys Thr Cys Arg Asn Pro Ser Gly His
1865 1870 1875

Cys Leu Val Asp Leu Pro Gly Leu Glu Gly Cys Tyr Pro Lys Cys
1880 1885 1890

Pro Pro Ser Gln Pro Phe Phe Asn Glu Asp Gln Met Lys Cys Val
1895 1900 1905

Ala Gln Cys Gly Cys Tyr Asp Lys Asp Gly Asn Tyr Tyr Asp Val
1910 1915 1920

Gly Ala Arg Val Pro Thr Ala Glu Asn Cys Gln Ser Cys Asn Cys
1925 1930 1935

Thr Pro Ser Gly Ile Gln Cys Ala His Ser Leu Glu Ala Cys Thr
1940 1945 1950

Cys Thr Tyr Glu Asp Arg Thr Tyr Ser Tyr Gln Asp Val Ile Tyr
1955 1960 1965

Asn Thr Thr Asp Gly Leu Gly Ala Cys Leu Ile Ala Ile Cys Gly
1970 1975 1980

Ser Asn Gly Thr Ile Ile Arg Lys Ala Val Ala Cys Pro Gly Thr
1985 1990 1995

Pro Ala Thr Thr Pro Phe Thr Phe Thr Thr Ala Trp Val Pro His
2000 2005 2010

Ser Thr Thr Ser Pro Ala Leu Pro Val Ser Thr Val Cys Val Arg
2015 2020 2025

Glu Val Cys Arg Trp Ser Ser Trp Tyr Asn Gly His Arg Pro Glu
2030 2035 2040

Pro Gly Leu Gly Gly Gly Asp Phe Glu Thr Phe Glu Asn Leu Arg
2045 2050 2055

Gln Arg Gly Tyr Gln Val Cys Pro Val Leu Ala Asp Ile Glu Cys
2060 2065 2070

Arg Ala Ala Gln Leu Pro Asp Met Pro Leu Glu Glu Leu Gly Gln
2075 2080 2085

Gln Val Asp Cys Asp Arg Met Arg Gly Leu Met Cys Ala Asn Ser
2090 2095 2100

Gln Gln Ser Pro Pro Leu Cys His Asp Tyr Glu Leu Arg Val Leu
2105 2110 2115

Cys Cys Glu Tyr Val Pro Cys Gly Pro Ser Pro Ala Pro Gly Thr
2120 2125 2130

Ser Pro Gln Pro Ser Leu Ser Ala Ser Thr Glu Pro Ala Val Pro
2135 2140 2145

Thr Pro Thr Gln Thr Thr Ala Thr Glu Lys Thr Thr Leu Trp Val

2150 2155 2160

Thr Pro Ser Ile Arg Ser Thr Ala Ala Leu Thr Ser Gln Thr Gly
2165 2170 2175

Ser Ser Ser Gly Pro Val Thr Val Thr Pro Ser Ala Pro Gly Thr
2180 2185 2190

Thr Thr Cys Gln Pro Arg Cys Gln Trp Thr Glu Trp Phe Asp Glu
2195 2200 2205

Asp Tyr Pro Lys Ser Glu Gln Leu Gly Gly Asp Val Glu Ser Tyr
2210 2215 2220

Asp Lys Ile Arg Ala Ala Gly Gly His Leu Cys Gln Gln Pro Lys
2225 2230 2235

Asp Ile Glu Cys Gln Ala Glu Ser Phe Pro Asn Trp Thr Leu Ala
2240 2245 2250

Gln Val Gly Gln Lys Val His Cys Asp Val His Phe Gly Leu Val
2255 2260 2265

Cys Arg Asn Trp Glu Gln Glu Gly Val Phe Lys Met Cys Tyr Asn
2270 2275 2280

Tyr Arg Ile Arg Val Leu Cys Cys Ser Asp Asp His Cys Arg Gly
2285 2290 2295

Arg Ala Thr Thr Pro Pro Pro Thr Thr Glu Leu Glu Thr Ala Thr
2300 2305 2310

Thr Thr Thr Thr Gln Ala Leu Phe Ser Thr Pro Gln Pro Thr Ser
2315 2320 2325

Ser Pro Gly Leu Thr Arg Ala Pro Pro Ala Ser Thr Thr Ala Val
2330 2335 2340

Pro Thr Leu Ser Glu Gly Leu Thr Ser Pro Arg Tyr Thr Ser Thr
2345 2350 2355

Leu Gly Thr Ala Thr Thr Gly Gly Pro Thr Thr Pro Ala Gly Ser
2360 2365 2370

Thr Glu Pro Thr Val Pro Gly Val Ala Thr Ser Thr Leu Pro Thr
2375 2380 2385

Arg Ser Ala Leu Pro Gly Thr Thr Gly Ser Leu Gly Thr Trp Arg
2390 2395 2400

Pro Ser Gln Pro Pro Thr Leu Ala Pro Thr Thr Met Ala Thr Ser
2405 2410 2415

Arg Ala Arg Pro Thr Gly Thr Ala Ser Thr Ala Ser Lys Glu Pro
2420 2425 2430

Leu Thr Thr Ser Leu Ala Pro Thr Leu Thr Ser Glu Leu Ser Thr
2435 2440 2445

Ser Gln Ala Glu Thr Ser Thr Pro Arg Thr Glu Thr Thr Met Ser
2450 2455 2460

Pro Leu Thr Asn Thr Thr Thr Ser Gln Gly Thr Thr Arg Cys Gln
2465 2470 2475

Pro Lys Cys Glu Trp Thr Glu Trp Phe Asp Val Asp Phe Pro Thr
2480 2485 2490

Ser Gly Val Ala Gly Gly Asp Met Glu Thr Phe Glu Asn Ile Arg
2495 2500 2505

Ala Ala Gly Gly Lys Met Cys Trp Ala Pro Lys Ser Ile Glu Cys
2510 2515 2520

Arg Ala Glu Asn Tyr Pro Glu Val Ser Ile Asp Gln Val Gly Gln
2525 2530 2535

Val Leu Thr Cys Ser Leu Glu Thr Gly Leu Thr Cys Lys Asn Glu
2540 2545 2550

Asp Gln Thr Gly Arg Phe Asn Met Cys Phe Asn Tyr Asn Val Arg
2555 2560 2565

Val Leu Cys Cys Asp Asp Tyr Ser His Cys Pro Ser Thr Pro Ala
2570 2575 2580

Thr Ser Ser Thr Ala Thr Pro Ser Ser Thr Pro Gly Thr Thr Trp
2585 2590 2595

Ile Leu Thr Lys Pro Thr Thr Thr Ala Thr Thr Thr Ala Ser Thr
2600 2605 2610

Gly Ser Thr Ala Thr Pro Thr Ser Thr Leu Arg Thr Ala Pro Pro
