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(54) **SECRETED AND CELL SURFACE GENES EXPRESSED IN BENIGN AND MALIGNANT COLORECTAL TUMORS**

SEZERNIERTE UND ZELLOBERFLÄCHENGENE, DIE IN GUTARTIGEN UND BÖSARTIGEN KOLOREKTALTUMOREN EXPRIMIERT WERDEN

GENES SECRETES ET GENES DE SURFACE CELLULAIRE EXPRIMES DANS DES TUMEURS BENIGNES ET MALIGNES DU COLON ET DU RECTUM

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Description

Technical Field of the Invention

[0001] The invention relates to the early detection of colorectal adenoma and carcinoma. In particular it relates to the detection of secreted or cell surface markers in easily collectible bodily samples.

Background of the Art

[0002] Colorectal cancer is the second leading cause of cancer death in the United States, with ~130,000 patients diagnosed each year and ~50,000 ultimately succumbing to the disease (1). Most colorectal cancers develop slowly, beginning as small benign colorectal adenomas which progress over several decades to larger and more dysplastic lesions which eventually become malignant. This gradual progression provides multiple opportunities for prevention and intervention. Indeed, benign adenomas can be detected and removed by simple colonoscopy and polypectomy, precluding the need for radical surgical and adjuvant treatments. It is therefore believed that early detection and removal of these benign neoplasms provides the best hope for minimizing morbidity and mortality from colorectal cancer. Various screening methods for detecting early colorectal tumors are available, such as fecal occult blood testing, sigmoidoscopy, and colonoscopy (reviewed in 2). However, none of these methods are optimal, and new approaches are needed.

BRIEF SUMMARY OF THE INVENTION

[0003] In a first embodiment a method is provided for detection of benign colorectal adenomas in presymptomatic patients, comprising the steps of:

isolating an mRNA sample from feces of a subject;
detecting renal dipeptidase mRNA in said mRNA sample;
comparing the amount of renal dipeptidase mRNA in said mRNA sample to amounts of renal dipeptidase mRNA in normal subjects, wherein an elevated amount of renal dipeptidase mRNA in said mRNA sample is an indicator of benign colorectal adenoma in the subject.

[0004] According to another embodiment of the invention a method is provided for detection of benign colorectal adenomas in presymptomatic patients, comprising the steps of:

isolating epithelial cells from blood of a subject;
isolating an mRNA sample from the epithelial cells;
detecting renal dipeptidase mRNA in said mRNA sample;
comparing the amount of renal dipeptidase RNA in said mRNA sample to amounts of renal dipeptidase mRNA in normal subjects, wherein an elevated amount of renal dipeptidase mRNA in said mRNA sample is an indicator of benign colorectal adenoma in the subject.

[0005] A third embodiment of the invention provides a method for detection of benign colorectal adenoma in a subject that has been administered an antibody which specifically binds to renal dipeptidase and wherein the antibody is labelled with a moiety which is detectable from outside of the subject, comprising:

detecting the moiety in the subject from outside of the subject, where an area of localization of the moiety within the subject but outside the proximal tubules of the kidney identifies benign colorectal adenoma.

[0006] According to another embodiment the invention provides a method for detection of benign colorectal adenomas in a subject that has been administered an inhibitor of renal dipeptidase wherein the inhibitor is labeled with a moiety which is detectable from outside the subjection, comprising detecting the moiety in the subject from outside of the subject, wherein an area of localization of the moiety within the subject but outside the proximal tubules of the kidney identifies benign colorectal adenoma.

[0007] Another embodiment of the invention provides a method for detection of benign colorectal adenomas in presymptomatic patients, comprising the steps of:

detecting renal dipeptidase in blood of a subject; and
comparing amount of renal dipeptidase in blood of the subject to the amount of renal dipeptidase in normal subjects, wherein an elevated amount of renal dipeptidase in the blood of the subject is an indicator of benign colorectal

adenoma in the subject.

[0008] Still another embodiment of the invention is a method for detection of benign colorectal adenomas in presymptomatic patients, comprising the steps of:

detecting renal dipeptidase in feces of a subject; and
comparing amount of renal dipeptidase in feces of the subject to the amount of renal dipeptidase in normal subjects, wherein an elevated amount of renal dipeptidase in the feces of the subject is an indicator of benign colorectal adenoma in the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] Fig. 1.A, Distribution of the fold changes of differentially expressed transcript tags. Transcripts in which the significance criterion was met ($p<0.05$, a total of 957 tags) in the comparisons between normal and adenoma or normal and cancer are plotted in the figure. The ratios of adenoma to normal and cancer to normal were plotted on a log scale. The shaded box in (Fig. 1.*A*) and enlarged in (Fig. 1.*B*) encloses the transcript tags detailed in Table 3. The two unlabeled dots correspond to tags whose differential expression could not be confirmed by quantitative PCR suggesting that the tags were derived from different transcripts than the ones indicated in Table 3.

[0010] Fig. 2. Quantitative PCR analysis of genes elevated in both adenomas and cancers. Quantitation of expression of genes in tumors and matched normal tissues from five patients (Pt) are shown as fold elevation over that in matched normal colonic mucosa. Each bar represents the average of three independent measurements. *TGFBI, LYS, RDP, MIC-1, REGA,* and *DEHL* are as described in Table 3.

[0011] Fig. 3. Quantitative PCR analysis of genes decreased in both adenomas and cancers. Quantitation of expression of genes in tumors and matched normal tissue from five patients (Pt) are shown as a fraction of matched normal. Each bar represents the average of three independent measurements. *CA2* and *DRA* are described in Table 4. Dual Specificity Phosphatase *(DUSP1),* and Acid Sphingomylenase-like phosphodiesterase *(ASML3a)* represented transcripts that were repressed but did not meet the stringent criteria required for inclusion in Table 4. SAGE data indicated that *DUSP1* was 5- and 76-fold repressed in adenomas and cancers, respectively. *ASML3a* was 15-fold repressed in both adenoma and cancer.

[0012] Fig. 4. Quantitative PCR analysis of mRNA from purified epithelial cells of genes elevated in both adenomas and cancers. Quantitation of expression of genes in the purified normal (N) or cancer (Ca) epithelial cells taken from two patients are shown as fold elevation over matched normal. Genes examined were the same as in Fig. 2.

[0013] Fig. 5A - Fig. 5E. *In-situ* hybridization analyses of elevated genes. Genes examined were *REGA* (Fig. 5A), TGFBI (Fig. 5B), *LYS* (Fig. 5C), *RDP* (Fig. 5D), and *MIC-1* (Fig. 5E). Positive cells appear red, arrows point to clusters of malignant epithelial cells, and arrow heads point to macrophages.

[0014] Fig. 6. Inhibitors of renal dipeptidase demonstrate inhibition constants ranging from 0.6 nM to 19.5 nM.

[0015] Fig. 7. A comparison of the inhibitors shown in Fig. 6. compares the inhibition rate as a function of concentration of inhibitor.

[0016] Fig. 8. Substrates of renal dipeptidase are shown.

[0017] Fig. 9 shows the difference in activity of renal dipeptidase found in adenomas, cancer, and metastases compared to normal colonic tissue.

DETAILED DESCRIPTION OF THE INVENTION

[0018] It is a finding of the present invention that particular genes are aberrantly and consistently expressed in both adenomas and carcinomas of the colon. Products of such genes provide cellular and serum markers for colorectal neoplasia. The ideal tumor marker would be expected to have several characteristics. First, it should be expressed at high levels in tumors and at greatly reduced levels in normal tissues. Second the elevated expression should occur early and remain elevated during the neoplastic process. Third, such a marker should be elevated in the majority of clinical samples. Fourth, the marker should be cell surface or secreted to facilitate its detection. We have identified Renal Dipeptidase as being a marker meeting all of these criteria and may therefore be especially useful as diagnostic tools for the early detection of benign colorectal adenoma in presymptomatic patients.

[0019] The marker can be found and detected in whole blood, serum, plasma, or fractions thereof. These are collectively referred to as "blood" herein. The marker can also be found in stool. Samples for testing can be feces or processed or fractionated feces. All such samples are referred to herein as "feces."

[0020] Inhibitors of Renal Dipeptidase can be used as affinity reagents for labeling. Preferably the inhibitors are those which bind irreversibly. Alternatively they are ones which bind and release, but release at a slow rate. Inhibitors with suitably slow release rates are those which have a binding half-life of greater than 30 minutes, or 1, 2, 3, 5, 8, or 10

hours. Many inhibitors of Renal Dipeptidase are known, including the commercially available Cilastatin, and phosphinic acid inhibitors. See Parsons et al., "A new class of potent, slowly reversibly dehydropeptidase inhibitors, "Biochemistry International, vol. 23, pp. 1107-1115, 1991. Inhibitors which covalently bind to and/or modify Renal Dipeptidase are also known and can be used. See Wu and Mobashery, "Targeting renal dipeptidase (dehydropeptidase I) for inactivation by mechanism-based inactivators, "J. Med. Chem., vol. 34, pp. 1914-1916,1991. Some inhibitors mimic transition states between substrates and product. Some useful inhibitors are shown in Fig. 6. These include inhibitors having halogen substitutions. Such inhibitors can be readily made using radioactive halogens for ready labeling of renal dipeptidase and easy detection. Inhibitors can be labeled using any detectable moiety known in the art, including but not limited to fluors and radioactive atoms.

[0021] RNA for the marker can be detected using any of the known techniques in the art. Preferably an amplification step will be used, because the amount of RNA for the marker is expected to be very small from the sources contemplated. Suitable techniques include RT-PCR, hybridization of copy mRNA (cRNA) to an array of nucleic acid probes, and Northern blotting.

[0022] Protein forms of the marker can be detected using any techniques known in the art. These include activity assays, immunological assays, binding to specific ligands, etc. Particularly suitable assays for Renal Dipeptidase include using L-L amino acid dipeptide substrates and L-D amino acid dipeptide substrates. Substrates which can be used for assaying renal dipeptidase are shown in Fig. 8, and include the generic structures for dipeptides and dehydrodipeptides. ε (DNP) -L-Lysine-D-Amp can also be used as a substrate, yielding a colored product. Such substrates can be labeled with detectable moieties, including but not limited to fluors and radioactive atoms. One particularly useful labeling scheme employs a substrate which is labeled with two moieties on opposite sides of the substrate cleavage site. One of the moieties is fluorescent and one of the moieties is a quencher. When the two moieties are close, as in an intact substrate, the fluorescence of the fluorescent moiety is quenched. Upon cleavage the quenching is released and an increase in fluorescence is observed.

[0023] As mentioned above, inhibitors can also be labeled and used for detecting the suitable marker. In addition, antibodies can be used to label protein forms of the marker. The antibodies can be labeled as is known in the art. Suitable radioactive atoms for use in labeling inhibitors, substrates, and antibodies include In-111, I-123, Tc-99m, Re-186, Re-188, Ga-67, Ga-68, T1-201, Fe-52, Pb-203, Co-58, Cu-64, I-124, I-125, I-131, At-210, Br-76, Br-77, and F-18 and others known in the art for such purposes. Contrast enhancement agents can also be attached to the substrates, inhibitors, or antibodies. Such agents include gadolinium. Moreover, imaging techniques can be used to detect such labels within the body. An example of an imaging technique which can be used is spiral computer tomography. For this technique, the detecting agent, such as inhibitor or antibody can be linked to a contrast enhancing agent.

[0024] Other detection means that can be used include gamma cameras, magnetic resonance imaging, planar scintigraphic imaging, SPECT imaging, PET imaging, and ultrasound imaging. Thus the marker can be detected both *in situ* in the body or *in vitro* in an isolated body sample.

[0025] Epithelial cells can be isolated from blood or other tissue samples to enrich for the marker or the mRNAs. Epithelial cells can be isolated, *inter alia,* by immunoaffinity techniques. Such a technique is described in more detail below.

[0026] Substrates of the marker can be administered to subjects and the reaction products measured in body samples. Inhibitors can be administered to subjects and the subject can be imaged to detect the inhibitor bound to the marker. Typical modes of administration of such agents can be any which is suitable, including but not limited to per os, intravenous, intramuscular, intraarterial, subdermal, transdermal, and rectal.

[0027] A high background of the marker may obscure detection of increased expression. In such a situation, one can use tumor-specific glycoforms as a means of distinguishing between the background marker and the marker that is due to the tumor. Tumor-specific glycoforms of Renal Dipeptidase bind to LPHA, an L lectin from Phaseolus vulgaris hemagglutinin, and thus can be distinguished on that basis. Other lectins such as with similar specificity for tumor-specific glycoforms, such as Sambucus Nigra Lectin isolated from Sambucus nigra (elderberry) bark can be used as well.

[0028] Normal subjects are used as a comparison to the test subjects to determine whether the amounts of the marker observed in the feces or blood are elevated. Preferably the normal subjects have been confirmed as tumor-free by colonoscopy. More preferably several samples are pooled or their individual values are averaged to arrive at a normal value.

[0029] Some of the most highly overexpressed genes found in colorectal adenomas and colorectal cancers are discussed below. Regenerating Islet Derived Pancreatic Stone Protein, encoded by the *REGA* gene, is a secreted polypeptide first found in pancreatic precipitates and stones from patients suffering from chronic pancreatitis (7). The cDNA encoding this protein was isolated from a random screen of genes highly expressed in a regenerating-islet derived cDNA library (8) and subsequently shown to be elevated in colorectal cancers (9). More recently, *REGA* was isolated in a hybridization-based screen for genes elevated in colorectal cancers and shown to be elevated in many colorectal adenocarcinomas (10). Consistent with these published observations, we observed a strong elevation in expression of *REGA* in unpurified tumors, and a similar elevation in one purified tumor. *In situ* hybridization experiments demonstrated *REGA* to be strongly expressed in the epithelial cells of the tumors, with no expression evident in the stroma (Fig. 5A).

[0030] TGFB-induced gene *(TGFBI)* encodes a small polypeptide of unknown function initially isolated through a differential display screen for genes induced in response to treatment with TGF β (11). The protein is expressed in the keratinocytes of the cornea (12) and, interestingly, germline mutations of this gene cause familial corneal dystrophies (13). *TGFBI* was previously shown to be among the most significantly elevated genes in colorectal cancers (4), and our new data show that it is expressed at high levels in adenomas as well. Quantitative PCR results demonstrated strong elevation both in unpurified tumors and purified tumor epithelial cells. Accordingly, *in situ* hybridization experiments revealed *TGFBI* to be expressed in many cell types, in both the stromal and epithelial compartments (Fig. 5B).

