candidates for immunotherapeutic targets.

SUMMARY ~~OF THE INVENTION~~

To verify the molecular role in colorectal carcinogenesis and find novel therapeutic targets for colorectal carcinoma (CRC) patients, we have performed expression profiles using a cDNA microarray consisting of 23040 genes (Lin YM, *et al.*, Oncogene 21: 4120-4128, 2002). Among the genes showing up-regulated expression in colorectal tumors, we have focused on *TOM34* (34kDa-Translocase of the outer mitochondrial membrane), because its expression levels were frequently enhanced in 16 of 20 CRC samples examined. Multiple tissue northern blotting analysis revealed that this gene was abundantly expressed in the testis and ovary, and weakly in the prostate, spleen and colon but not in any of 11 other normal adult tissues examined. Immunohistochemical staining of TOM34 showed significant accumulation in CRC tissues compared with their corresponding non-cancerous mucosae.

In the present ~~invention~~disclosure, it was confirmed that human TOM34 is frequently up-regulated in CRCs, and that it is expressed in the testis and the ovary but not in other 15 normal adults tissues examined. Since suppression of this TOM34 by siRNA markedly reduced the growth of colon cancer cells, the gene product may be a potential therapeutic target for human tumors as well as a useful diagnostic marker.

Furthermore, it was considered that TOM34 might serve as a TAA (Tumor Associated Antigen) which could induce significant cellular immune response against colon cancer. To examine this hypothesis, we stimulated PBMC harvested from healthy volunteers with peptides derived from TOM34 and successfully obtained peptide-specific CTL clones which also recognize and kill the tumor cells expressing the antigen.

Specificaly, transfection of colon cancer HCT116 and RKO cells with small-interfering RNA (siRNA) specific to *TOM34*, effectively suppressed its expression, and dramatically inhibited the cell growth.

Furthermore, we examined the peptides with sequences derived from TOM34 for their potential to serve as antigenic epitope peptides and have explored a novel epitope peptide for effective cancer immunotherapy which could induce significant cellular immune response against colon cancer. Peripheral Blood Mononuclear Cells (PBMC) of healthy donors were stimulated using the peptides having partial sequences of TOM34. Those peptides were chosen that were predicted to bind to HLA-A*2402. We have successfully identified a specific antigen peptide whose sequence derived from TOM34 that could induce cytotoxic T lymphocyte (CTL) cell lines showing specific cytotoxicity against cells that

present the antigen in HLA-A24-restricted manner. CTL clones were established from these CTL cell lines. Further analysis of the CTL clones showed that they had potent cytotoxic activity not only against the peptide-loaded target cells but also against the cells that endogenously express TOM34. Furthermore, cold target inhibition assay indicated that the CTL clones specifically recognized the antigen peptide in the complex of MHC Class I molecule. These results strongly suggest that the peptide is a HLA-A24-restricted epitope peptide which can induce potent and specific immune response against colon cancer cells expressing TOM34.

These findings suggest that TOM34 is involved in the growth of cancer cells, and may contribute to the development of novel anticancer drugs and/or diagnosis for CRCs.

The present ~~invention~~disclosure is based on the discovery of a pattern of gene expression of TOM34. The nucleotide sequence and amino acid sequence of TOM34 are set forth in SEQ ID NO:60 and 61, respectively. These sequence are also availabale from Genbank Accession NO. AB085681.

Accordingly, the present ~~invention~~disclosure provides a method of diagnosing or determining a predisposition to colon cancer in a subject by determining an expression level of TOM34 in a patient-derived biological sample, such as tissue sample. A normal cell is one obtained from colon tissue. An alteration, *e.g.*, an increase in the level of expression of a gene as compared to a normal control level of the gene, indicates that the subject suffers from or is at risk of developing colon cancer.