2615 2620 2625

Pro Lys Val Leu Thr Thr Thr Ala Thr Thr Pro Thr Val Thr Ser
2630 2635 2640

Ser Lys Ala Thr Pro Ser Ser Ser Pro Gly Thr Ala Thr Ala Leu
2645 2650 2655

Pro Ala Leu Arg Ser Thr Ala Thr Thr Pro Thr Ala Thr Ser Val
2660 2665 2670

Thr Pro Ile Pro Ser Ser Ser Leu Gly Thr Thr Trp Thr Arg Leu
2675 2680 2685

Ser Gln Thr Thr Thr Pro Thr Ala Thr Met Ser Thr Ala Thr Pro
2690 2695 2700

Ser Ser Thr Pro Glu Thr Ala His Thr Ser Thr Val Leu Thr Ala
2705 2710 2715

Thr Ala Thr Thr Thr Gly Ala Thr Gly Ser Val Ala Thr Pro Ser
2720 2725 2730

Ser Thr Pro Gly Thr Ala His Thr Thr Lys Val Pro Thr Thr Thr
2735 2740 2745

Thr Thr Gly Phe Thr Ala Thr Pro Ser Ser Ser Pro Gly Thr Ala
2750 2755 2760

Leu Thr Pro Pro Val Trp Ile Ser Thr Thr Thr Thr Pro Thr Thr
2765 2770 2775

Arg Gly Ser Thr Val Thr Pro Ser Ser Ile Pro Gly Thr Thr His
2780 2785 2790

Thr Ala Thr Val Leu Thr Thr Thr Thr Thr Thr Val Ala Thr Gly
2795 2800 2805

Ser Met Ala Thr Pro Ser Ser Ser Thr Gln Thr Ser Gly Thr Thr
2810 2815 2820

His Thr Pro Pro Val Pro Asn Thr Met Ala Thr Thr His Gly Arg
2825 2830 2835

Ser Leu Pro Pro Ser Ser Pro His Thr Val Arg Thr Ala Trp Thr
2840 2845 2850

Ser Ala Thr Ser Gly Ile Leu Gly Thr Thr His Ile Thr Glu Pro
2855 2860 2865

Ser Thr Val Thr Ser His Thr Leu Ala Ala Thr Thr Gly Thr Thr
2870 2875 2880

Gln His Ser Thr Pro Ala Leu Ser Ser Pro His Pro Ser Ser Arg
2885 2890 2895

Thr Thr Glu Ser Pro Pro Ser Pro Gly Thr Thr Thr Pro Gly His
2900 2905 2910

Thr Thr Ala Thr Ser Arg Thr Thr Ala Thr Ala Thr Pro Ser Lys
2915 2920 2925

Thr Arg Thr Ser Thr Leu Leu Pro Ser Ser Pro Thr Ser Ala Pro
2930 2935 2940

Ile Thr Thr Val Val Thr Met Gly Cys Glu Pro Gln Cys Ala Trp
2945 2950 2955

Ser Glu Trp Leu Asp Tyr Ser Tyr Pro Met Pro Gly Pro Ser Gly
2960 2965 2970

Gly Asp Phe Asp Thr Tyr Ser Asn Ile Arg Ala Ala Gly Gly Ala
2975 2980 2985

Val Cys Glu Gln Pro Leu Gly Leu Glu Cys Arg Ala Gln Ala Gln
2990 2995 3000

Pro Gly Val Pro Leu Arg Glu Leu Gly Gln Val Val Glu Cys Ser
3005 3010 3015

Leu Asp Phe Gly Leu Val Cys Arg Asn Arg Glu Gln Val Gly Lys
3020 3025 3030

Phe Lys Met Cys Phe Asn Tyr Glu Ile Arg Val Phe Cys Cys Asn
3035 3040 3045

Tyr Gly His Cys Pro Ser Thr Pro Ala Thr Ser Ser Thr Ala Met
3050 3055 3060

Pro Ser Ser Thr Pro Gly Thr Thr Trp Ile Leu Thr Glu Leu Thr
3065 3070 3075

Thr Thr Ala Thr Thr Thr Glu Ser Thr Gly Ser Thr Ala Thr Pro
3080 3085 3090

Ser Ser Thr Pro Gly Thr Thr Trp Ile Leu Thr Glu Pro Ser Thr
3095 3100 3105

Thr Ala Thr Val Thr Val Pro Thr Gly Ser Thr Ala Thr Ala Ser
3110 3115 3120

Ser Thr Gln Ala Thr Ala Gly Thr Pro His Val Ser Thr Thr Ala
3125 3130 3135

Thr Thr Pro Thr Val Thr Ser Ser Lys Ala Thr Pro Phe Ser Ser
3140 3145 3150

Pro Gly Thr Ala Thr Ala Leu Pro Ala Leu Arg Ser Thr Ala Thr
3155 3160 3165

Thr Pro Thr Ala Thr Ser Phe Thr Ala Ile Pro Ser Ser Ser Leu
3170 3175 3180

Gly Thr Thr Trp Thr Arg Leu Ser Gln Thr Thr Thr Pro Thr Ala

3185 3190 3195

Thr Met Ser Thr Ala Thr Pro Ser Ser Thr Pro Glu Thr Val His
3200 3205 3210

Thr Ser Thr Val Leu Thr Thr Thr Ala Thr Thr Thr Gly Ala Thr
3215 3220 3225

Gly Ser Val Ala Thr Pro Ser Ser Thr Pro Gly Thr Ala His Thr
3230 3235 3240

Thr Lys Val Leu Thr Thr Thr Thr Thr Gly Phe Thr Ala Thr Pro
3245 3250 3255

Ser Ser Ser Pro Gly Thr Ala Arg Thr Leu Pro Val Trp Ile Ser
3260 3265 3270

Thr Thr Thr Thr Pro Thr Thr Arg Gly Ser Thr Val Thr Pro Ser
3275 3280 3285

Ser Ile Pro Gly Thr Thr His Thr Pro Thr Val Leu Thr Thr Thr
3290 3295 3300

Thr Thr Thr Val Ala Thr Gly Ser Met Ala Thr Pro Ser Ser Ser
3305 3310 3315

Thr Gln Thr Ser Gly Thr Thr His Thr Pro Pro Val Pro Asn Thr
3320 3325 3330

Thr Ala Thr Thr His Gly Arg Ser Leu Ser Pro Ser Ser Pro His
3335 3340 3345

Thr Val Arg Thr Ala Trp Thr Ser Ala Thr Ser Gly Thr Leu Gly
3350 3355 3360

Thr Thr His Ile Thr Glu Pro Ser Thr Gly Thr Ser His Thr Pro