[0031] Lysozyme *(LYS,* 1,4-β-N-acetylmuramidase, EC 3.2.1.17) is an enzyme with bacteriolytic activity (14) capable of cleaving β-1,4 glycosidic bonds found in the cell walls of gram-positive bacteria. The enzyme is expressed in the secretory granules of monocytes, macrophages and leukocytes, as well as in the Paneth cells of the gastrointestinal tract. Fecal lysozyme levels are dramatically elevated in patients with inflammatory bowel disease (15, 16), and serum lysozyme activity is significantly elevated in patients with sarcoidosis (17), both of which are diseases characterized by aberrant chronic inflammation. Furthermore, lysozyme immunoreactivity has been observed in the epithelial cells of both adenomas and carcinomas of the large intestine (18). In our study, the degree of elevation of expression of *LYS* varied from 4-fold to 55-fold in the unpurified samples. In contrast, the degree of elevation of expression of *LYS* observed in purified epithelial cells was only 2-5 fold. This suggested that a substantial portion of the expression for this gene in the tumors could have been derived from non-epithelial cells. Consistent with this hypothesis, *in situ* hybridization experiments revealed that the majority of *LYS* mRNA was present in a stromal component that appeared to be macrophages (Fig. 5C). The expression of *LYS* in the macrophage compartment of colorectal tumors was also supported by its high representation in a SAGE library constructed from hematopoietic cells (CD45+, CD64+, CD14+) purified from colorectal tumors (602 *LYS* tags/56,643 total tags) (6).

[0032] The gene identified in the current study is renal dipeptidase (*RDP*). *RDP* is a GPI-anchored enzyme whose major site of expression is the epithelial cells of the proximal tubules of the kidney (reviewed in (19)). The enzyme has been extensively analyzed with respect to its catalytic mechanism and inhibition kinetics by a variety of synthetic inhibitors. *RDP* is unique among the dipeptidases in that it can cleave amide bonds in which the C-terminal partner is a D amino acid, providing excellent opportunity for the development of specific probes for its detection *in vivo.* Quantitative PCR revealed *RDP* to be markedly elevated in both unpurified and purified tumor epithelial cells, and *in situ* hybridization experiments showed that *RDP* was exclusively localized to epithelial cells of colorectal tumors (Fig. 5D).

[0033] Macrophage Inhibitory Cytokine (MIC-1) is a small polypeptide of 16 kDa first isolated from a differential screen for genes that were induced upon macrophage activation (20). Concurrently, it was identified in the IMAGE database by a search for molecules homologous to the Bone Morphogenic Protein/TGF β family of growth and differentiation factors (21). In addition to being highly expressed in activated macrophages, *MIC-1* has been noted to be highly expressed in placenta and the epithelial cells of normal prostate. In the current study, we found *MIC-1* expression to be elevated between 7 and 133 fold in the unpurified tumors. As observed for *LYS,* the purified tumor cells demonstrated significant but less elevation of expression of *MIC-1* (5 to 7-fold) indirectly implicating stromal expression to be partly responsible for the dramatic elevation seen in some tumors. Consistent with this hypothesis, *in situ* hybridization experiments revealed expression in both the epithelium of the tumor, and in a cell type resembling infiltrating macrophages (Fig. 5E).

Examples

Example 1: SAGE

[0034] In an effort to identify potential molecular markers of early colorectal tumors, we have here analyzed gene expression in benign and malignant colorectal tumors in an unbiased and comprehensive fashion. We used SAGE to analyze global gene expression in normal, benign and malignant colorectal tissue. SAGE is a gene expression profiling method that associates individual mRNA transcripts with 15-base tags derived from specific positions near their 3' termini (3). The abundance of each tag provides a quantitative measure of the transcript level present within the mRNA population studied. SAGE is not dependent on pre-existing databases of expressed genes, and therefore provides an unbiased view of gene expression profiles. For the current study, SAGE libraries derived from two samples of normal colonic epithelium, two colorectal adenomas, and two colorectal cancers were analyzed. These libraries contained a combined total of 290,394 transcript tags representing 21,343 different transcripts (Table 1).

Tables 1-Summary of SAGE data

SAGE Library	Total number of tags observed	Number of different transcripts observed *
Normal Colorectal Epithelium		
NC-1	49,610	9,359

(continued)

SAGE Library	Total number of tags observed	Number of different transcripts observed *
NC-2	48,479	9,610
Adenomas		
Ad-A	52,573	11,167
Ad-B	42,661	9,483
Cancers		
Tu-98	41,371	9,780
Tu-102	55,700	11,039
Total	**290,394**	**21,343**

* To minimize the effect of potential sequencing errors, only tags observed more than once in a given SAGE library were counted to give a conservative estimate of the minimum number of different transcripts analyzed.

[0035] Two comparisons were performed, one between the adenoma and normal samples, and one between the cancer and normal samples. These comparisons revealed 957 transcript tags that were differentially expressed more than 2-fold between normal and tumor tissue (Table 2). A comparison of the fold change in adenomas versus cancers revealed that many transcripts were similarly elevated or repressed in both adenomas and cancers although the magnitude often varied (Fig. 1A). Indeed the majority (79%) of comparisons were in quadrants of the plot indicative of concordant elevation.

Table 2 - *Differentially expressed transcripts in benign and malignant tumor colorectal tissue*

Fold change in expression	Elevated in adenomas[a]	Elevated in cancers[a]	Elevated in both adenomas and cancer[a]	Repressed in adenomas[b]	Repressed in cancers[b]	Repressed in both adenomas and cancers[b]	Total transcripts differentially expressed
2	346	170	50	313	380	192	957
4	263	119	23	225	270	117	735
10	160	79	10	134	157	58	462
20	49	40	9	72	52	23	181

[a]Elevated transcripts showed a significantly different ($P<0.05$) tag count between normal and tumor tissue, were expressed in both tumor tissues analyzed, and had an expression level that was higher in the tumors than in the normals by the fold indicated in column one. For the purposes of calculation, 0.5 was substituted for the denominator when no tags were detected in the normal samples.

[b]Repressed transcripts showed a significantly different ($P<0.05$) tag count between normal and tumor tissue, were expressed in both normal tissues analyzed and had an expression level that was lower in the tumors than in the normals by the fold indicated in column one.

[0036] From both practical and biological perspectives, those changes showing the greatest magnitude were deemed the most interesting. In this regard, 49 tags were identified to be elevated by ≥20-fold in the adenomas and 40 were elevated by ≥20-fold in the cancers (Table 2). Conversely, there were 72 transcripts that were decreased by ≥20-fold in adenomas and 52 decreased by ≥20-fold in the cancers (Table 2).

[0037] There were nine transcripts that were elevated by ≥20-fold in both adenomas and cancers (Fig. 1B and Table 3) and 23 that were repressed by ≥20-fold (Table 4). We were especially interested in genes whose products were predicted to be secreted or displayed on the cell surface, as these would be particularly suitable for the development of serologic or imaging tests for presymptomatic neoplasia, respectively. We were able to identify six such genes *(TGFBI, LYS, RDP, MIC-1, REGA* and *DEHL)* from among those whose transcript tags were elevated in both adenoma and carcinoma SAGE libraries.

Table 3. Transcripts most elevated in adenomas and cancers[a]

	Normal		Adenomas		Cancers		
Tag Sequence	NC-1	NC-2	AD-A1	AD-B2	Tu-98	Tu-102	Transcript name
ATGTAAAAAA	**0**	**0**	**26**	**32**	**2**	**12**	**Lysozyme (LYS)**
TAATTTTTGC	**0**	**1**	**99**	**12**	**20**	**37**	**Differentially Expressed in Hematopoietic Lineages *(DEHL)***
GTGTGTTTGT	**0**	**0**	**17**	**29**	**17**	**15**	**Transforming Growth Factor, Beta-Induced *(TGFBI)***
GTGCTCATTC	**0**	**0**	**13**	**7**	**2**	**10**	**Macrophage Inhibitory Cytokine, 1 *(MIC-1)***
TTCCAGCTGC	0	0	7	6	2	9	Adaptor-related Protein Complex 2, alpha 2 subunit[b]
ACCATTGGAT	0	0	3	10	3	9	Interferon Induced Transmembrane Protein 1 (9-27)[b]
TTTCCACTAA	**0**	**0**	**8**	**4**	**4**	**6**	**Regenerating Islet-Derived 1 alpha *(REGA)***
CAAGGACCAG	**0**	**0**	**5**	**6**	**10**	**12**	**Renal Dipeptidase, (*RDP*)**
AGGACCATCG	0	0	8	2	1	18	Defensin, Alpha 5, Paneth cell-specific[c]

[a] These tags displayed at least twenty fold elevation in both neoplastic states. The numbers given are the raw tag counts for each tag observed in each library. Transcript name provides a description of matching UniGene cluster (Build 3/13/01). Rows shown in bold are genes confirmed by quantitative PCR to be differentially expressed.
[b]Differential expression could not be confirmed by quantitative PCR suggesting that the tag was derived from a different transcript than the one indicated.
[c] Not tested.

Table 4. Transcripts most repressed in adenomas and cancers[a]

	Normal		Adenoma		Cancer			
Tag Sequence	NC-1	NC2	AD-A1	AD-B	Tu-981	Tu-102	UNI ID	Transcript name
GTCATCACCA	35	22	0	0	0	0	32966	Guanylate Cyclase Activator 2B
CCTTCAAATC	29	17	0	0	1	0	23118	Carbonic Anhydrase I
TCTGAATTAT	24	16	0	0	1	0	50964	Carcinoembryonic Antigen-Related Cell Adhesion Molecule 1
TTATGGTGTG	11	17	0	0	0	0	271499	ESTs
CTGGCAAAGG	14	22	1	0	0	0	72789	hypothetical protein FLJ20217
AGGTGACTGG	10	14	0	0	0	0		No Match
CTTATGGTCC	36	11	0	1	1	0	179608	Retinol Dehydrogenase Homolog
ATGATGGCAC	12	32	1	0	1	0	84072	Transmembrane 4 Superfamily Member 3
GTCCGAGTGC	17	3	0	0	0	0	3337	Transmembrane 4 Superfamily Member 1
ATTTCAAGAT	**35**	**21**	**0**	**2**	**1**	**0**	**155097**	**Carbonic Anhydrase II *(CA2)***
CAAGAGTTTC	14	2	0	0	0	0	183617	ESTs
GCCATCCTCC	9	13	0	1	0	0		No Match
ACCCAACTGC	12	3	0	0	0	0	232604	Homo sapiens cDNA: FLJ22675 fis, clone HSI10553

(continued)

	Normal		Adenoma		Cancer			
Tag Sequence	NC-1	NC2	AD-A1	AD-B	Tu-981	Tu-102	UNI ID	Transcript name
GCCCACGTCA	7	8	0	0	0	0		No Match
TTTGGTTTCA	2	13	0	0	0	0		No Match
CTCAGAACTT	18	3	1	0	0	0	194710	N-acetylglucosaminyl transferase 3, mucin type
CCAACACCAG	9	19	1	0	0	1	181165	Eukaryotic Translation Elongation Factor I Alpha I
GCCACATACT	3	9	0	0	0	0	4984	KIAA0828 protein
GTATTGGGGC	5	7	0	0	0	0		No Match
CCGGCTTGAG	7	4	0	0	0	0	2722	Inositol 1,4,5-trisphosphate 3-Kinase A
GATATGTAAA	1	10	0	0	0	0	227059	Chloride Channel, Calcium Activated, Family Member 4
CATAGGTTTA	**66**	**39**	**4**	**1**	**5**	**0**	**1650**	**Solute Carrier Family 26, member 3 *(DRA)***
GTCCTGAACA	7	3	0	0	0	0	78546	ATPase, Ca++ Transporting, Plasma Membrane 1

[a] These tags displayed at least twenty fold decrease in both neoplastic states. The numbers given are the raw tag counts for each tag observed in each library. Transcript name provides a description of matching UniGene cluster (Build 3/13/01). Rows shown in bold are genes that were tested and confirmed by quantitative PCR to be differentially expressed.

[0038] SAGE. For the initial SAGE[3] of benign tumors, fresh adenomas were obtained from surgical specimens derived from FAP patients. Adenomas from FAP patients were employed because of the ready availability of small lesions and the certainty of inactivation of the APC pathway which initiates the formation of the majority of sporadic tumors. After histopathological verification of the neoplastic nature of the lesion (>70% neoplastic cells), total RNA was isolated by solubilizing the tissue in RNAgents Lysis Buffer (Promega, Madison, WI) followed by ultracentrifugation over a cesium chloride gradient. mRNA selection was performed from the purified total RNA using oligo(dT) cellulose (Life Technologies, Gaithersburg, MD). Two adenoma SAGE libraries were prepared as described (3, 4) and sequenced to a total depth of over 90,000 transcript tags. For SAGE of normal and malignant tissues, four previously described normal (NC-1 and NC-2) and primary cancer (Tu-98 and Tu-102) SAGE libraries were employed (4). In collaboration with the Cancer Genome Anatomy Project (CGAP) (5), the analyses of these libraries was extended from a total of 123,046 transcripts in the previously published work to 195,160 transcripts in the current work. Tags were extracted from the raw sequence data and, after excluding repeated ditags, linker sequences, and tags from the polymorphic Major Histocompatibility loci, the resulting tag libraries were compared and statistical analysis performed using SAGE software, version 4.0. Data from the libraries are publicly available at the Uniform Resource Locator (URL) address for the http file type found on the www host server that has a domain name of ncbi.nlm.nih.gov, and a path to the directory SAGE, and detailed SAGE protocols are available at the Uniform Resource Locator (URL) address for the http file type found on the www host server that has a domain name of sagenet.org, and file name of sage_protocol.htm.

Example 2: RT-PCR

[0039] To verify the increased expression of these six genes, we used quantitative RT-PCR techniques to analyze the expression in seven colorectal neoplasms (three sporadic adenomas and four sporadic cancers) and matched normal colonic mucosa. For these assays, specific primers were developed that resulted in amplification from cDNA but not genomic DNA. Controls were provided by similar quantitative PCR assays of a gene whose expression was found to be very similar in the SAGE libraries of normal and neoplastic colon (β-amyloid precursor protein). The quantitative PCR experiments verified that five of the six selected genes *(TGFBI, LYS, RDP, MIC-1, REGA)* were expressed at significantly

higher levels in every neoplastic sample analyzed compared to patient-matched normal mucosa (Fig. 2). Several tumors exhibited ≥20-fold higher levels of the studied transcripts compared to their patient-matched normal colonic mucosa, as predicted by SAGE. Another control was provided by the quantitative PCR analysis of four genes whose expression was observed to be reduced in the SAGE libraries prepared from adenomas and cancers compared to those from normal colonic mucosa. As shown in Fig. 3, the quantitative PCR confirmed the lower levels of expression of each of these genes, emphasizing that the dramatic elevations in expression observed in Fig. 2 represented gene-specific phenomena.

[0040] Quantitative PCR. Tumors were collected, snap frozen, and stored at -80°C. They were verified to be predominantly composed of neoplastic cells by histopathological analysis. mRNA was isolated from tumors, and patient-matched normal colonic mucosa using QuickPrep reagents (Amersham Pharmacia Biotech UK, Buckinghamshire, England), and single-stranded cDNA was synthesized using Superscript II (Life Technologies, Gaithersburg, MD). Quantitative PCR was performed using an iCycler (Bio-Rad, Hercules, CA), and threshold cycle numbers determined using iCycler software, version 2.1. Reactions were performed in triplicate and threshold cycle numbers averaged. All genes examined were normalized to a control gene (β-amyloid precursor protein, shown by SAGE to be expressed at equivalent levels in all colorectal samples), and fold induction calculated according to the formula $2^{(Rt-Et)}/2^{(Rn-En)}$ where Rt is the threshold cycle number for the Reference gene observed in the tumor, Et is the threshold cycle number for the Experimental gene observed in the tumor, Rn is the threshold cycle number for the Reference gene observed in the normal, and En is the threshold cycle number for the Experimental gene observed in the normal. The primers used for quantitative PCR were obtained from GeneLink (Hawthorne, NY), and their sequences are available upon request.