In a first aspect, there is provided a method of screening for a compound for treating or preventing colon cancer, said method comprising the steps of:

a) contacting a test compound with a polypeptide encoded by a polynucleotide of TOM34;

b) detecting the binding activity between the polypeptide and the test compound; and

c) selecting the test compound that binds to the polypeptide.

In a second aspect, there is provided a method of screening for a compound for treating or preventing colon cancer, said method comprising the steps of:

a) contacting a candidate compound with a cell expressing TOM34; and

b) selecting the candidate compound that reduces the expression level of TOM34 as compared to the expression level of TOM34 detected in the absence of the test compound.

In a third aspect, there is provided a method of screening for a compound for treating or preventing colon cancer, said method comprising the steps of:

a) contacting a test compound with a polypeptide encoded by a polynucleotide of TOM34;

b) detecting the biological activity of the polypeptide of step (a); and

c) selecting the test compound that suppresses the biological activity of the polypeptide encoded by the polynucleotide of TOM34 as compared to the biological activity of said polypeptide detected in the absence of the test compound.

In a fourth aspect, there is provided a method of screening for compound for treating or preventing colon cancer, said method comprising the steps of:

a) contacting a candidate compound with a cell into which a vector, comprising the transcriptional regulatory region of TOM34 and a reporter gene that is expressed under the control of the transcriptional regulatory region, has been introduced;

b) measuring the expression or activity of said reporter gene; and

c) selecting the candidate compound that reduces the expression or activity of said reporter gene.

In a fifth aspect, there is provided a kit when used for detecting colon cancer comprising a detection reagent which binds to (a) TOM34, or (b) polypeptides encoded by TOM34 and a positive control reagent for colon cancer.

In a sixth aspect, there is provided an isolated decapeptide comprising the amino acid sequence of SEQ ID NO: 7.

In a seventh aspect, there is provided an isolated peptide having cytotoxic T cell inducibility, wherein the peptide consists of the amino acid sequence of SEQ ID NO: 7, in which 1 or 2 amino acids are substituted or added.

In an eighth aspect, there is provided a pharmaceutical composition for treating or preventing colon cancer, wherein said composition comprises a pharmaceutically effective amount of the peptide as defined above as active ingredient.

When used in the context of the present ~~invention~~disclosure the term "predisposition to colon cancer " encompasses a state of a subject of being predisposed to, having a tendency, prevalence, inclination or susceptibility to colon cancer. Moreover, said term also encompasses that a subject is at a risk of acquiring colon cancer.

In the context of the present ~~invention~~disclosure, the phrase "control level" refers to a protein expression level detected in a control sample. A control level can be a single expression pattern derived from a single reference population or from a plurality of expression patterns. For example, the control level can be a database of expression patterns from previously tested cells. A "normal control level" refers to a level of gene expression detected in a normal, healthy individual or in a population of individuals known not to be suffering from colon cancer. A normal individual is one with no clinical symptoms of colon cancer.

An increase in the expression level of TOM34 detected in a test sample as compared to a normal control level indicates that the subject (from which the sample was obtained)

suffers from or is at risk of developing CRC.

According to the present ~~invention~~disclosure, gene expression level is deemed "altered" when gene expression is increased 10%, 25%, 50% as compared to the control level. Alternatively, an expression level is deemed "increased" when gene expression is increased by at least 0.1, at least 0.2, at least 1, at least 2, at least 5, or at least 10 or more fold as compared to a control level. Expression is determined by detecting hybridization, *e.g.*, TOM34 probe to a gene transcript of the patient-derived tissue sample.

In the context of the present ~~invention~~disclosure, the patient-derived tissue sample is any tissue obtained from a test subject, *e.g.*, a patient known to or suspected of having colon cancer. For example, the tissue may contains an epithelial cell. More particularly, the tissue may be an epithelial cell from a colorectal carcinoma.