3365 3370 3375

Ala Ala Thr Thr Gly Thr Thr Gln His Ser Thr Pro Ala Leu Ser
3380 3385 3390

Ser Pro His Pro Ser Ser Arg Thr Thr Glu Ser Pro Pro Ser Pro
3395 3400 3405

Gly Thr Thr Thr Pro Gly His Thr Arg Ala Thr Ser Arg Thr Thr
3410 3415 3420

Ala Thr Ala Thr Pro Ser Lys Thr Arg Thr Ser Thr Leu Leu Pro
3425 3430 3435

Ser Ser Pro Thr Ser Ala Pro Ile Thr Thr Val Val Thr Met Gly
3440 3445 3450

Cys Glu Pro Gln Cys Ala Trp Ser Glu Trp Leu Asp Tyr Ser Tyr
3455 3460 3465

Pro Met Pro Gly Pro Ser Gly Gly Asp Phe Asp Thr Tyr Ser Asn
3470 3475 3480

Ile Arg Ala Ala Gly Gly Ala Val Cys Glu Gln Pro Leu Gly Leu
3485 3490 3495

Glu Cys Arg Ala Gln Ala Gln Pro Gly Val Pro Leu Arg Glu Leu
3500 3505 3510

Gly Gln Val Val Glu Cys Ser Leu Asp Phe Gly Leu Val Cys Arg
3515 3520 3525

Asn Arg Glu Gln Val Gly Lys Phe Lys Met Cys Phe Asn Tyr Glu
3530 3535 3540

Ile Arg Val Phe Cys Cys Asn Tyr Gly His Cys Pro Ser Thr Pro
3545 3550 3555

Ala Thr Ser Ser Thr Ala Thr Pro Ser Ser Thr Pro Gly Thr Thr
3560 3565 3570

Trp Ile Leu Thr Glu Gln Thr Thr Ala Ala Thr Thr Thr Ala Thr
3575 3580 3585

Thr Gly Ser Thr Ala Ile Pro Ser Ser Thr Pro Gly Thr Ala Pro
3590 3595 3600

Pro Pro Lys Val Leu Thr Ser Thr Ala Thr Thr Pro Thr Ala Thr
3605 3610 3615

Ser Ser Lys Ala Thr Ser Ser Ser Ser Pro Arg Thr Ala Thr Thr
3620 3625 3630

Leu Pro Val Leu Thr Ser Thr Ala Thr Lys Ser Thr Ala Thr Ser
3635 3640 3645

Phe Thr Pro Ile Pro Ser Phe Thr Leu Gly Thr Thr Gly Thr Leu
3650 3655 3660

Pro Glu Gln Thr Thr Thr Pro Met Ala Thr Met Ser Thr Ile His
3665 3670 3675

Pro Ser Ser Thr Pro Glu Thr Thr His Thr Ser Thr Val Leu Thr
3680 3685 3690

Thr Lys Ala Thr Thr Thr Arg Ala Thr Ser Ser Met Ser Thr Pro
3695 3700 3705

Ser Ser Thr Pro Gly Thr Thr Trp Ile Leu Thr Glu Leu Thr Thr
3710 3715 3720

Ala Ala Thr Thr Thr Ala Ala Thr Gly Pro Thr Ala Thr Pro Ser
3725 3730 3735

Ser Thr Pro Gly Thr Thr Trp Ile Leu Thr Glu Pro Ser Thr Thr
3740 3745 3750

Ala Thr Val Thr Val Pro Thr Gly Ser Thr Ala Thr Ala Ser Ser
3755 3760 3765

Thr Arg Ala Thr Ala Gly Thr Leu Lys Val Leu Thr Ser Thr Ala
3770 3775 3780

Thr Thr Pro Thr Val Ile Ser Ser Arg Ala Thr Pro Ser Ser Ser
3785 3790 3795

Pro Gly Thr Ala Thr Ala Leu Pro Ala Leu Arg Ser Thr Ala Thr
3800 3805 3810

Thr Pro Thr Ala Thr Ser Val Thr Ala Ile Pro Ser Ser Ser Leu
3815 3820 3825

Gly Thr Ala Trp Thr Arg Leu Ser Gln Thr Thr Thr Pro Thr Ala
3830 3835 3840

Thr Met Ser Thr Ala Thr Pro Ser Ser Thr Pro Glu Thr Val His
3845 3850 3855

Thr Ser Thr Val Leu Thr Thr Thr Thr Thr Thr Thr Arg Ala Thr
3860 3865 3870

Gly Ser Val Ala Thr Pro Ser Ser Thr Pro Gly Thr Ala His Thr
3875 3880 3885

Thr Lys Val Pro Thr Thr Thr Thr Thr Gly Phe Thr Ala Thr Pro
3890 3895 3900

Ser Ser Ser Pro Gly Thr Ala Leu Thr Pro Pro Val Trp Ile Ser
3905 3910 3915

Thr Thr Thr Thr Pro Thr Thr Arg Gly Ser Thr Val Thr Pro Ser
3920 3925 3930

Ser Ile Pro Gly Thr Thr His Thr Ala Thr Val Leu Thr Thr Thr
3935 3940 3945

Thr Thr Thr Val Ala Thr Gly Ser Met Ala Thr Pro Ser Ser Ser
3950 3955 3960

Thr Gln Thr Ser Gly Thr Thr His Thr Pro Pro Val Pro Asn Thr
3965 3970 3975

Thr Ala Thr Thr His Gly Arg Ser Leu Pro Pro Ser Ser Pro His
3980 3985 3990

Thr Val Arg Thr Ala Trp Thr Ser Ala Thr Ser Gly Ile Leu Gly
3995 4000 4005

Thr Thr His Ile Thr Glu Pro Ser Thr Val Thr Ser His Thr Pro
4010 4015 4020

Ala Ala Thr Thr Ser Thr Thr Gln His Ser Thr Pro Ala Leu Ser
4025 4030 4035

Ser Pro His Pro Ser Ser Arg Thr Thr Glu Ser Pro Pro Ser Pro
4040 4045 4050

Gly Thr Thr Thr Pro Gly His Thr Arg Gly Thr Ser Arg Thr Thr
4055 4060 4065

Ala Thr Ala Thr Pro Ser Lys Thr Arg Thr Ser Thr Leu Leu Pro
4070 4075 4080

Ser Ser Pro Thr Ser Ala Pro Ile Thr Thr Val Val Thr Thr Gly
4085 4090 4095

Cys Glu Pro Gln Cys Ala Trp Ser Glu Trp Leu Asp Tyr Ser Tyr
4100 4105 4110

Pro Met Pro Gly Pro Ser Gly Gly Asp Phe Asp Thr Tyr Ser Asn