Example 3: Expression in isolated epithelial cells

[0041] The quantitative PCR data obtained from mRNA isolated from whole tumors provided independent evidence that SAGE provided an accurate indication of gene expression changes in colorectal neoplasia. However, neither analysis identified the cell types responsible for the increased expression. Non-neoplastic stromal cells within tumors may be considerably different than those in normal colonic mucosa (6), and the epithelial derivation of gene expression differences cannot reliably be concluded without direct supporting evidence. We therefore sought to determine if the epithelial cells of cancers express elevated levels of the six genes depicted in Fig. 2. First, we affinity-purified cancerous and patient-matched normal epithelial cells from fresh surgical specimens using immunomagnetic beads directed to the pan epithelial marker Ber-EP4, prepared cDNA and performed quantitative PCR analysis to determine the expression levels of the elevated genes as above. Elevated expression was observed in the purified tumor epithelial cells for each of the six genes examined (Fig. 4), demonstrating that at least some of the increased expression was derived from epithelial cells. However, relative expression of *LYS* was not as prominent or reproducible in the purified epithelial cells as in the mRNA from the unfractionated tumors, suggesting that other cell types might have contributed transcripts from this gene.

[0042] Epithelial cell immunoaffinity purification. Tumor epithelial cells were purified using a modification of the procedure previously developed for the isolation of tumor endothelial cells (6). In brief, fresh surgical specimens of tumor and matched normal tissue were obtained and digested with collagenase and the resulting material filtered through a nylon mesh to obtain single cell suspensions. The cells were then bound to a mixture of anti-CD14 and anti-CD45 immunomagnetic beads (Dynal, Oslo, Norway) to deplete the population of hematopoetic cells (negative selection). The remaining cell suspension was then incubated with anti-Ber-EP4 immunomagnetic beads to isolate epithelial cells (positive selection). Purified cells were lysed directly on the beads and mRNA purified using QuickPrep reagents (Amersham Pharmacia Biotech UK, Buckinghamshire, England).

Example 4: *In situ* hybridization in multiple tumors

[0043] We performed *in situ* hybridization to RNA in frozen sections of tumors for five of the genes showing the most consistent elevation. *DEHL* was found to be elevated in only five of the nine tumors examined and was not investigated further. To increase the sensitivity of detection, we generated several RNA probes for each tested gene using *in vitro* transcription techniques. The results obtained are discussed below in conjunction with brief overviews of each of the five genes of interest.

[0044] *In situ* Hybridization. Non-radioactive *in situ* hybridization was performed as described (6). For each gene analyzed, a cocktail of anti-sense probes made through *in vitro* transcription were employed to increase sensitivity. The primers used to generate templates for the synthesis of the *in situ* riboprobes were obtained from GeneLink (Hawthorne, NY), and their sequences are available upon request.

[0045] The results summarized above show that although a large number of tags are observed in the colorectal tissues analyzed, only a small fraction (957/21,343, <5%) were expressed differentially in benign or malignant neoplastic tissues. A similarly small fraction of genes (66/4000, 1.7%) were found to be aberrantly expressed in colorectal neoplasms using oligonucleotide arrays (22). Analysis of these differentially expressed genes not only has the potential to provide insights into the biology of human neoplasia but also may have clinically useful applications. One of the most exciting potential

applications concerns the identification of genes whose products provide cellular and serum markers for colorectal neoplasia. In the current study, we identified several genes that appeared to meet all of these criteria and may therefore be especially useful as diagnostic tools for the early detection of presymptomatic colorectal neoplasia. Indeed, the product of one of these genes (MIC-1), has recently been found to be elevated in the serum of patients with colorectal and other cancers, providing further validation of this approach (24).

TABLE 5

Tag_Sequence	NC1	NC2	AD1	AD2	CA1	CA2	UNI ID	Description
AAAAGAAACT	1	3	33	52	7	16	172182	poly(A)-binding protein, cytoplasmic 1
AACGAGGAAT	8	0	24	26	17	23		
AAGAAGATAC	6	6	28	25	21	34	184776	ribosomal protein L23a
AATAGGTCCA	12	9	32	36	22	22	113029	ribosomal protein S25
ACAACTCAAT	1	1	7	7	8	6	244125	EST
ACAACTCAAT	1	1	7	7	8	6	75922	brain protein 13
ACATCATCGA	10	18	50	66	34	46	182979	ribosomal protein L12
ACCATTGGAT	0	0	3	10	3	9	146360	interferon induced transmembrane protein 1 (9-27)
ACCTGTATCC	5	3	20	6	26	35	182241	interferon induced transmembrane protein 3 (1-8U)
ACTCCAAAAA	9	12	21	65	21	37	133230	ribosomal protein S15
AGCACCTCCA	37	37	108	81	57	108	75309	eukaryotic translation elongation factor 2
AGGACCATCG	0	0	8	2	1	18		
AGGGCTTCCA	26	41	74	108	50	85	29797	ribosomal protein L10
ATGGCTGGTA	18	46	79	75	81	136	182426	ribosomal protein S2
ATGTAAAAAA	0	0	26	32	2	12	178112	DNA segment, single copy probe LNS-CAI/LNS-CAII (deleted in polyposis
ATGTAAAAAA	0	0	26	32	2	12	234734	lysozyme (rena) amyloidosis)
ATGTAAAAAA	0	0	26	32	2	122	3715	Sjogren syndrome antigen B (autoantigen La)
ATTCTCCAGT	8	20	20	48	43	28	234518	ribosomal protein L23
CAAGGACCAG	0	0	5	6	10	12	109	dipeptidase 1 (renal)
CAATAAATGT	8	6	40	76	33	67	179779	ribosomal protein L37
CAGCTCACTG	4	17	9	35	21	24	158675	ribosomal protein L14
CATTTGTAAT	48	27	102	57	36	125		
CCTAGCTGGA	16	27	58	45	48	66	182937	peptidylprolyl isomerase A (cyclophilin A)
CCTTCGAGAT	6	12	13	29	7	41	76194	ribosomal protein S5
CTCCTCACCT	7	13	38	36	24	75	242908	lecithin-cholesterol acyltransferase
CTGACTTGTG	0	0	1	20	9	2	77961	major histocompatibility complex, class I, B
CTGGGTTAAT	14	24	34	83	42	112	126701	ribosomal protein S19
CTGTTGATTG	13	3	60	38	32	27	249495	heterogeneous nuclear ribonucleoprotein A1
CTGTTGGTGA	9	19	37	59	31	62	3463	ribosomal protein S23
GAAAAATGGT	7	12	49	47	25	27	181357	laminin receptor 1 (67kD, ribosomal protein SA)
GAGTCAGGAG	2	C	8	6	9	7	181271	CG1-120 protein
GCATAATAGG	11	16	22	54	50	21	184108	ribosomal protein L21 (gene or pseudogene)
GCATTTAAAT	1	2	10	18	12	7	261802.	eukaryotic translation elongation factor 1 beta 1

(continued)

Tag_Sequence	NC1	NC2	AD1	AD2	CA1	CA2	UNI ID	Description
GCATTTAAAT	1	2	10	18	12	7	275959	eukaryotic translation elongation factor 1 beta 2
GCATTTGACA	2	5	27	17	9	20	172129	Homo sapiens cDNA: FLJ21409 fis, clone COL03924
GCTTTTAAGG	2	8	14	32	16	17	8102	ribosomal protein S20
GGACCACTGA	18	39	76	57	48	83	119598	ribosomal protein L3
GGGGGTAACT	1	2	8	11	14	13	99969	fusion, derived from t(12;16) malignant liposarcoma
GTGCGCTGAG	0	0	75	0	20	18	277477	major histocompatibility complex, class I, C
GTGCTCATTC	0	0	13	7	2	10	116577	prostate differentiation factor
GTGCTCATTC	0	0	13	7	2	10	25945	ESTs
GTGTGTTTGT	0	0	17	29	17	15	118787	transforming growth factor, beta-induced, 68kD
GTTCGTGCCA	1	13	18	43	24	18	179606	nuclear RNA helicase, DECD variant of DEAD box family
GTTCGTGCCA	1	13	18	43	24	18	179666	uncharacterized hypothalamus protein HSMNP1
TAATAAAGGT	4	11	37	62	24	27	151604	ribosomal protein S8
TAATTTTTGC	0	1	99	12	20	37	273321	differentially expressed in hematopoietic lineages
TCACAAGCAA	10	7	17	21	13	38	146763	nascent-polypeptide-associated complex alpha polypeptide
TCAGATCTTT	14	32	37	108	31	87	75344	ribosomal protein S4, X-linked
TCCTGCCCCA	1	5	10	14	7	16	171814	parathymosin
TGAAATAAAA	0	2	2	14	13	11	173205	nucleophosmin (nucleolar phosphoprotein 823, numatrin) Human DNA sequence from clone RP5-1179L24 on chromosome 6q24.3-25.3. Contains the 3' end of the gene for a novel protein similar to mouse
TGAAATAAAA	0	2	2	14	13	11	192822	phospholipase C neighboring protein PNG, ESTs, STSs and GSSs
TGATGTCTGG	0	0	2	6	8	2	83883	transmembrane, prostate androgen induced MRNA
TGTAATCAAT	2	3	13	11	8	11	249495	heterogeneous nuclear ribonucleoprotein A1
TTACCATATC	10	5	22	30	26	22	300141	ribosomal protein L39,
TTATGGGATC	6	4	24	37	36	47	5662	guanine nucleotide binding protein (G protein), beta polypeptide 2-like 1
TTCAATAAAA	8	14	79	111	36	50	177592	ribosomal protein, large, P1
TTCCAGCTGC	0	0	7	6	2	9	12442	ESTs, Weakly similar to
TTCCAGCTGC	0	0	7	6	2	9	19121	adaptor-related protein complex 2, alpha 2 subunit:
TTCCAGCTGC	G	0	7	6	2	9	227277	sine oculis homeobox (Drosophila) homolog 3 regenerating islet-derived 1 alpha (pancreatic Stone protein, pancreatic
TTTCCACTAA	0	0	8	4	4	6	1032	thread protein)
TTCCACTAA	0	0	8	4	4	6	289088	heat shock 90kD protein 1, alpha

(continued)

Tag_Sequence	NC1	NC2	AD1	AD2	CA1	CA2	UNI ID	Description
TTTTTAATGT	0	2	12	18	8	7	161307	H3 histone, family 3A

TABLE 6

Tag_Sequence	NC1	NC2	AD1	AD2	CA1	CA2	UNI ID	Description
AAATCTGGCA	16	15	2	4	2	0	430	plastin 1 (1 isoform)
AACGTGCAGG	29	31	13	6	7	8	160786	argininosuccinate synthetase
AAGAAAGCTC	20	6	0	2	1	5	25264	DKFZP434N126 protein
AAGAAAGCTC	20	6	0	2	1	5	1011	anterior gradient 2 (Xenepus laevis) homolog
AAGAAGCAGG	8	16	3	4	1	3	11441	chromosome 1 open reading frame 8
AAGGTAGCAG	15	16	2	4	2	4	104125	adenylyl cyclase-associated protein
AATAAAGGCT	25	11	3	7	3	4	179735	ras homolog gene family, member C
AATAGTTTCC	7	16	2	3	6	1	272620	pregnancy specific beta-1-glycoprotein 9
AATCACAAAT	18	45	1	4	14	3	74466	carcinoembryonic antigen-related cell adhesion molecule 7
AATGAGAAGG	11	3	0	0	1	0	198248	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 1
ACAATTGGTC	0	10	0	0	0	0	155097	carbonic anhydrase II
ACACCCATCA	2	27	1	0	5	3	110445	CG1-97 protein
ACAGGGTGAC	25	13	1	8	7	6	174050	endothelial differentiation-related factor 1
ACATTGGGTG	377	334	67	34	96	33	275086	PR domain containing 10
ACATTGGGTG	377	334	67	34	96	33	5241	fatty acid binding protein 1, liver
ACCCAACTGC	12	3	0	0	0	0	232604	Homo sapiens cDNA: FLJ22675 fis, clone HSI10553
ACCCACGTCA	22	10	3	5	3	1	198951	jun B proto-oncogene
ACCCCCCCGC	44	38	7	13	16	6	229413	ESTs
ACCCCCCCGC	44	38	7	13	16	6	2780	jun D proto-oncogene
ACCTGCATCC	0	12	0	0	0	0		
ACCTGGGGAG	35	11	1	3	4	3	131748	ESTs, Moderately similar to 1-acylglycerol-3-phosphate O-acyltransferase 2 (lysophosphatidic acid
ACCTGGGGAG	35	11	1	3	4	3	209119	acyltransferase, beta)
ACGGTCCAGG	5	12	0	0	0	1	72924	cytidine deaminase
ACTCTTGTTG	7	2	0	0	0	0	5378	spondin 1, (f-spondin) extracellular matrix protein
ACTGTGGCGG	17	34	9	12	9	4	112242	ESTs
AGAATAGCTT	44	67	3	9	11	30	24133	ESTs
AGCAGGAGCA	50	14	6	3	7	6	178292	KIAAC180 protein
AGCAGGAGCA	50	14	6	3	7	6	738	early growth response 1
AGCCCGACCA	16	S	2	4	1	3	104114	H.sapiens HCG I mRNA
AGGATGGTCC	34	19	5	3	6	8	71779	Homo sapiens DNAfrom chromosome 19, cosmid F21856
AGGCCAAGGG	21	6	3	1	3	4	76057	galactose-4-epimerase, UDP-

(continued)