The present ~~invention~~disclosure further provides methods of identifying an agent that inhibits the expression or activity of TOM34, by contacting a test cell expressing TOM34 with a test compound and determining the expression level of TOM34 or the activity of its gene product. The test cell may be an epithelial cell, such as an epithelial cell obtained from a colorectal carcinoma. A decrease in the expression level of TOM34 or the activity of its gene product as compared to a control level or activity of the gene or gene product indicates that the test compound is an inhibitor of TOM34 and may be used to reduce a symptom of colon cancer.

The present ~~invention~~disclosure also provides a kit comprising a detection reagent which binds to TOM34 nucleic acids or polypeptides.

Therapeutic methods of the present ~~invention~~disclosure include a method of treating or preventing colon cancer in a subject including the step of administering to the subject an antagonist or inhibitor of TOM34 which is, for example, an antisense composition or an antibody composition. The antagonist or inhibitor can either act on the nucleic acid or protein level so as to reduce or inhibit TOM34 expression or activity.

In the context of the present ~~invention~~disclosure, the antisense composition reduces the expression of the specific target gene. For example, the antisense composition may contain a nucleotide which is complementary to TOM34 sequence. Alternatively, the present method may include the steps of administering to a subject a small interfering RNA (siRNA) composition. In the context of the present ~~invention~~disclosure, the siRNA composition reduces the expression of TOM34. In yet another method, the treatment or prevention of colon cancer in a subject may be carried out by administering to a subject a ribozyme composition. In the context of the present ~~invention~~disclosure, the nucleic acid-specific ribozyme composition reduces the

expression of TOM34. Actually, the inhibition effect of the siRNA for TOM34 was confirmed. For example, it has been clearly shown that the siRNA for TOM34 inhibit cell proliferation of colon cancer cells in the examples section. Thus, in the present ~~invention~~disclosure, TOM34 is a preferable therapeutic target of the colon cancer.

The present ~~invention~~disclosure also includes vaccines and vaccination methods. For example, a method of treating or preventing colon cancer in a subject may involve administering to the subject a vaccine containing a polypeptide encoded by a nucleic acid of TOM34 or an immunologically active fragment of such a polypeptide. In the context of the present ~~invention~~disclosure, an immunologically active fragment is a polypeptide that is shorter in length than the full-length naturally-occurring protein yet which induces an immune response analogous to that induced by the full-length protein. For example, an immunologically active fragment should be at least 8 residues in length and capable of stimulating an immune cell such as a T cell or a B cell. Immune cell stimulation can be measured by detecting cell proliferation, elaboration of cytokines (*e.g.*, IL-2), or production of an antibody.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this ~~invention~~disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present ~~invention~~disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference herein in their entirety. Nothing herein is to be construed as an admission that the ~~invention~~present disclosure is not entitled to antedate such disclosure by virtue of prior ~~invention~~disclosure.

In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

One advantage of the methods described herein is that the disease is identified prior to detection of overt clinical symptoms of colon cancer. Other features and advantages of the ~~invention~~present disclosure will be apparent from the following detailed description, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the expression level of *TOM34* gene in various tissues. (A) Semi-quantitative RT-PCR analysis of *TOM34* in colon cancer tissues and their corresponding non-cancerous mucosa. T, tumor tissue; N, normal tissue. Expression of *GAPDH* served as an internal control. (B) Multiple-tissue northern blot analysis

CLAIMS

1. A method of diagnosing colon cancer or a predisposition for developing colon cancer in a subject, comprising determining a level of expression of TOM34 in a patient-derived biological sample, wherein an increase in said sample expression level as compared to a normal control level of said gene indicates that said subject suffers from or is at risk of developing colon cancer.

2. The method of claim 1, wherein said sample expression level is at least 10% greater than said normal control level.

3. The method of claim 1, wherein gene expression level is determined by a method selected from the group consisting of:
a) detecting mRNA of TOM34,
b) detecting a protein encoded by TOM34, and
c) detecting a biological activity of TOM34.