4115 4120 4125

Ile Arg Ala Ala Gly Gly Ala Val Cys Glu Gln Pro Leu Gly Leu
4130 4135 4140

Glu Cys Arg Ala Gln Ala Gln Pro Gly Val Pro Leu Arg Glu Leu
4145 4150 4155

Gly Gln Val Val Glu Cys Ser Leu Asp Phe Gly Leu Val Cys Arg
4160 4165 4170

Asn Arg Glu Gln Val Gly Lys Phe Lys Met Cys Phe Asn Tyr Glu
4175 4180 4185

Ile Arg Val Phe Cys Cys Asn Tyr Gly His Cys Pro Ser Thr Pro
4190 4195 4200

Ala Thr Ser Ser Thr Ala Thr Pro Ser Ser Thr Pro Gly Thr Thr
4205 4210 4215

Trp Ile Leu Thr Lys Leu Thr Thr Thr Ala Thr Thr Thr Glu Ser

4220 4225 4230

Thr Gly Ser Thr Ala Thr Pro Ser Ser Thr Pro Gly Thr Thr Trp
4235 4240 4245

Ile Leu Thr Glu Pro Ser Thr Thr Ala Thr Val Thr Val Pro Thr
4250 4255 4260

Gly Ser Thr Ala Thr Ala Ser Ser Thr Gln Ala Thr Ala Gly Thr
4265 4270 4275

Pro His Val Ser Thr Thr Ala Thr Thr Pro Thr Val Thr Ser Ser
4280 4285 4290

Lys Ala Thr Pro Phe Ser Ser Pro Gly Thr Ala Thr Ala Leu Pro
4295 4300 4305

Ala Leu Arg Ser Thr Ala Thr Thr Pro Thr Ala Thr Ser Phe Thr
4310 4315 4320

Ala Ile Pro Ser Ser Ser Leu Gly Thr Thr Trp Thr Arg Leu Ser
4325 4330 4335

Gln Thr Thr Thr Pro Thr Ala Thr Met Ser Thr Ala Thr Pro Ser
4340 4345 4350

Ser Thr Pro Glu Thr Ala His Thr Ser Thr Val Leu Thr Thr Thr
4355 4360 4365

Ala Thr Thr Thr Arg Ala Thr Gly Ser Val Ala Thr Pro Ser Ser
4370 4375 4380

Thr Pro Gly Thr Ala His Thr Thr Lys Val Pro Thr Thr Thr Thr
4385 4390 4395

Thr Gly Phe Thr Val Thr Pro Ser Ser Ser Pro Gly Thr Ala Arg
4400 4405 4410

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        35                  40                  45

Asn Arg Gln Cys Gln Gln Leu Ser Gln His Val Arg Thr Gly Ser Ala
    50                  55                  60
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Met Tyr Leu Ser Cys Ser Thr Asn Pro Glu Met Thr Ser Ile Glu Ser
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Ser Val Thr Ser Asp Thr Pro Gly Val Ser Ser Thr Arg Met Thr Pro
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Thr Glu Ser Arg Thr Thr Ser Glu Ser Thr Ser Asp Ser Thr Thr Leu
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Phe Pro Ser Ser Thr Glu Asp Thr Ser Ser Pro Thr Thr Pro Glu Gly
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Thr Asp Val Pro Met Ser Thr Pro Ser Glu Glu Ser Ile Ser Ser Thr
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Gly Gly Gly Lys Arg Phe Ser His Ser Gly Asn Gln Leu Asp Gly Pro
        35                  40                  45

Ile Thr Ala Leu Arg Val Arg Val Asn Thr Tyr Tyr Ile Val Gly Leu
    50                  55                  60

Gln Val Arg Tyr Gly Lys Val Trp Ser Asp Tyr Val Gly Gly Arg Asn
65                  70                  75                  80

Gly Asp Leu Glu Glu Ile Phe Leu His Pro Gly Glu Ser Val Ile Gln
                85                  90                  95

Val Ser Gly Lys Tyr Lys Trp Tyr Leu Lys Lys Leu Val Phe Val Thr
            100                 105                 110

Asp Lys Gly Arg Tyr Leu Ser Phe Gly Lys Asp Ser Gly Thr Ser Phe
        115                 120                 125

Asn Ala Val Pro Leu His Pro Asn Thr Val Leu Arg Phe Ile Ser Gly
    130                 135                 140

Arg Ser Gly Ser Leu Ile Asp Ala Ile Gly Leu His Trp Asp Val Tyr
145                 150                 155                 160

Pro Thr Ser Cys Ser Arg Cys
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```

Claims

1. A method for detecting gastric cancer, comprising:

   (i) providing a blood, plasma or serum sample;
   (ii) detecting the protein levels of human zymogen granule protein 16 ("ZG16") in said sample; and
   (iii) comparing the amount of ZG16 in said sample with a value obtained from one or more control samples not having gastric cancer,

   wherein the overexpression of ZG16 in the biological sample is indicative of gastric cancer.