Tag_Sequence	NC1	NC2	AD1	AD2	CA1	CA2	UNI ID	Description
AGGTGACTGG	10	14	0	0	0	0		
AGTGGGCTCA	3	8	0	1	0	0		
ATACTCCACT	82	59	0	0	10	3	778	guanylate cyclase activator 1B (retina)
ATATAATCTG	18	14	1	4	5	5	621	lectin, galactoside-binding, soluble, 3 (galectin 3)
ATCGTGGCGG	193	92	19	25	20	10	5372	claudin 4
ATGACGCTCA	22	18	6	5	3	4	8254	hypothetical protein PR00899
ATGATGGCAC	12	32	1	0	1	0	84072	transmembrane 4 superfamily member 3
ATGCGGAGTC	14	13	5	3	2	4	25527	tight junction protein 3 (zona occludens 3)
ATGCGGGAGA	38	39	15	22	5	6	109748	Homo sapiens CAC-1 mRNA, partial cds
ATGGCACGGA	6	21	1	1	3	0	81097	cytochrome c oxidase subunit VIII
ATGGTCTACG	10	5	0	0	1	0	96593	hypothetical protein
ATGGTGGGGG	25	30	11	9	1	2	1665	zinc finger protein homologous to Zfp-36 in mouse
ATGTGCGTGG	38	8	11	3	7	7	56937	suppression of tumorigenicity 14 (colon carcinoma, matriptase, epithin)
ATGTGGGCTC	7	2	0	0	0	0	151641	glycoprotein A repetitions predominant
ATGTGGGCTC	7	2	0	0	0	0	27018	Ris
ATTGGAGTGC	136	85	15	36	37	19	220529	carcinoembryonic antigen-related cell adhesion molecule 5
ATTTCAAGAT	35	21	0	2	1	0	155097	carbonic anhydrase II
ATTTCAAGAT	35	21	0	2	1	0	24453	ESTs
CAAATAAAAG	9	12	2	0	1	1	185055	BENE protein
CAAGAGTTTC	14	2	0	0	0	0	183617	ESTs
CACCCCTGAT 73	16	9	58	58	13	36	173724	creatine kinase, brain
CAGTGCGTTC	12	3	0	0	3	0	8302	four and a half LIM domains 2
CATAGGTTTA	66	39	4	1	5	0	1650	solute carrier family 26, member 3
CCAAAGCTAT	42	16	14	12	6	11	84072	transmembrane 4 superfamily member 3
CCAACACCAG	9	19	1	0	0	1	181165	eukaryotic translation elongation factor 1 alpha 1
CCACTGCACC	21	19	5	5	6	14		
CCAGGGGAGA	45	66	24	22	10	20	278613	interferon, alpha-inducible protein 27
CCATTCCACT	13	1	2	0	0	0		
CCCAACGCGC	106	1	3	5	0	2	272572	hemoglobin, alpha 2
CCCCCCAAGC	25	27	3	15	4	8	31265	ESTs, Weakly similar to
CCCCCGCGGA	33	25	6	11	4	12	95697	liver-specific bHLH-Zip transcription factor
CCCCCTGCAT	5	4	0	0	0	0		
CCCGCCTCTT	C	40	3	3	16	1	mito	Tag matches mitochondrial sequence
CCCTCCCGAA	89	54	9	14	18	7	5940	hypothetical protein FLJ20063
CCGCTGCACT	127	102	55	46	37	30		

(continued)

Tag_Sequence	NC1	NC2	AD1	AD2	CA1	CA2	UNI ID	Description
CCGGCTTGAG	7	4	0	0	0	0	2722	inositol 1,4,5-trisphosphate 3-kinase A
CCTCCAGCTA	715	458	142	125	131	147	242463	keratin 8
CCTCCAGTAC	20	8	2	3	2	4		
CCTGCCCCCC	20	30	6	3	11	4	861	mitogen-activated protein kinase 3
CCTGCTGCAG	7	34	0	1	6	9	102482	mucin 5, subtype B, tracheobronchial
CCTGCTTGTC	20	23	0	3	0	5	268171	ESTs, Weakly similar to epididymis-specific, whey-acidic protein type, four-disulfide core; putative
CCTGCTTGTC	20	23	0	3	0	5	2719	ovarian carcinoma marker procollagen-praline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), beta polypeptide (protein disulfide isomerase; thyroid hormone binding
CCTGGAAGAG	30	26	11	4	12	16	75655	protein p55)
CCTGTCTGCC	14	22	0	1	1	1	107139	hypothetical protein anti-oxidant protein 2 (non-selenium glutathione peroxidase, acidic calcium-
CCTGTGACAG	22	27	0	4	3	1	120	independent phospholipase A2)
CCTTCAAATC	29	17	0	0	1	0	23118	carbonic anhydrase I
CGAGGGGCCA	110	47	18	18	12	32	182485	actinin, alpha 4
CGCTGTGGGG	58	53	19	8	8	6	7486	protein expressed in thyroid
CGGACTCACT	20	45	5	14	14	10	284134	serologically defined colon cancer antigen 28
CGGACTCACT	20	45	5	14	14	10	84700	similar to phosphatidylcholine transfer protein 2
CGGGAGTCGG	28	30	13	2	9	1	236720	ESTs, Weakly similar to
CGGTGGGACC	7	14	1	1	3	3	99175	Homo sapiens cDNA: FLJ21606 fis, clone COL07302
CGTGGGTGGG	1	10	1	0	0	1	202833	heme oxygenase (decycling) 1
CTACCCTCAC	172	90	30	53	36	58	14376	actin, gamma 1
CTCAGAACTT	18	3	1	0	0	0	194710	glucosaminyl (N-acetyl) transferase 3, mucin type
CTGAACCTCC	5	15	2	0	0	0	4205	hypothetical protein FL120124
CTGACCTGTG	88	130	48	18	16	46	77961	major histocompatibility complex, class 1, B
CTGGATCTGG	21	21	3	9	5	10	75658	phosphorylase, glycogen; brain
CTGGCAAAGG	14	22	1	0	0	0		
CTGGCCCTCG	186	52	1	3	15	14	1406	trefoil factor 1 (breast cancer, estrogen-inducible sequence expressed in)
CTGGCCCTCG	186	52	1	3	15	14	166184	Intersectin 2
CTGCCCCTCG	136	52	1	3	15	14	7720	dynein, cytoplasmic, heavy polypeptide 1
CTGGCTATCC	7	3	0	0	0	1	10784	hypotherical protein FLJ20037

(continued)

Tag_Sequence	NC1	NC2	AD1	AD2	CA1	CA2	UNI ID	Description
CTGGGCCTCT	22	22	2	2	3	3	50868	solute carrier family 22 (organic cation transporter), member 1-like
CTGTACTTGT	9	5	1	1	0	0	75678	FBJ murine osteosarcoma viral oncogene homolog B
CTGTGTGGCT	0	12	1	0	0	0	127610	acyl-Coenzyme A dehydrogenase, C-2 to C-3 short chain
CTGTGTGGCT	0	12	1	0	0	0	54277	DNA segment on chromosome X (unique) 9928 expressed sequence
CTTACAAGCA	21	13	2	3	4	3	mito	Tag matches mitochondrial sequence
CTTAGAGGGG	16	22	0	1	1	1	155191	villin 2 (ezrin)
CTTATGGTCC	36	11	0	1	1	0	179608	retinol dehydrogenase homolog
CTTCCAGCTA	64	31	22	20	14	19	217493	annexin A2
CTTCTTGCCC	29	2	2	2	0	1	251577	hemoglobin, alpha 1
CTTGACATAC	18	20	4	4	0	0	171695	dual specificity phosphatase 1
CTTGATTCCC	26	9	5	0	2	5	77266	quiescin Q6
GACATCAAGT	198	87	23	14	47	17	182265	keratin 19
GACCAGCCCA	23	21	3	2	12	5	75799	protease, serine, 8 (prostasin)
GACCAGTGGC	21	44	4	0	2	0	143131	glycoprotein A33 (transmembrane)
GACGCGGCGC	30	47	10	17	12	17	301684	RNA POLYMERASE I AND TRANSCRIPT RELEASE FACTOR
GAGAGCTCCC	5	11	3	0	2	1	mito	Tag matches mitochondrial sequence
GAGCACCGTG	7	4	1	0	0	1		
GATATGTAAA	1	10	0	0	0	0		
GATCCCAACT	9	29	5	7	1	1	118786	metallothionein 2A
GATGAATCCG	12	14	2	2	1	2	283552	ESTs, Weakly similar to
GATGACCCCC	42	49	4	3	3	3	mito	Tag matches mitochondrial sequence
GCAAGAAAGT	48	0	0	4	0	1	155376	hemoglobin, beta
GCACAGGTCA	5	9	1	0	1	1		
GCACCCTTTC	13	5	0	0	1	0		
GCACCTGTCG	2	9	0	0	1	0	109059	mitochondrial ribosomal protein L12 alanyl (membrane) aminopeptidase (aminopeptidase N, aminopeptidase M,
GCACCTGTCG	2	9	0	0	1	0	1239	microsomal aminopeptidase, CD13, p150) ems1 sequence (mammary tumor and squamous cell carcinoms-associated
GCAGCTCCTG	13	47	3	2	7	3	119257	(p80/85 src substrate)
GCAGGAGGTG	2	13	0	0	0	1	11441	chromosome 1 open reading frame 8
GCAGGAGGTG	2	13	0	0	0	1	76040	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum Protein retention receptor 1

(continued)

Tag_Sequence	NC1	NC2	AD1	AD2	CA1	CA2	UNI ID	Description
GCAGGGCCTC	128	165	41	69	39	51	92323	FXYD domain-containing ion transport regulator 3
GCCACATACT	3	9	0	0	0	0	4984	KIAA0828 protein
GCCACGTGGA	19	16	5	2	7	1	103665	villin-like
GCCAGACACC	19	9	3	4	1	2	3804	DKFZP564C1940 protein
GCCAGGTTGC	14	5	1	1	1	1	42824	hypothetical protein FLJ10718
GCCAGGTTGC	14	5	1	1	1	1	55682	eukaryotic translation initiation factor 3, subunit 7 (zeta, 66/67kD)
GCCAGGTTGC	14	5	1	1	1	1	78996	proliferating cell nuclear antigen
GCCATCCTCC	9	13	0	1	0	0		
GCCCACACAG	1 5	0	1	1	0	0	1690	heparin-binding growth factor binding protein
GCCCACGTCA	7	8	0	0	0	0		
GCCCAGGGCC	4	44	2	1	2	1	10326	coatomer protein complex, subunit epsilon
GCCCAGGGCC	4	44	2	1	2	1	229417	EST, Moderately similar to
GCCCAGGGCC	4	44	2	1	2	1	229546	EST
GCCCAGGTCA	519	447	136	128	58	22	154903	ESTs, Weakly similar to
GCCCAGTGGC	51	0	8	15	2	5	143131	glycoprotein A33 (transmembrane)
GCCGACCAGG	46	47	15	8	19	9	75741	amiloride binding protein 1 (amine oxidase (copper-containing))
GCCGGGTGGG	207	149	18	24	68	67	74631	basigin
GCCGTGGAGA	32	23	4	11	7	7	80680	major vault protein
GCCTGGCCAT	26	34	4	6	10	14	5662	guanine nucleotide binding protein (G protein), beta polypeptide 2-like 1
GCCTGGCCAT	26	34	4	6	10	14	63042	DKFZpS64J157 protein
GCGAAACCCT	167	566	123	43	64	98		
GCGAAACTCG	5	9	1	0	0	0		
GCGCAGAGGT	2	16	1	0	0	1	108124	ribosomal protein L41
GCTCTTCCCC	9	2	1	1	2	0	33455	peptidyl arginine deiminase, type II
GCTGCCCTTG	44	6	13	6	6	18	272897	Tubulin, alpha, brain-specific
GCTGCCCTTG	44	6	13	6	6	18	27824	tubulin, alpha, ubiquitous
GCTGGCACAT	15	14	1	0	6	C	179704	meprin A, alpha (PABA peptide hydrolase)
GCTGGCCCCG	5	11	0	0	0	1	8185	CGI-44 protein; sulfide dehydrogenase like (yeast)
GCTGTGCCTG	36	42	2	4	11	8	58247	proteass, serine, 4 (trypsin 4, brain)
GCTTGGGGAT	11	8	2	0	0	0	5394	myosin, heavy polypeptide-like (110kD)
GGAACAGGGG	1	13	1	0	0	2	102336	Rho GTPase activating protein 8
GGAACAGGGG	1	13	1	0	0	2	272972	hyperthetical protein FLJ20185
GGAACAGGGG	1	13	1	0	0	2	77961	major histocompatibility complex, class 1, B
GGAACTGTGA	90	84	10	18	10	2	38972	tetraspan 1

(continued)

Tag_Sequence	NC1	NC2	AD1	AD2	CA1	CA2	UNI ID	Description
GGAAGAGCAC	21	11	1	1	2	5	75268	sialyltransferase 4C (beta-galactosidase alpha-2,3-sialytransferase)
GGAGGCCGAG	13	9	5	0	2	5	301342	ESTs, Weakly similar to
GGAGGCGCTC	5	11	1	1	0	1	33455	peptidyl arginine deiminase, type II
GGATGGCTTA	25	5	2	1	1	1	64179	hypothetical protein
GGCACCGTGC	22	44	8	10	8	4	120912	ESTs
GGCCCTGCAG	14	7	1	0	5	1	105463	sir2-related protein type 6
GGCTCGGGAT	15	11	2	4	3	5	2575	calpain 1, (mu/l) large subunit
GGCTGCCGC	13	11	4	3	5	2	180958	ESTs
GGCTGCCTGC	13	11	4	3	5	2	197314	ESTs
GGCTGGGCCT	46	25	14	5	10	8	144102	EST
GGCTGGGCCT	46	25	14	5	10	8	14846	Homo sapiens mRNA; cDNA DKFZp564D016 (from clone DKFZp564D016)
GGCTGGGCCT	46	25	14	5	10	8	73919	clathrin, light polypeptide (Lcb)
GGGAAGCAGA	32	17	18	4	8	9		
GGGACGAGTG	20	6	1	3	6	1	3337	transmembrane 4 superfamily member 1
GGGCGCTGTG	11	27	3	6	4	5	8372	ubiquinol-cytochrome c reductase (6.4kD) subunit
GGGGCAGGGC	48	64	27	10	15	31	119140	eukaryotic translation initiation factor SA
GGTGAAGAGG	16	32	5	3	10	9	233950	serine protease inhibitor, Kunitz type 1
GTAGCAGGTG	24	27	11	7	7	7	140452	cargo selection protein (mannose 6 phosphate receptor binding protein)
GTATTGGGGC	5	7	0	0	0	0		
GTCATCACCA	35	22	0	0	0	0	107382	KIAA1517 protein
GTCATCACCA	35	22	0	0	0	0	257045	Homo sapiens cDNA: FLJ23415 fis, clone HEP20738
GTCATCACCA	35	22	0	0	0	0	32966	guanylate cyclase activator 2B (uroguanylin)
GTCATCACCA	35	22	0	0	0	0	68877	cytochrome b-245, alpha polypeptide
GTCCGAGTGC	17	3	0	0	0	0	3337	transmembrane 4 superfamily member 1
GTCCTGAACA	7	3	0	0	0	0	78546	ATPase, Ca++ transporting, plasma membrane 1
GTCCTGAACA	7	3	0	0	0	0	8256	DKFZP434D1335 protein
GTGCACTGAG	118	45	14	7	12	13	181244	major histocompatibility complex, class 1, A
GTGCACTGAG	118	45	14	7	12	13	277477	major histocompatibility complex, class 1, C
GTGCCTGAGA	18	15	2	6	7	3	77886	lamin A/C
GTGGCGGGAA	3	15	1	0	4	0		
GTGGGGGCGC	5	22	2	0	1	0	254105	enolase 1, (alpha)
GTGGTGGCAG	29	11	1	1	10	3	194691	rotinoic acid induced 3
GTGGTTCACG	4	5	0	0	0	0	272088	ESTs, Moderately similar to