4. The method of claim 1, wherein said patient-derived biological sample comprises an epithelial cell.

5. The method of claim 1, wherein said patient-derived biological sample comprises a colon cancer cell.

6. The method of claim 1, wherein said patient-derived biological sample comprises an epithelial cell from a colon cancer cell.

7. A method of screening for a compound for treating or preventing colon cancer, said method comprising the steps of:
a) contacting a test compound with a polypeptide encoded by a polynucleotide of TOM34;
b) detecting the binding activity between the polypeptide and the test compound; and
c) selecting the test compound that binds to the polypeptide.

8. A method of screening for a compound for treating or preventing colon cancer, said method comprising the steps of:
a) contacting a candidate compound with a cell expressing TOM34; and
b) selecting the candidate compound that reduces the expression level of TOM34 as compared to the expression level of TOM34 deteced in the absence of the test compound.

9. The method of claim 8, wherein said cell comprises a colon cancer cell.

10. A method of screening for a compound for treating or preventing colon cancer, said

method comprising the steps of:

a) contacting a test compound with a polypeptide encoded by a polynucleotide of TOM34;

b) detecting the biological activity of the polypeptide of step (a); and

c) selecting the test compound that suppresses the biological activity of the polypeptide encoded by the polynucleotide of TOM34 as compared to the biological activity of said polypeptide detected in the absence of the test compound.

11. A method of screening for compound for treating or preventing colon cancer, said method comprising the steps of:

a) contacting a candidate compound with a cell into which a vector, comprising the transcriptional regulatory region of TOM34 and a reporter gene that is expressed under the control of the transcriptional regulatory region, has been introduced;

b) measuring the expression or activity of said reporter gene; and

c) selecting the candidate compound that reduces the expression or activity of said reporter gene.

12. A kit comprising a detection reagent which binds to (a) TOM34, or (b) polypeptides encoded by TOM34.

13. A method of treating or preventing colon cancer in a subject comprising administering to said subject an antisense composition, said antisense composition comprising a nucleotide sequence complementary to a coding sequence of TOM34.

14. A method of treating or preventing colon cancer in a subject comprising administering to said subject an siRNA composition, wherein said siRNA composition reduces the expression of TOM34.

15. The method of claim 14, wherein said siRNA comprises the sense strand comprising a nucleotide sequence of SEQ ID NO: 48 or SEQ ID NO: 52.

16. The method of claim 15, wherein said siRNA has the general formula 5'-[A]-[B]-[A']-3', wherein [A] is a ribonucleotide sequence corresponding to a sequence of SEQ ID NO: 48 or SEQ ID NO: 52, [B] is a ribonucleotide loop sequence consisting of 3 to 23 nucleotides, and [A'] is a ribonucleotide sequence consisting of the complementary sequence of [A].

17. A method of treating or preventing colon cancer in a subject comprising the step of administering to said subject a pharmaceutically effective amount of an antibody, or immunologically active fragment thereof, that binds to a protein of TOM34.

18. A method of treating or preventing colon cancer in a subject comprising administering to said subject a vaccine comprising (a) a polypeptide encoded by a nucleic acid of TOM34, (b) an immunologically active fragment of said polypeptide, or (c) a polynucleotide encoding the polypeptide or said immunologically active fragment of (b).

19. The method of claim 18, wherein said immunologically active fragment is either or both of (i) decapeptide comprising the amino acid sequence of SEQ ID NO: 7, and (ii) a peptide having cytotoxic T cell inducibility, wherein the peptide comprises the amino acid sequence of SEQ ID NO: 7, wherein 1, 2, or several amino acids are substituted or added.

20. A method of inducing an anti-tumor immunity, said method comprising the step of contacting with an antigen presenting cell a polypeptide, a polynucleotide encoding the polypeptide or a vector comprising the polynucleotide, wherein the polypeptide is encoded by TOM34 or an immunologically active fragment of said polypeptide.