2. The method of claim 1, comprising detecting the level of one or more further GTM family members.

3. The method of claim 2, wherein:

   (i) said method further comprises detecting the levels of a further GTM family member selected from the group consisting of mucin 5AC ("MUC5AC"), and mucin 17 ("MUC17"); and/ or
   (ii) the one or more further GTM family member is selected from MUC5AC, MUC17, ZG16, carboxypeptidase N, polypeptide 2, 83 kDa chain ("CPN2"), matrix metalloproteinase 12 ("MMP12"), inhibin ("INHBA"), insulin-like growth factor 7 ("IGFBP7"), gamma-glutamyl hydrolase ("GGH"), leucine proline enriched proteoglycan ("LEPRE1"), cystatin S ("CST4"), secreted frizzled-related protein 4 ("SFRP4"), asporin ("ASPN"), cell growth regulator with EF hand domain 1 ("CGREF1"), kallikrein 10 (KLK10), tissue inhibitor of metalloproteinase 1

("TIMP1"), secreted acidic cysteine-rich protein ("SPARC"), transforming growth factor, β-induced ("TGFBI"), EGF-containing fibulin-like extracellular matrix protein 2 ("EFEMP2"), lumican ("LUM"), stannin ("SNN"), secreted phosphoprotein 1 ("SPP1"), chondroitin sulfate proteoglycan 2 ("CSPG2"), N-acylsphingosine amidohydrolase ("ASAH1"), serine protease 11 ("PRSS11"), secreted frizzled-related protein 2 ("SFRP2"), phospholipase A2, group XIIB ("PLA2G12B"), spondin 2, extracellular matrix protein ("SPON2"), olfactomedin 1 ("OLFM1"), thrombospondin repeat containing 1 ("TSRC1"), thrombospondin 2 ("THBS2"), adlican, cystatin SA ("CST2"), cystatin SN ("CST1 "), lysyl oxidase-like enzyme 2 ("LOXL2"), thyroglobulin ("TG"), transforming growth factor beta1 ("TGFB1"), serine or cysteine proteinase inhibitor Clade H, member 1 ("SERPINH1"), serine or cysteine proteinase inhibitor Clade B, member 5 ("SERPINB5"), matrix metalloproteinase 2 ("MMP2"), proprotein convertase subtilisin/kexin type 5 ("PCSK5"), hyaluronan glycoprotein link protein 4 ("HAPLN4"), CA19-9, CA72-4, pepsinogen and CEA; and/or
(iii) the GTM markers tested comprise MUC5AC, MUC17, ZG16, cystatin SN, serpinH1 and serpinB5.

4. The method of any one of claims 1 to 3, wherein:

(i) said step of detecting is carried out by detecting the levels of a GTM protein; or
(ii) said step of detecting is carried out by detecting the levels of a GTM peptide.

5. The method of claim 4 wherein said step of detecting is carried out using an antibody directed against said GTM, optionally wherein said antibody is a monoclonal antibody, or a polyclonal antiserum.

6. The method of claim 4 or 5, wherein said step of detecting is carried out using a sandwich-type immunoassay method, or using an antibody chip.

7. Use of an antibody specific for human zymogen granule protein 16 ("ZG16") for detecting gastric cancer in a blood, plasma or serum sample.

Patentansprüche

1. Verfahren zum Nachweis von Magenkrebs, welches umfasst:

(i) Bereitstellen einer Blut-, Plasma- oder Serumprobe;
(ii) Nachweisen des Proteinlevels des humanen Zymogen-Granule-Protein 16 ("ZG16") in der Probe, und
(iii) Vergleichen der Menge von ZG16 in der Probe mit einem Wert, der aus ein oder mehr Nicht-Magenkrebs-Kontrollproben erhalten wurde,

wobei die Überexpression von ZG16 in der biologischen Probe Magenkrebs anzeigt.

2. Verfahren gemäß Anspruch 1, umfassend Nachweisen des Levels von ein oder mehr weiteren GTM-Familienmitgliedern.

3. Verfahren gemäß Anspruch 2, wobei:

(i) das Verfahren ferner Nachweisen des Levels eines weiteren GTM-Familienmitglieds umfasst, ausgewählt aus der Gruppe, bestehend aus Mucin 5AC ("MUC5AC") und Mucin 17 ("MUC17"), und/oder
(ii) die ein oder mehr weiteren GTM-Familienmitglieder ausgewählt werden aus MUC5AC, MUC17, ZG16, Carboxypeptidase N, Polypeptid 2, 83-kDa-Kette ("CPN2"), Matrix-Metalloproteinase 12 ("MMP12"), Inhibin ("INHBA"), Insulinähnlichem Wachstumsfaktor 7 ("IGFBP7"), gamma-Glutamylhydrolase ("GGH"), Leucin-Prolin-reichem Proteoglycan ("LEPRE1"), Cystatin S ("CST4"), sezerniertem frizzled-verwandtem Protein 4 ("SFRP4"), Asporin ("ASPN"), Zellwachstumsregulator mit EF-Hand-Domäne 1 ("CGREF1"), Kallikrein 10 (KLK10), Gewebeinhibitor von Metalloproteinase 1 ("TIMP1"), sezerniertem saurem Cystein-reichem Protein ("SPARC"), transformierendem Wachstumsfaktor, β-induziert ("TGFBI"), EGF-haltigem Fibulin-ähnlichem extrazellulärem Matrixprotein 2 ("EFEMP2"), Lumican ("LUM"), Stannin ("SNN"), sezerniertem Phosphoprotein 1 ("SPP1"), Chondroitinsulfatproteoglycan 2 ("CSPG2"), N-Acylsphingosinamidohydrolase ("ASAH1"), Serinprotease 11 ("PRSS11"), sezerniertem frizzled-verwandtem Protein 2 ("SFRP2"), Phospholipase A2, Gruppe XIIB ("PLA2G12B"), Spondin 2, extrazellulärem Matrixprotein ("SPON2"), Olfactomedin 1 ("OLFM1"), Thrombospondin-Repeat-haltig 1 ("TSRC1"), Thrombospondin 2 ("THBS2"), Adlican, Cystatin SA ("CST2"), Cystatin SN