(continued)

Tag_Sequence	NC1	NC2	AD1	AD2	CA1	CA2	UNI ID	Description
GTGGTTCACG	4	5	0	0	0	0	62192	coagulation factor III (thromboplastin, tissue factor)
GTGTTGGGGG	21	19	8	6	1	3	55016	hypothetical protein FLJ21935
GTTTAGAGGG	5	16	0	0	2	1	181874	interferon-induced protein with tetratricopeptide repeats 4
TAAATTGCAA	103	59	8	17	5	3	56205	Insulin induced gene 1
TAAGGCCTTT	6	9	1	0	0	1	20149	deleted in lymphocytic leukemia, 1
TAAGGCCTTT	6	9	1	0	0	1	42945	acid sphingomyelinase-like phosphodiesterase
TAATCCCAGC	37	33	8	7	16	13		
TAATTTGCAT	25	2	1	1	2	0	79368	epithelial membrane protein 1
TACGGTGTGG	7	13	2	2	1	0	105460	DKFZP56400823 protein
TACTCGGCCA	10	5	0	0	1	0	79474	tyrosine 3-monooxygenase/ tryptophan 5-monooxygenase activation protein, epsilon polypeptide
TACTGTGGAT	4	11	0	2	2	1	21537	protein phosphatase 1, catalytic subunit, beta isoform
TAGACTAGCA	31	27	6	6	22	4	100090	tetraspan 3
TAGGATGGGG	24	30	4	7	6	1	76941	ATPase, Na+/K+ transporting, beta 3 polypeptide
TATGATGAGC	13	21	2	2	1	5	205126	Homo sapiens cDNA: FLJ22667 fis, clone HS108385
TCACACTGCC	26	7	3	1	3	4	81008	filamin B, beta (actin-binding protein-278)
TCACCGGTCA	118	75	10	10	5	6	290070	galsolin (amyloidosis, Finnish type)
TCAGAGCGCT	5	21	0	7	0	1	92323	FXYD domain-containing ion transport regulator 3
TCAGCTGCAA	56	16	0	0	9	3	284199	mucin 12
TCAGCTGCAA	56	16	0	0	9	3	301888	Homo sapiens cDNA FLJ11205 fis, clone PLACE1007843
TCGGAGCTGT	21	20	9	6	3	0	4055	G protein-coupled receptor kinase-interactor 1
TCTGAATTAT	24	16	0	0	1	0	50964	carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)
TGACTAATTG	7	9	2	0	0	3	293380	ESTs
TGAGTGACAG	9	68	0	6	3	7	205126	Homo sapiens cDNA: FLJ22667 fis, clone HSI08385
TGAGTGACAG	9	68	0	6	3	7	271688	ESTs
TGATCTCTGT	6	7	1	0	1	1	30738	hypothetical protein FLJ10407
TGCAGCACGA	6	185	5	30	24	16	110309	major histocompatibility complex, class 1, F
TGCAGCGCCT	16	9	1	1	1	1	77 573	uridine phosphorylase
TGCCGCCCGC	14	5	2	2	1	0	202097	procollagen C-encopeptidase enhancer
TCCTCCTACC	140	113 3	70	22	17	22	111732	Fc fragment of IgG binding protein

(continued)

Tag_Sequence	NC1	NC2	AD1	AD2	CA1	CA2	UNI ID	Description
TGCTCCTACC	140	113	70	22	17	22	301256	Homo sapiens chromosome 19, cosmid R30669
TGGCCATCTG	30	24	8	7	3	4	184052	PP1201 protein
TGGCGCGTGT	25	8	0	0	9	5	25640	claudin 3
TGGCTACTTA	6	9	1	0	1	2	117950	multifunctional polypeptide similar to SAICAR synthetase and AIR carboxylase
TGGGGAGAGG	43	18	20	7	3	7	288998	S100-type calcium binding protein A14
TTAACCCCTC	34	14	5	9	1	5	78224	ribonuclease, RNase A family, 1 (pancreatic)
TTATGGTGTG	11	17	0	0	0	0	271499	ESTs
TTCCACTAAC	29	9	7	4	5	5	79706	plectin 1, intermediate filament binding protein, 500kD
TTCCGCGTTC	5	16	0	0	2	2	137274	ESTs, Weakly similar to
TTCTGGTGCG	8	2	0	0	1	0	119251	ubiquinol-cytochrome c reductase core protein I
TTCTGTAGCC	13	23	4	2	4	2	5541	ATPase, Ca++ transporting, ubiquitous
TTGGACCTGG	33	31	7	18	12	16	89761	ATP synthase, H+ transporting, mitochondrial F1 complex, delta subunit
TTGGGGTTTC	111	184	50	81	67	50	62954	ferritin, heavy polypeptide 1
TTTAACGGCC	93	67	36	35	11	30	mito	Tag matches mitochondrial sequence
TTTCCTCTCA	21	8	6	2	4	3	184510	stratifin
TTTCCTCTCA	21	8	6	2	4	3	303400	ESTs
TTTCTCGTCG	10	16	2	3	0	2	1686	guanine nucleotide binding protein (G protein), alpha 11 (Gq class)
TTTGGTTTCA	2	13	0	0	0	0		carcinoembryonic antigen-related cell adhesion molecule 1 (biliary
TTTTCTGCAT	8	7	1	0	1	2	50964	glycoprotein)
TTTTCTGCAT	8	7	1	0	1	2	77318	platelet-activating factor acetylhydrolase, isoform Ib, alpha subunit (45kD)
TTTTTACTGA	32	19	10	10	8	1	111577	integral membrane protein 2C

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Claims

1. A method for detection of benign colorectal adenomas in presymptomatic patients, comprising the steps of:

   isolating an mRNA sample from feces of a subject;
   detecting renal dipeptidase mRNA in said mRNA sample;
   comparing the amount of renal dipeptidase mRNA in said mRNA sample to amounts of renal dipeptidase mRNA in normal subjects, wherein an elevated amount of renal dipeptidase mRNA in said mRNA sample is an indicator

of benign colorectal adenoma in the subject.

2. A method for detection of benign colorectal adenomas in presymptomatic patients, comprising the steps of:

isolating epithelial cells from blood of a subject;
isolating an mRNA sample from the epithelial cells;
detecting renal dipeptidase mRNA in said mRNA sample;
comparing the amount of renal dipeptidase mRNA in said mRNA sample to amounts of renal dipeptidase mRNA in normal subjects, wherein an elevated amount of renal dipeptidase mRNA in said mRNA sample is an indicator of benign colorectal adenoma in the subject.

3. The method of claim 1 or claim 2, further comprising the step of:

identifying the patient as having a benign colorectal adenoma if an elevated amount of renal dipeptidase mRNA in said mRNA sample is observed.

4. The method of claim 1 or claim 2, wherein renal dipeptidase mRNA is detected by RT-PCR.

5. The method of claim 1 or claim 2, wherein renal dipeptidase mRNA is detected by hybridization of copy mRNA to a nucleic acid array.

6. A method for detection of benign colorectal adenoma in a subject that has been administered an antibody which specifically bind to renal dipeptidase and wherein the antibody is labelled with a moiety which is detectable from outside of the subject, comprising:

detecting the moiety in the subject from outside of the subject, wherein an area of localization of the moiety within the subject but outside the proximal tubules of the kidney identifies benign colorectal adenoma.

7. A method for detection of benign colorectal adenomas in a subject that has been administered an inhibitor of renal dipeptidase wherein the inhibitor is labelled with a moiety which is detectable from outside the subject, comprising detecting the moiety in the subject from outside of the subject, wherein an area of localization of the moiety within the subject but outside the proximal tubules of the kidney identifies benign colorectal adenoma.

8. The method of claim 6 or claim 7, wherein the moiety is fluor.

9. The method of claim 6 or claim 7, wherein the moiety is a radioactive atom.

10. The method of claim 6 or claim 7, wherein the moiety is a contrast agent for spiral computer tomography.

11. The method of claim 7, wherein the inhibitor binds irreversibly to renal dipeptidase.

12. The method of claim 7, wherein the inhibitor binds and slowly releases from renal dipeptidase with a half-life of greater than 4 hours.

13. A method for detection of benign colorectal adenomas in presymptomatic patients, comprising the steps of:

detecting renal dipeptidase in blood of a subject; and
comparing amount of renal dipeptidase in blood of the subject to the amount of renal dipeptidase in normal subjects, wherein an elevated amount of renal dipeptidase in the blood of the subject is an indicator of benign colorectal adenoma in the subject.

14. A method for detection of benign colorectal adenomas in presymptomatic patients, comprising the steps of:

detecting renal dipeptidase in feces of a subject; and
comparing amount of renal dipeptidase in feces of the subject to the amount of renal dipeptidase in normal subjects, wherein an elevated amount of renal dipeptidase in the feces of the subject is an indicator of benign colorectal adenoma in the subject.

15. The method of claim 13 further comprising:

determining if renal dipeptidase detected in the blood comprises a tumor-specific glycoform of renal dipeptidase.

16. The method of claim 14 further comprising:

determining if renal dipeptidase detected in the feces comprises a tumor-specific glycoform of renal dipeptidase.

17. The method of claim 13 further comprising:

determining if renal dipeptidase detected in the blood binds to L lectin from *Phaseolus vulgaris* hemagglutinin.

18. The method of claim 14 further comprising:

determining if renal dipeptidase detected in the feces binds to L lectin from *Phaseolus vulgaris* hemagglutinin.

19. The method of claim 13 further comprising:

determining if renal dipeptidase detected in the blood binds to L lectin from *Sambucus nigra* hemagglutinin.

20. The method of claim 14 further comprising:

determining if renal dipeptidase detected in the feces binds to L lectin from *Sambucus nigra* hemagglutinin.

Patentansprüche

1. Ein Verfahren zum Erfassen von gutartigen kolorektalen Adenomen in präsymptomatischen Patienten, welches die folgenden Schritte umfasst:

Isolieren einer mRNA-Probe aus den Fäzes eines Subjekts;
Erfassen der mRNA der renalen Dipeptidase in der mRNA-Probe;
Vergleichen der Menge an mRNA der renalen Dipeptidase in der mRNA-Probe mit Mengen der mRNA der renalen Dipeptidase in normalen Subjekten, wobei eine erhöhte Menge an mRNA der renalen Dipeptidase in der mRNA-Probe ein Indikator für ein gutartiges kolorektales Adenom in dem Subjekt ist.

2. Ein Verfahren zum Erfassen von gutartigen kolorektalen Adenomen in präsymptomatischen Patienten, welches die folgenden Schritte umfasst:

Isolieren epithelialer Zellen aus dem Blut eines Subjekts;
Isolieren einer mRNA-Probe aus den epithelialen Zellen;
Erfassen der mRNA der renalen Dipeptidase in der mRNA-Probe;
Vergleichen der Menge an mRNA der renalen Dipeptidase in der mRNA-Probe mit Mengen der mRNA der renalen Dipeptidase in normalen Subjekten, wobei eine erhöhte Menge an mRNA der renalen Dipeptidase in der mRNA-Probe ein Indikator für ein gutartiges kolorektales Adenom in dem Subjekt ist.

3. Das Verfahren nach Anspruch 1 oder 2, welches ferner den folgenden Schritt umfasst:

Identifizieren des Patienten als Träger eines gutartigen kolorektalen Adenoms,
wenn eine erhöhte Menge an mRNA der renalen Dipeptidase in der mRNA-Probe beobachtet wird.

4. Das Verfahren nach Anspruch 1 oder 2, wobei die mRNA der renalen Dipeptidase mittels RT-PCR bestimmt wird.

5. Das Verfahren nach Anspruch 1 oder 2, wobei die mRNA der renalen Dipeptidase durch Hybridisieren von Kopie-mRNA an ein Nukleinsäurearray erfasst wird.

6. Ein Verfahren zum Erfassen eines gutartigen kolorektalen Adenoms in einem Subjekt, welchem ein Antikörper verabreicht wurde, der spezifisch an renale Dipeptidase bindet, und wobei der Antikörper mit einer Markierung

versehen ist, welche von der Außenseite des Subjekts detektierbar ist, umfassend:

Detektieren der Markierung in dem Subjekt von der Außenseite des Subjekts,
wobei ein Lokalisierungsbereich der Markierung innerhalb des Subjekts, jedoch außerhalb der proximalen Tubuli der Niere ein gutartiges kolorektales Adenom identifiziert.

7. Ein Verfahren zum Erfassen von gutartigen kolorektalen Adenomen in einem Subjekt, welchem ein renaler Dipeptidase-Inhibitor verabreicht wurde, wobei der Inhibitor mit einer Markierung versehen ist, welche von der Außenseite des Subjekts detektierbar ist, umfassend das Detektieren der Markierung in dem Subjekt von der Außenseite des Subjekts, wobei ein Lokalisierungsbereich der Markierung innerhalb des Subjekts, jedoch außerhalb der proximalen Tubuli der Niere ein gutartiges kolorektales Adenom identifiziert.

8. Das Verfahren nach Anspruch 6 oder 7, wobei die Markierung Fluor ist.

9. Das Verfahren nach Anspruch 6 oder 7, wobei die Markierung ein radioaktives Atom ist.

10. Das Verfahren nach Anspruch 6 oder 7, wobei die Markierung ein Kontrastmittel für die Spiral-Computertomografie ist.

11. Das Verfahren nach Anspruch 7, wobei der Inhibitor irreversibel an renale Dipeptidase bindet.

12. Das Verfahren nach Anspruch 7, wobei der Inhibitor an renale Dipeptidase bindet und mit einer Halbwertszeit von größer als 4 Stunden sich langsam wieder davon löst.

13. Ein Verfahren zum Erfassen von gutartigen kolorektalen Adenomen in präsymptomatischen Patienten, welches die folgenden Schritte umfasst:

Erfassen von renaler Dipeptidase im Blut eines Subjekts; und
Vergleichen der Menge an renaler Dipeptidase im Blut des Subjekts mit der Menge an renaler Dipeptidase in normalen Subjekten, wobei eine erhöhte Menge an renaler Dipeptidase in dem Blut des Subjekts ein Indikator für ein gutartiges kolorektales Adenom in dem Subjekt ist.

14. Ein Verfahren zum Erfassen von gutartigen kolorektalen Adenomen in präsymptomatischen Patienten, welches die folgenden Schritte umfasst:

Erfassen der renalen Dipeptidase in Fäzes eines Subjekts; und
Vergleichen der Menge an renaler Dipeptidase in Fäzes des Subjekts mit der Menge an renaler Dipeptidase in normalen Subjekten, wobei eine erhöhte Menge an renaler Dipeptidase in den Fäzes des Subjekts ein Indikator für ein gutartiges kolorektales Adenom in dem Subjekt ist.

15. Das Verfahren nach Anspruch 13, ferner umfassend:

Bestimmen, ob renale Dipeptidase, die in dem Blut detektiert wird, eine tumorspezifische Glykoform der renalen Dipeptidase umfasst.

16. Das Verfahren nach Anspruch 14, ferner umfassend:

Bestimmen, ob renale Dipeptidase, die in den Fäzes detektiert wird, eine tumorspezifische Glykoform der renalen Dipeptidase umfasst.

17. Das Verfahren nach Anspruch 13, ferner umfassend:

Bestimmen, ob renale Dipeptidase, die in dem Blut detektiert wird, an L-Lektin aus *Phaseolus vulgaris* Hämagglutinin bindet.

18. Das Verfahren nach Anspruch 14, ferner umfassend:

Bestimmen, ob renale Dipeptidase, die in den Fäzes detektiert wird, an L-Lektin aus *Phaseolus vulgaris* Hämag-

glutinin bindet.

19. Das Verfahren nach Anspruch 13, ferner umfassend:

Bestimmen, ob renale Dipeptidase, die in dem Blut detektiert wird, an L-Lektin aus *Sambucus nigra* Hämagglutinin bindet.

20. Das Verfahren nach Anspruch 14, ferner umfassend:

Bestimmen, ob renale Dipeptidase, die in den Fäzes detektiert wird, an L-Lektin aus *Sambucus nigra* Hämagglutinin bindet.

Revendications

1. Procédé de détection d'adénomes colorectaux bénins chez des patients présymptomatiques, comprenant les étapes consistant à :

isoler un échantillon d'ARNm à partir des selles d'un sujet ;
détecter l'ARNm de la dipeptidase rénale dans ledit échantillon d'ARNm ;
comparer la quantité d'ARNm de la dipeptidase rénale dans ledit échantillon d'ARNm aux quantités d'ARNm de la dipeptidase rénale chez des sujets normaux, où une quantité élevée d'ARNm de la dipeptidase rénale dans ledit échantillon d'ARNm est un indicateur d'adénome colorectal bénin chez le sujet.

2. Procédé de détection d'adénomes colorectaux bénins chez des patients présymptomatiques, comprenant les étapes consistant à :

isoler des cellules épithéliales à partir du sang d'un sujet ;
isoler un échantillon d'ARNm à partir des cellules épithéliales ;
détecter l'ARNm de la dipeptidase rénale dans ledit échantillon d'ARNm ;
comparer la quantité d'ARNm de la dipeptidase rénale dans ledit échantillon d'ARNm aux quantités d'ARNm de la dipeptidase rénale chez des sujets normaux, où une quantité élevée d'ARNm de la dipeptidase rénale dans ledit échantillon d'ARNm est un indicateur d'adénome colorectal bénin chez le sujet.

3. Procédé selon la revendication 1 ou la revendication 2, comprenant en outre l'étape consistant à :

identifier le patient comme étant atteint d'un adénome colorectal bénin si une quantité élevée d'ARNm de la dipeptidase rénale dans ledit échantillon d'ARNm est observée.

4. Procédé selon la revendication 1 ou la revendication 2, dans lequel l'ARNm de la dipeptidase rénale est détecté par RT-PCR.

5. Procédé selon la revendication 1 ou la revendication 2, dans lequel l'ARNm de la dipeptidase rénale est détecté par hybridation de copie d'ARNm à une puce d'acide nucléique.

6. Procédé de détection d'un adénome colorectal bénin chez un sujet auquel il a été administré un anticorps qui se lie spécifiquement à la dipeptidase rénale et où l'anticorps est marqué par un radical qui est détectable depuis l'extérieur du sujet, comprenant :

la détection du radical chez le sujet depuis l'extérieur du sujet, où une zone de localisation du radical au sein du sujet mais en dehors des tubules proximaux rénaux identifie un adénome colorectal bénin.

7. Procédé de détection d'adénomes colorectaux bénins chez un sujet auquel il a été administré un inhibiteur de la dipeptidase rénale où l'inhibiteur est marqué par un radical qui est détectable depuis l'extérieur du sujet, comprenant la détection du radical chez le sujet depuis l'extérieur du sujet, où une zone de localisation du radical au sein du sujet mais en dehors des tubules proximaux rénaux identifie un adénome colorectal bénin.

8. Procédé selon la revendication 6 ou la revendication 7, dans lequel le radical est du fluor.

9. Procédé selon la revendication 6 ou la revendication 7, dans lequel le radical est un atome radioactif.

10. Procédé selon la revendication 6 ou la revendication 7, dans lequel le radical est un agent de contraste pour tomodensitométrie spiralée.

11. Procédé selon la revendication 7, dans lequel l'inhibiteur se lie de manière irréversible à la dipeptidase rénale.

12. Procédé selon la revendication 7, dans lequel l'inhibiteur se lie et se libère lentement de la dipeptidase rénale avec une demi-vie supérieure à 4 heures.

13. Procédé de détection d'adénomes colorectaux bénins chez des patients présymptomatiques, comprenant les étapes consistant à :

détecter la dipeptidase rénale dans le sang d'un sujet ; et
comparer la quantité de dipeptidase rénale dans le sang du sujet à la quantité de dipeptidase rénale chez des sujets normaux, où une quantité élevée de dipeptidase rénale dans le sang du sujet est un indicateur d'adénome colorectal bénin chez le sujet.

14. Procédé de détection d'adénomes colorectaux bénins chez des patients présymptomatiques, comprenant les étapes consistant à :

détecter la dipeptidase rénale dans les selles d'un sujet ; et
comparer la quantité de dipeptidase rénale dans les selles du sujet à la quantité de dipeptidase rénale chez des sujets normaux, où une quantité élevée de dipeptidase rénale dans les selles du sujet est un indicateur d'adénome colorectal bénin chez le sujet.

15. Procédé selon la revendication 13 comprenant en outre l'étape consistant à :

déterminer si la dipeptidase rénale détectée dans le sang comprend une glycoforme à spécificité tumorale de la dipeptidase rénale.

16. Procédé selon la revendication 14 comprenant en outre l'étape consistant à :

déterminer si la dipeptidase rénale détectée dans les selles comprend une glycoforme à spécificité tumorale de la dipeptidase rénale.

17. Procédé selon la revendication 13 comprenant en outre l'étape consistant à :

déterminer si la dipeptidase rénale détectée dans le sang se lie à la lectine L provenant de l'hémagglutinine de *Phaseolus vulgaris.*

18. Procédé selon la revendication 14 comprenant en outre l'étape consistant à :

déterminer si la dipeptidase rénale détectée dans les selles se lie à la lectine L provenant de l'hémagglutinine de *Phaseolus vulgaris.*

19. Procédé selon la revendication 13 comprenant en outre l'étape consistant à :

déterminer si la dipeptidase rénale détectée dans le sang se lie à la lectine L provenant de l'hémagglutinine de *Sambucus nigra.*

20. Procédé selon la revendication 14 comprenant en outre l'étape consistant à :

déterminer si la dipeptidase rénale détectée dans les selles se lie à la lectine L provenant de l'hémagglutinine de *Sambucus nigra.*

FIG. 1A

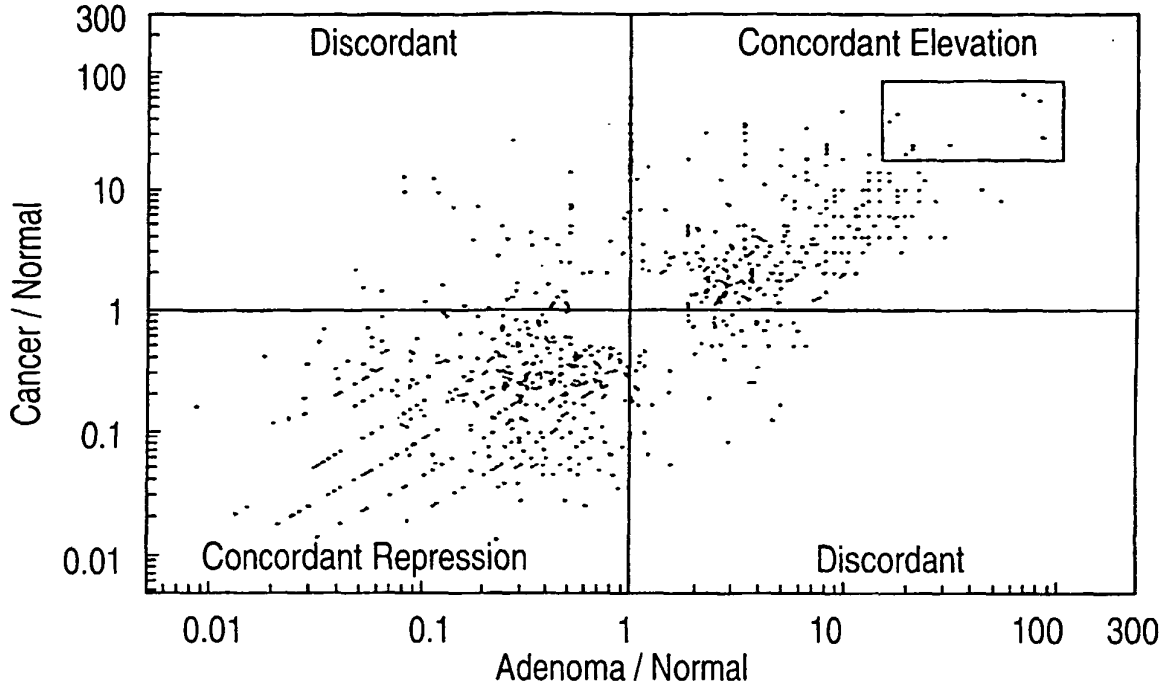

Discordant
Concordant Elevation
Concordant Repression
Discordant
Cancer / Normal
Adenoma / Normal
300
100
10
1
0.1
0.01
0.01
0.1
1
10
100
300

FIG. 1B

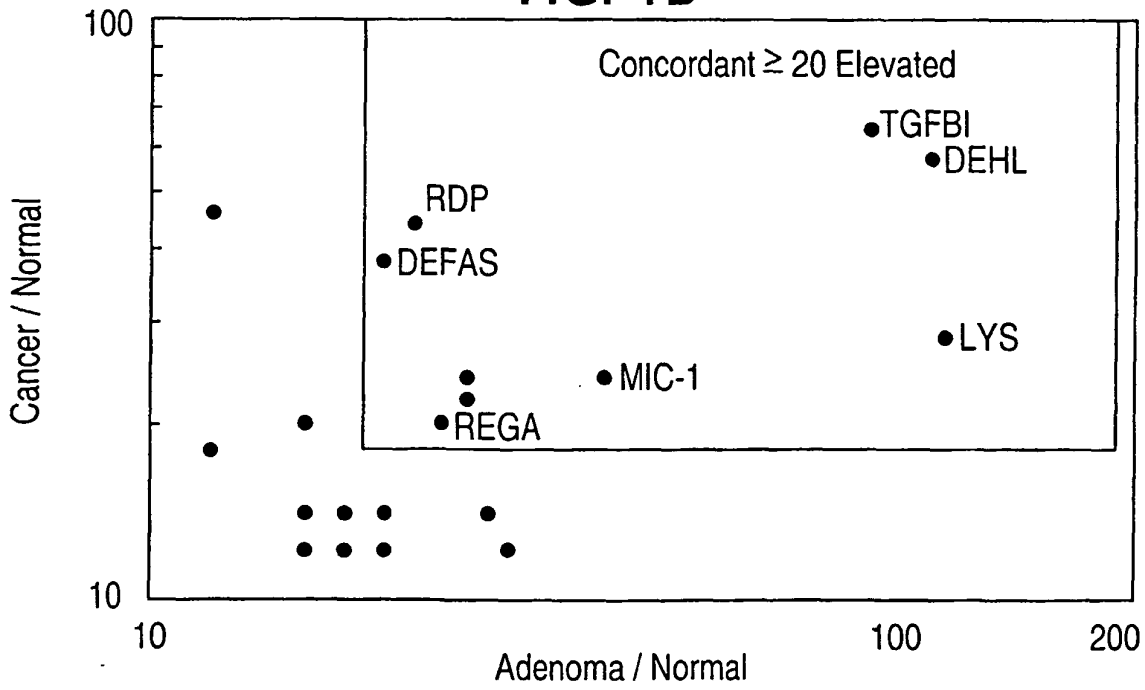

Concordant ≥ 20 Elevated
TGFBI
DEHL
RDP
DEFAS
LYS
MIC-1
REGA
Cancer / Normal
Adenoma / Normal
100
10
10
100
200