("CST1"), Lysyloxidase-ähnlichem Enzym 2 ("LOXL2"), Thyroglobulin ("TG"), transformierendem Wachstumsfaktor beta 1 ("TGFB1"), Serin- oder Cysteinproteinaseinhibitor Clade H, Mitglied 1 ("SERPINH1"), Serin- oder Cysteinproteinaseinhibitor Clade B, Mitglied 5 ("SERPINB5"), Matrixmetalloproteinase 2 ("MMP2"), Proproteinkonvertase Subtilisin/Kexin Typ 5 ("PCSK5"), Hyaluronanglycoprotein-Verbindungsprotein 4 ("HAPLN4"), CA19-9, CA72-4, Pepsinogen und CEA, und/oder
(iii) die getesteten GTM-Marker MUC5AC, MUC17, ZG16, Cystatin SN, SerpinH1 und SerpinB5 umfassen.

4. Verfahren gemäß einem der Ansprüche 1 bis 3, wobei:

(i) der Schritt des Nachweisens durch Nachweisen des Levels eines GTM-Proteins durchgeführt wird, oder
(ii) der Schritt des Nachweisens durch Nachweisen des Levels eines GTM-Peptids durchgeführt wird.

5. Verfahren gemäß Anspruch 4, wobei der Schritt des Nachweisens durch Verwendung eines Antikörpers durchgeführt wird, der gegen das GTM gerichtet ist, wobei der Antikörper gegebenenfalls ein monoklonaler Antikörper oder ein polyklonales Antiserum ist.

6. Verfahren gemäß Anspruch 4 oder 5, wobei der Schritt des Nachweisens mit Hilfe eines Sandwich-Immunassayverfahrens oder mit Hilfe eines Antikörperchips durchgeführt wird.

7. Verwendung eines Antikörpers, spezifisch für humanes Zymogen-Granule-Protein 16 ("ZG16") zum Nachweis von Magenkrebs in einer Blut-, Plasma- oder Serumprobe.

Revendications

1. Procédé de détection d'un cancer de l'estomac, comportant les étapes suivantes :

i) prendre un échantillon de sang, de plasma ou de sérum,
ii) déterminer le niveau de protéine ZG16 (protéine de granules de zymogène 16) humaine dans cet échantillon,
iii) et comparer la quantité de ZG16 présente dans cet échantillon avec une valeur obtenue à partir d'un ou de plusieurs échantillons de témoins sans cancer de l'estomac,

étant entendu qu'une surexpression de ZG16 dans l'échantillon biologique constitue un indice de la présence d'un cancer de l'estomac.

2. Procédé conforme à la revendication 1, qui comporte le fait de déterminer le niveau ou les niveaux d'un ou de plusieurs autre(s) membre(s) de la famille de protéines GTM.

3. Procédé conforme à la revendication 2,

i) lequel procédé comporte en outre le fait de déterminer le niveau d'un autre membre de la famille GTM choisi dans l'ensemble formé par la mucine 5AC (MUC5AC) et la mucine 17 (MUC17) ;
ii) et/ou dans lequel procédé l'autre ou les autres membre(s) de la famille GTM est ou sont choisi(s) parmi les suivants : MUC5AC, MUC17, ZG16, CPN2 (polypeptide 2 de carboxypeptidase N à chaîne de 83 kDa), MMP12 (métalloprotéase de matrice 12), INHBA (inhibine), IGFBP7 (facteur de croissance de type insuline 7), GGH (gamma-glutamyle hydrolase), LEPRE1 (protéoglycane enrichi en leucine et proline), CST4 (cystatine S), SFRP4 (protéine apparentée à Frizzled sécrétée 4), ASPN (asporine), CGREF1 (régulateur de croissance cellulaire à motif main EF 1), KLK10 (kallikréine 10), TIMP1 (inhibiteur tissulaire de métallo-protéinase 1), SPARC (protéine sécrétée acide riche en cystéine), TGFBI (facteur de croissance transformant β-induit), EFEMP2 (protéine de matrice extracellulaire de type fibuline contenant de l'EGF 2), LUM (lumicane), SNN (stannine), SPP1 (phospho-protéine sécrétée 1), CSPG2 (protéoglycane à chondroïtine-sulfate 2), ASAH1 (N-acyl-sphingosine amidohydrolase), PRSS11 (protéase à sérine 11), SFRP2 (protéine apparentée à Frizzled sécrétée 2), PLA2G12B (phospholipase A2, groupe XII-B), SPON2 (protéine de matrice extracellulaire spondine 2), OLFM1 (olfactomédine 1), TSRC1 (thrombospondine à motifs répétés 1), THBS2 (thrombospondine 2), adlicane, CST2 (cystatine SA), CST1 (cystatine SN), LOXL2 (enzyme de type lysyl-oxydase 2), TG (thyroglobuline), TGFB1 (facteur de croissance transformant β1), SERPINH1 (inhibiteur de protéase à sérine ou à cystéine, clade H, membre 1), SERPINB5 (inhibiteur de protéase à sérine ou à cystéine, clade B, membre 5), MMP2 (métalloprotéase de matrice 2), PCSK5 (proprotéine convertase de type subtilisine/kexine 5), HAPLN4

(protéine de liaison entre hyaluronane et protéoglycane 4), CA19-9, CA72-4, pepsinogène et CEA ;
iii) et/ou les marqueurs GTM testés comprennent MUC5AC, MUC17, ZG16, cystatine SN, SERPINH1 et SERPINB5.