FIG. 2

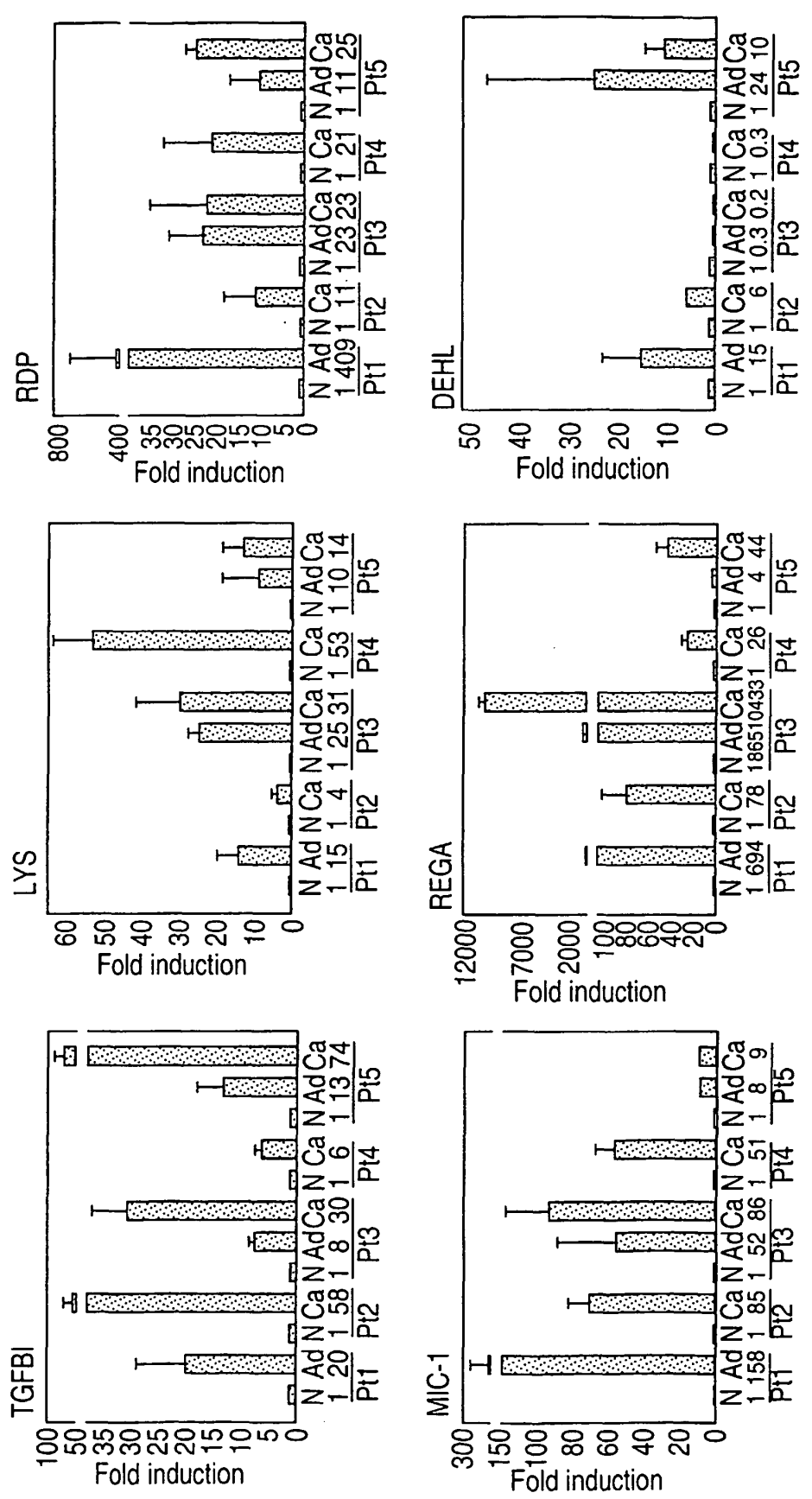

RDP
Fold induction
N Ad N Ca N Ad Ca N Ca N Ad Ca
1 409 1 11 1 23 23 1 21 1 11 25
Pt1 Pt2 Pt3 Pt4 Pt5
DEHL
Fold induction
N Ad N Ca N Ad Ca N Ca N Ad Ca
1 15 1 6 1 0.3 0.2 1 0.3 1 24 10
Pt1 Pt2 Pt3 Pt4 Pt5
LYS
Fold induction
N Ad N Ca N Ad Ca N Ca N Ad Ca
1 15 1 4 1 25 31 1 53 1 10 14
Pt1 Pt2 Pt3 Pt4 Pt5
REGA
Fold induction
N Ad N Ca N Ad Ca N Ca N Ad Ca
1 694 1 78 1 865 104 331 26 1 4 44
Pt1 Pt2 Pt3 Pt4 Pt5
TGFBI
Fold induction
N Ad N Ca N Ad Ca N Ca N Ad Ca
1 20 1 58 1 8 30 1 6 1 13 74
Pt1 Pt2 Pt3 Pt4 Pt5
MIC-1
Fold induction
N Ad N Ca N Ad Ca N Ca N Ad Ca
1 158 1 85 1 52 86 1 51 1 8 9
Pt1 Pt2 Pt3 Pt4 Pt5

FIG. 3

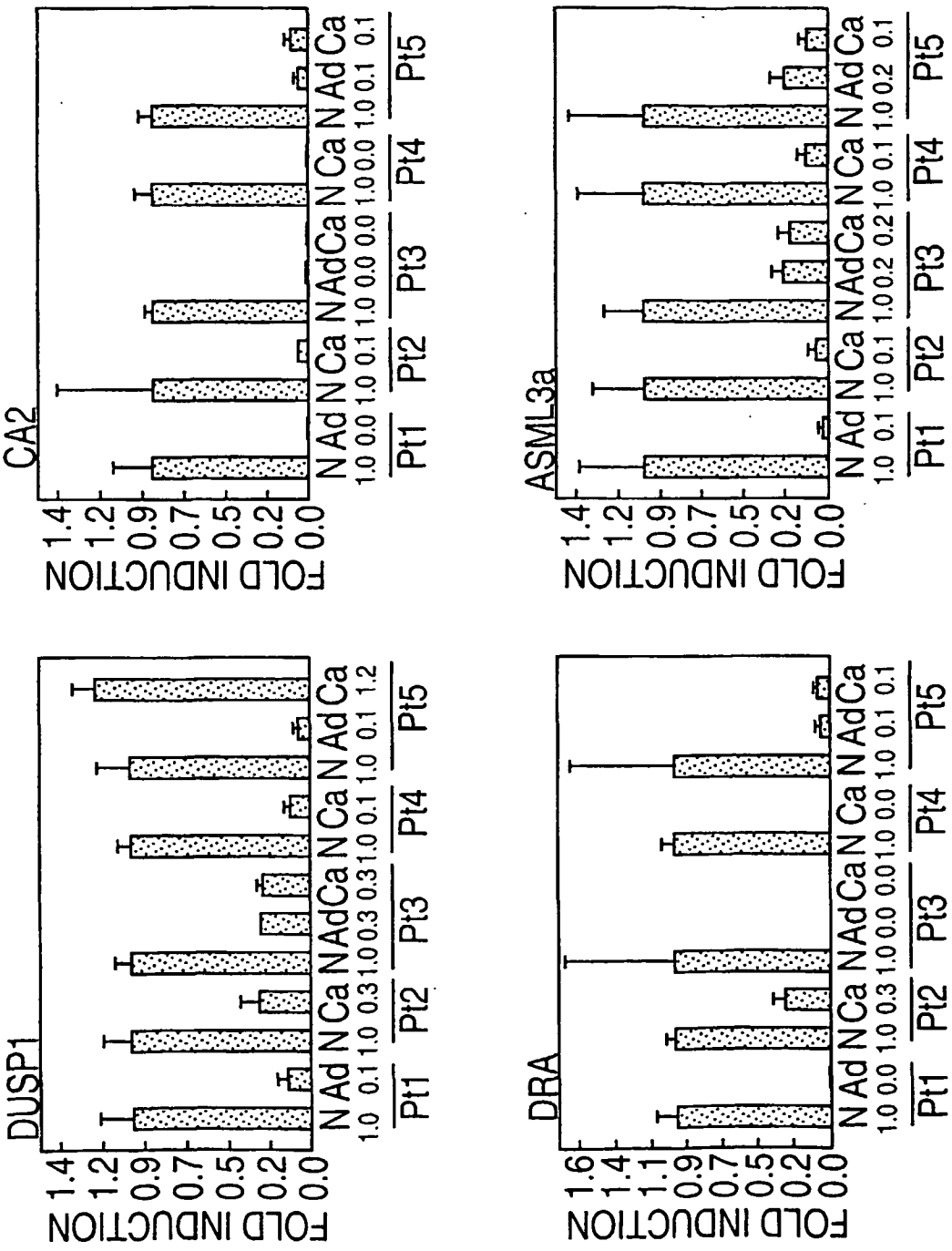
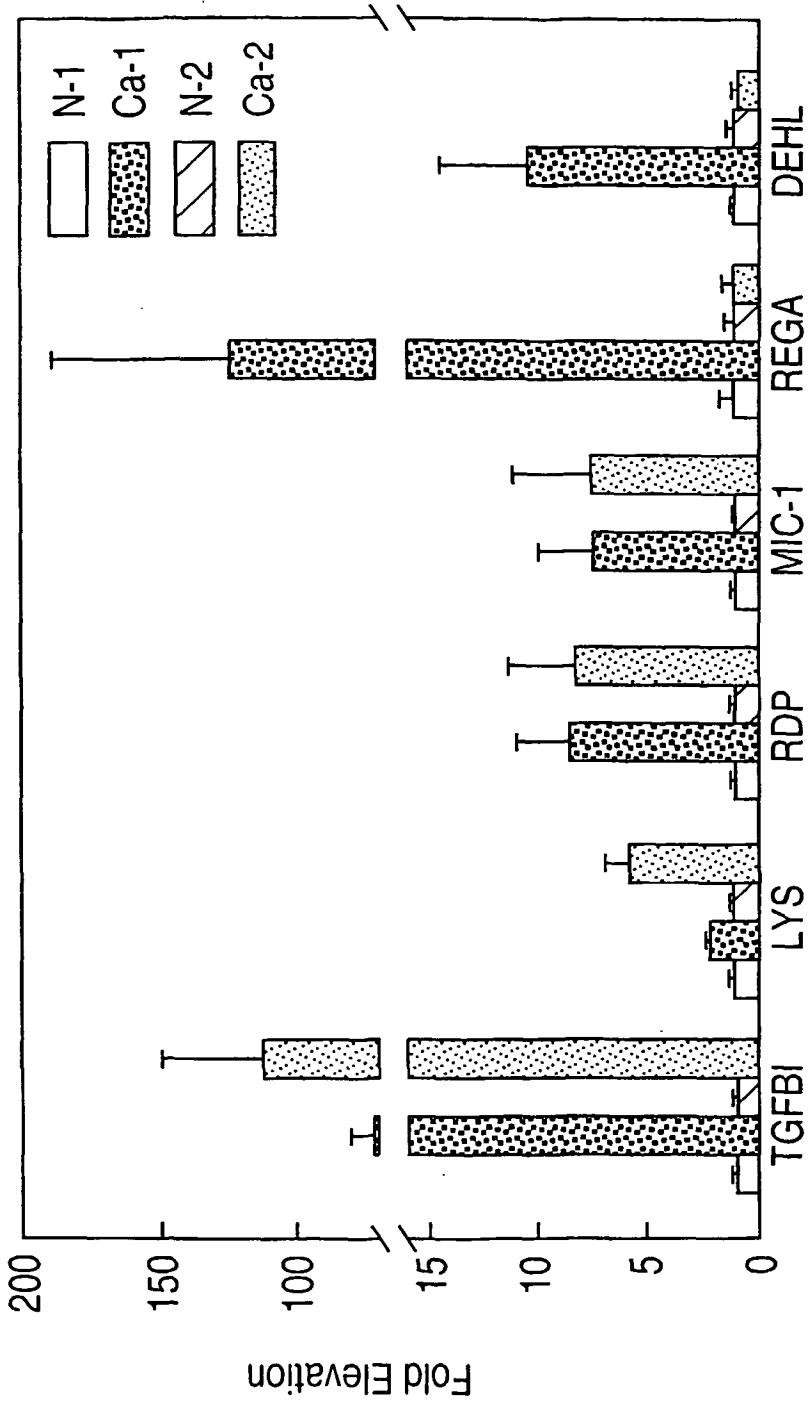

CA2
FOLD INDUCTION
1.4
1.2
0.9
0.7
0.5
0.2
0.0
N Ad N Ca N Ad Ca N Ad N Ca N Ad Ca
1.0 0.0 1.0 0.1 1.0 0.0 0.0 1.0 0.0 1.0 0.1 0.1
Pt1 Pt2 Pt3 Pt4 Pt5
ASML3a
FOLD INDUCTION
1.4
1.2
0.9
0.7
0.5
0.2
0.0
N Ad N Ca N Ad Ca N Ad N Ca N Ad Ca
1.0 0.1 1.0 0.1 1.0 0.2 0.2 1.0 0.1 1.0 0.2 0.1
Pt1 Pt2 Pt3 Pt4 Pt5
DUSP1
FOLD INDUCTION
1.4
1.2
0.9
0.7
0.5
0.2
0.0
N Ad N Ca N Ad Ca N Ad N Ca N Ad Ca
1.0 0.1 1.0 0.3 1.0 0.3 0.3 1.0 0.1 1.0 0.1 1.2
Pt1 Pt2 Pt3 Pt4 Pt5
DRA
FOLD INDUCTION
1.6
1.4
1.1
0.9
0.7
0.5
0.2
0.0
N Ad N Ca N Ad Ca N Ad N Ca N Ad Ca
1.0 0.0 1.0 0.3 1.0 0.0 0.0 1.0 0.0 1.0 0.1 0.1
Pt1 Pt2 Pt3 Pt4 Pt5

FIG. 4


N-1
Ca-1
N-2
Ca-2
Fold Elevation
200
150
100
15
10
5
0
TGFBI
LYS
RDP
MIC-1
REGA
DEHL

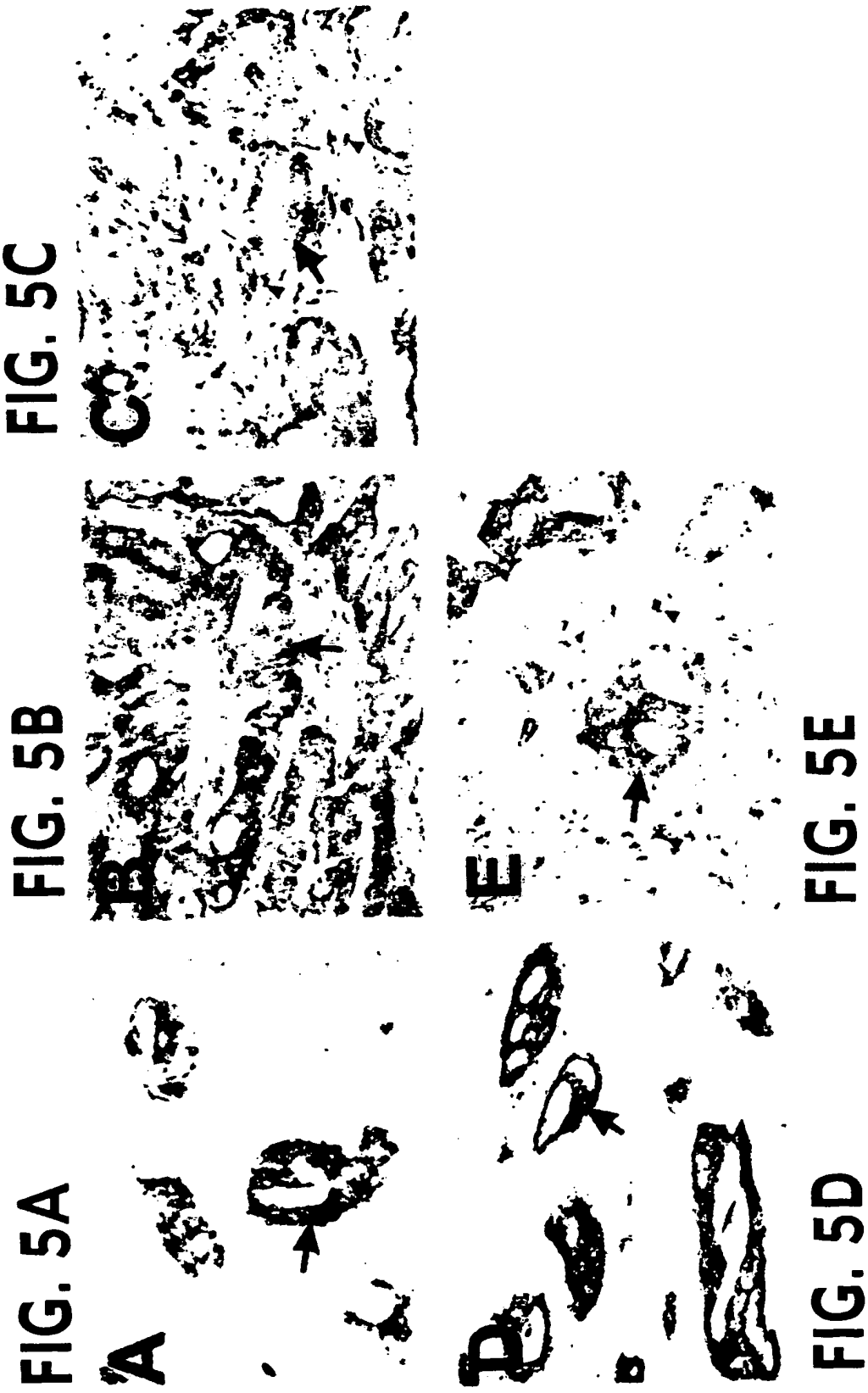


FIG. 5A

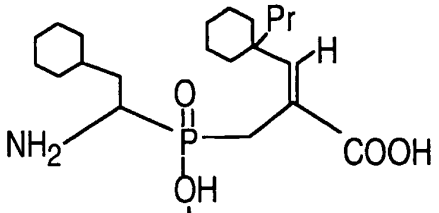
FIG. 5B

FIG. 5C

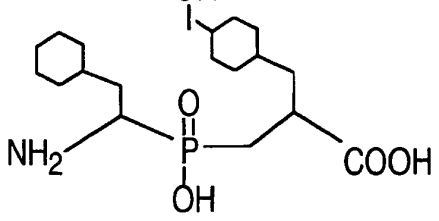
FIG. 5D

FIG. 5E

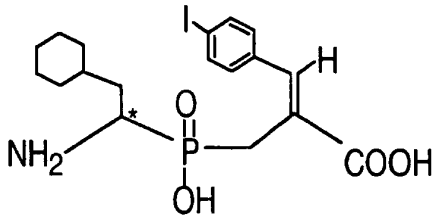
FIG.6



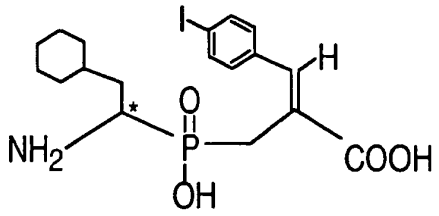
G-89 ≡ 10 nM



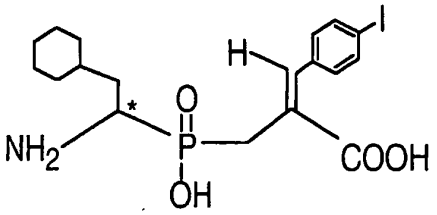
G-90 ≡ 17.6 nM



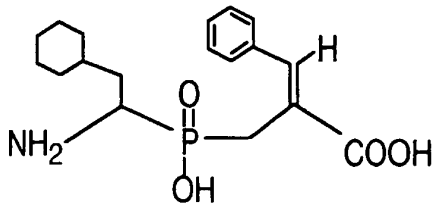
G-132 ≡ 0.8nM
*(RACEMATE)