4. Procédé conforme à l'une des revendications 1 à 3, dans lequel

i) on réalise ladite étape de détermination en déterminant le niveau d'une protéine GTM,
ii) ou l'on réalise ladite étape de détermination en déterminant le niveau d'un peptide GTM.

5. Procédé conforme à la revendication 4, dans lequel on réalise ladite étape de détermination en utilisant un anticorps dirigé contre ledit ou ladite GTM, étant entendu que cet anticorps, en option, est un anticorps monoclonal, ou un sérum polyclonal.

6. Procédé conforme à la revendication 4 ou 5, dans lequel on réalise ladite étape de détermination en utilisant un procédé par immuno-test de type sandwich ou en utilisant une puce à anticorps.

7. Utilisation d'un anticorps spécifique de la protéine ZG16 (protéine de granules de zymogène 16) humaine pour détecter un cancer de l'estomac en travaillant sur un échantillon de sang, de plasma ou de sérum.

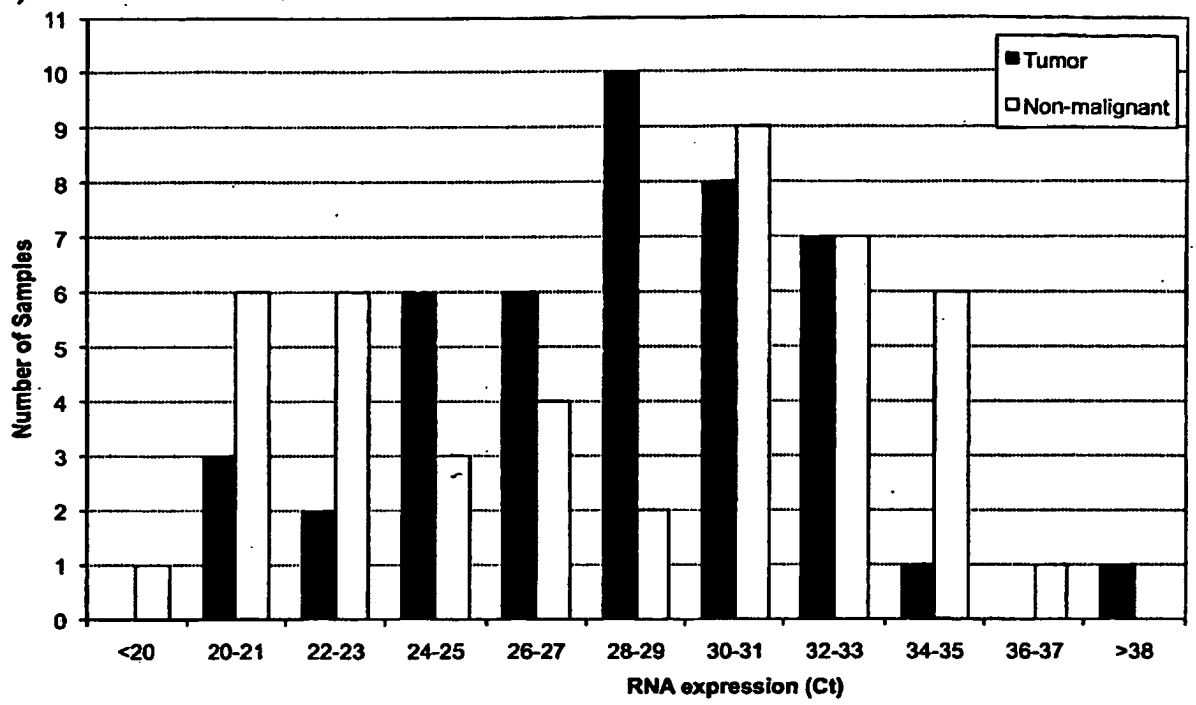
name	symbol	MWG oligo	Unigene mRNA ref sequence	Protein ref sequence	Rank intensity (tumor)	Rank Intensity (non-malignant)
Mucin 5, subtypes A and C	MUC5AC	30K#B: 7561	NM_017511	NP_059981	404	328
Mucin 17, cell surface associated	MUC17	30K#C: 0346	NM_001040105	NP_001035 194	2043	1485
Zymogen granule protein 16	ZG16	30K#C: 0156	NM_152338	NP_689551	3606	3249
carcinoembryonic antigen-related cell adhesion molecule 5	CEACAM5	30K#B: 5440	NM_004363	NP_004354	4873	7668

Figure 1

Gastric cancer patients		Controls	
Stage T1	2	Colorectal cancer Stage T2	1
Stage T2	15	Colorectal cancer Stage T3	9
Stage T3	13	Colorectal cancer Stage T4	1
Stage T4	1	Colorectal cancer Post-surgery	7
Gastrointestinal stromal tumours	2	Non-malignant disease	10
		Healthy	13
TOTAL	**33**	**TOTAL**	**41**

Figure 2

(a)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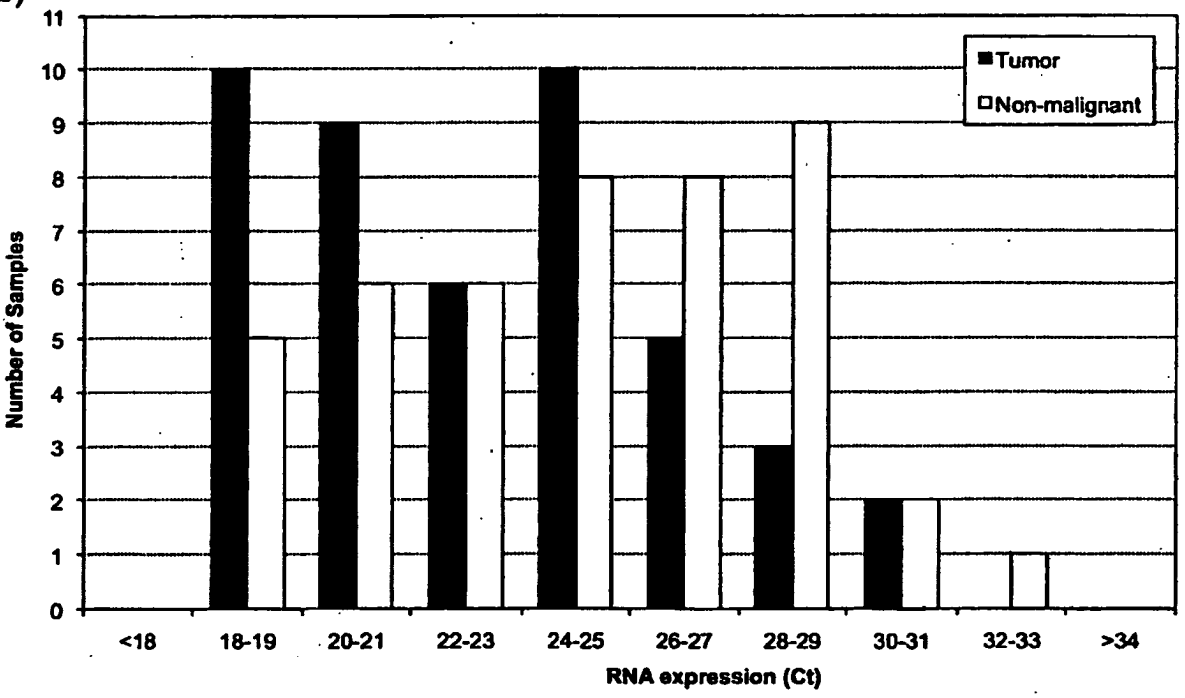
RNA expression (Ct)