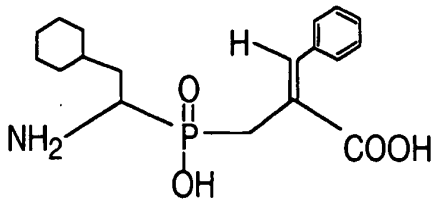
G-133 ≡ 0.6nM
*(PURE STEREOISOMER)
(L?)



G-136 ≡ 2.3nM
*(RACEMATE)



G-110 ≡ 1.2nM



G-111 ≡ 19.5nM

FIG. 7

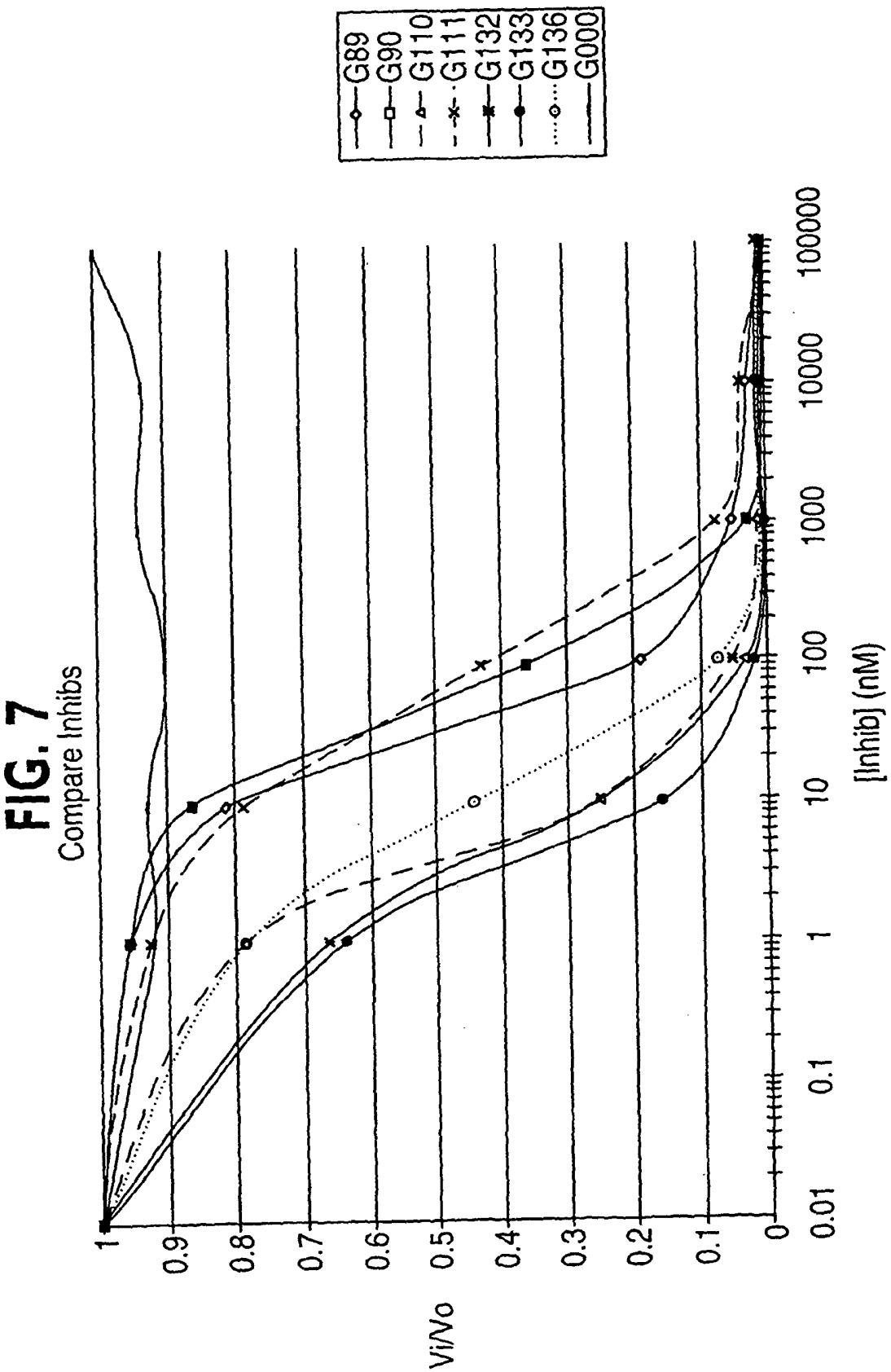

Compare Inhibs
G89
G90
G110
G111
G132
G133
G136
G000
Vi/Vo
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.01
0.1
1
10
100
1000
10000
100000
[Inhib] (nM)

FIG. 8

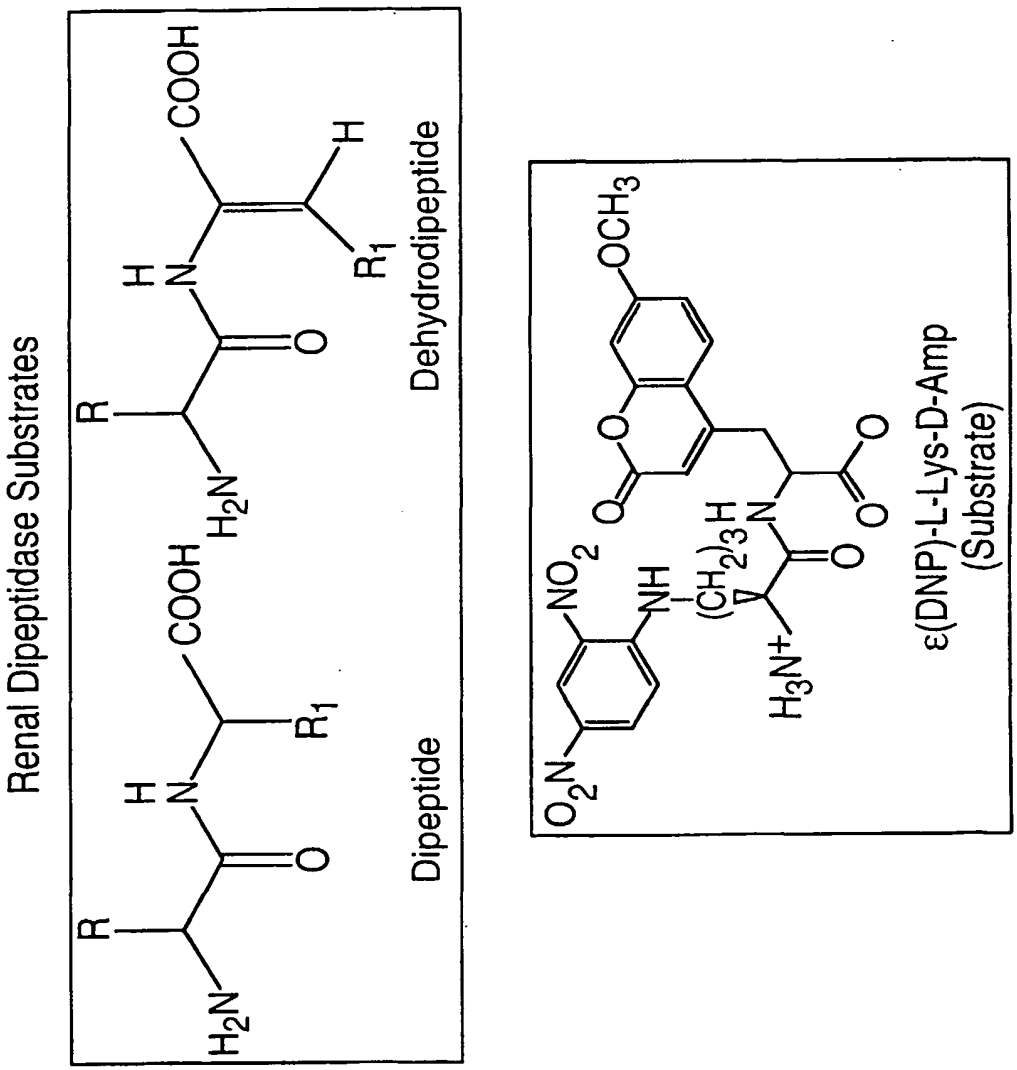
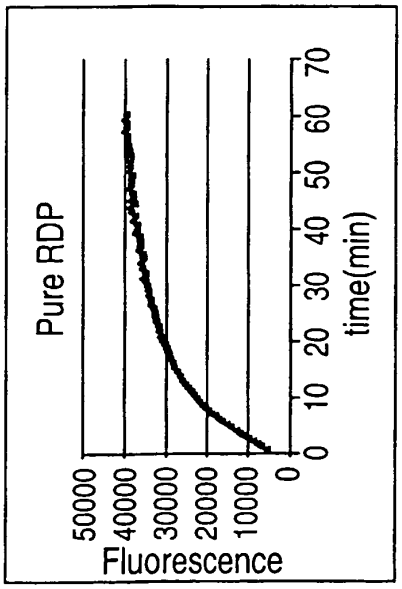
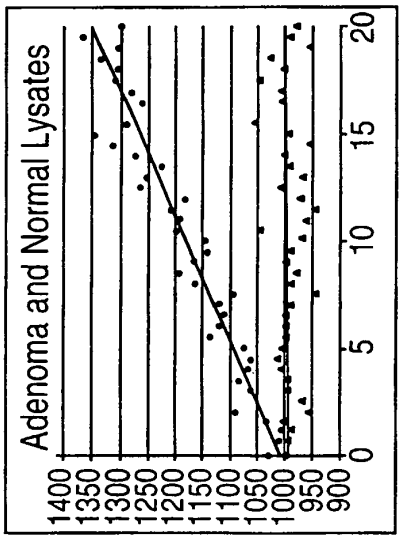
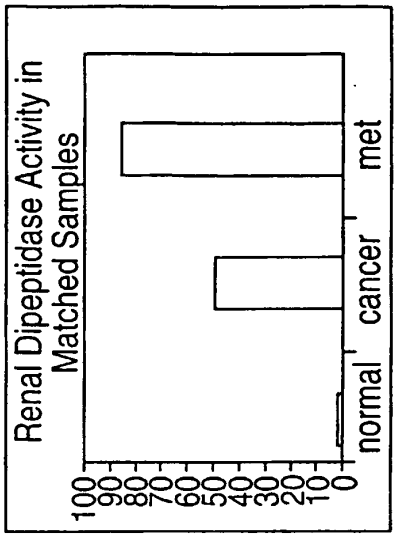

Renal Dipeptidase Substrates
Dipeptide
Dehydrodipeptide
ε(DNP)-L-Lys-D-Amp
(Substrate)

FIG. 9

Renal Dipeptidase Enzyme Activity in Human Tumors







REFERENCES CITED IN THE DESCRIPTION

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专利名称(译)	分泌的和细胞表面基因在良性和恶性结肠直肠肿瘤中表达		
公开(公告)号	EP1430071B1	公开(公告)日	2011-04-06
申请号	EP2002773302	申请日	2002-09-09
[标]申请(专利权)人(译)	医学的约翰霍普金斯大学医学院		
申请(专利权)人(译)	医学的约翰霍普金斯大学医学院		
当前申请(专利权)人(译)	医学的约翰霍普金斯大学医学院		
[标]发明人	BUCKHAULTS PHILLIP KINZLER KENNETH W VOGELSTEIN BERT		
发明人	BUCKHAULTS, PHILLIP KINZLER, KENNETH, W. VOGELSTEIN, BERT		
IPC分类号	C07H21/04 C12N9/48 C07K14/495 G01N33/53 A61K49/00 A61K51/00 C07K14/47 C07K14/705 C12N15/09 C12Q1/37 C12Q1/68 G01N21/78 G01N37/00		
CPC分类号	C07K14/495 C07K14/47 C07K14/705		
代理机构(译)	汤布林，ADRIAN GEORGE		
优先权	60/317494 2001-09-07 US 60/383805 2002-05-30 US		
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外部链接	Espacenet		

摘要(译)

基因表达的系列分析（SAGE）用于鉴定编码在结肠直肠的良性和恶性肿瘤中表达的分泌或细胞表面蛋白的转录物。从正常，腺瘤和癌性结肠上皮分析总共290,394个标签。在观察到的21,343种不同转录物中，发现957种在正常和腺瘤之间或在正常和癌症之间差异表达。 49个转录物在腺瘤中升高≥20倍，40个转录物在癌症中升高≥20倍，并且9个转录物在两者中升高≥20倍。预测六种这九种转录物（TGFBI，LYS，RDP，MIC-1，REGA和DEHL）的产物被分泌或驻留在细胞表面上，并且更详细地分析这些。通过对这六种基因中的每一种进行定量PCR分析来确认SAGE预测的异常表达水平。此外，通过原位杂交和通过PCR分析从原发性肿瘤纯化的上皮细胞免疫亲和来鉴定负责表达升高的细胞类型。