(b)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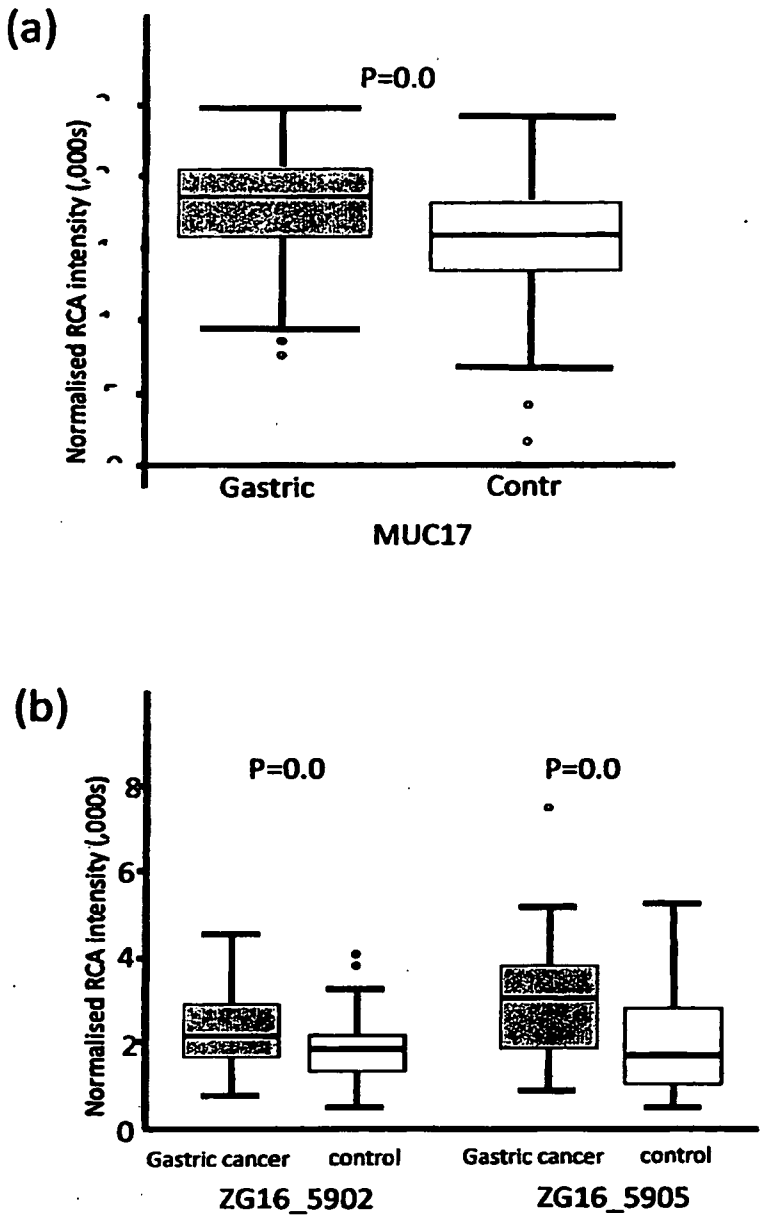
RNA expression (Ct)

Figure 3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P=0.0
Normalised RCA intensity (,000s)
Gastric
Contr
MUC17
(b)
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Normalised RCA intensity (,000s)
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2
4
6
8
Gastric cancer
control
Gastric cancer
control
ZG16_5902
ZG16_5905

Figure 4

REFERENCES CITED IN THE DESCRIPTION

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专利名称(译)	用于检测胃癌的标记物		
公开(公告)号	EP2430193B1	公开(公告)日	2016-03-09
申请号	EP2010775152	申请日	2010-05-14
[标]申请(专利权)人(译)	环太平洋生物技术有限公司		
申请(专利权)人(译)	PACIFIC EDGE生物科技有限公司		
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发明人	GUILFORD, PARRY JOHN		
IPC分类号	C12Q1/68 G01N33/53 G01N33/50 G01N33/68		
CPC分类号	G01N33/57446 C12Q1/6886 C12Q2600/158 C12Q2600/16 G01N2333/47 G01N2333/4725 G16B25/00		
优先权	577012 2009-05-15 NZ		
其他公开文献	EP2430193A4 EP2430193A1		
外部链接	Espacenet		

摘要(译)

肿瘤的早期检测是患有肿瘤（包括胃肿瘤）的患者存活的主要决定因素。 GTM基因家族的成员可以在胃肿瘤组织中差异表达，因此可以用作检测胃癌和其他类型癌症的标志物。本发明提供了用于检测肿瘤的新型GTM，包括胃肿瘤，特别是人酶原颗粒蛋白16（ZG16）。 GTM可以单独使用或与其他已知的GTM一起使用，以提供用于检测肿瘤（包括胃肿瘤）的新特征。