21. The method of claim 20, wherein said immunologically active fragment is either or both of (i) a decapeptide comprising the amino acid sequence of SEQ ID NO: 7, and (ii) a peptide having cytotoxic T cell inducibility, wherein the peptide comprises the amino acid sequence of SEQ ID NO: 7, wherein 1, 2, or several amino acids are substituted or added.

22. The method of claim 20, wherein the method further comprises the step of administering the antigen presenting cell to a subject.

23. A method of treating or preventing colon cancer in a subject, said method comprising the step of administering a compound obtained by a method according to any one of claims 7-11.

24. A composition for treating or preventing colon cancer, said composition comprising a pharmaceutically effective amount of an antisense polynucleotide or siRNA against a polynucleotide of TOM34.

25. The composition of claim 24, wherein said siRNA comprises the sense strand comprising a nucleotide sequence of SEQ ID NO: 48 or SEQ ID NO: 52.

26. The composition of claim 25, wherein said siRNA has the general formula 5'-[A]-[B]-[A']-3', wherein [A] is a ribonucleotide sequence corresponding to a sequence of SEQ ID NO: 48 or SEQ ID NO: 56, [B] is a ribonucleotide loop sequence consisting of 3 to 23 nucleotides, and [A'] is a ribonucleotide sequence consisting of the complementary

sequence of [A].

27. A composition for treating or preventing colon cancer, said composition comprising a pharmaceutically effective amount of an antibody or fragment thereof that binds to a protein encoded by TOM34.

28. A composition for treating or preventing colon cancer, said composition comprising as an active ingredient a pharmaceutically effective amount of a compound selected by a method of any one of claims 7-11, and a pharmaceutically acceptable carrier.

29. A decapeptide comprising the amino acid sequence of SEQ ID NO: 7.

30. A peptide having cytotoxic T cell inducibility, wherein the peptide comprises the amino acid sequence of SEQ ID NO: 7, wherein 1, 2, or several amino acids are substituted or added.

31. The peptide of claim 30, wherein the second amino acid from the N terminus is phenylalanine, tyrosine, methionine, or tryptophan.

32. The peptide of claim 30, wherein the C-terminal amino acid is phenylalanine, leucine, isoleucine, tryptophan, or methionine.

33. A pharmaceutical composition for treating or preventing colon cancer, wherein said composition comprises pharmaceutically effective amount of the peptide of claim 29 or 30 as active ingredient.

CLAIMS

1. A method of screening for a compound for treating or preventing colon cancer, said method comprising the steps of:
a) contacting a test compound with a polypeptide encoded by a polynucleotide of TOM34;
b) detecting the binding activity between the polypeptide and the test compound; and
c) selecting the test compound that binds to the polypeptide.

2. A method of screening for a compound for treating or preventing colon cancer, said method comprising the steps of:
a) contacting a candidate compound with a cell expressing TOM34; and
b) selecting the candidate compound that reduces the expression level of TOM34 as compared to the expression level of TOM34 detected in the absence of the test compound.

3. The method of claim 2, wherein said cell comprises a colon cancer cell.

4. A method of screening for a compound for treating or preventing colon cancer, said method comprising the steps of:
a) contacting a test compound with a polypeptide encoded by a polynucleotide of TOM34;
b) detecting the biological activity of the polypeptide of step (a); and
c) selecting the test compound that suppresses the biological activity of the polypeptide encoded by the polynucleotide of TOM34 as compared to the biological activity of said polypeptide detected in the absence of the test compound.

5. A method of screening for compound for treating or preventing colon cancer, said method comprising the steps of:
a) contacting a candidate compound with a cell into which a vector, comprising the transcriptional regulatory region of TOM34 and a reporter gene that is expressed under the control of the transcriptional regulatory region, has been introduced;
b) measuring the expression or activity of said reporter gene; and
c) selecting the candidate compound that reduces the expression or activity of said reporter gene.

6. A kit when used for detecting colon cancer comprising a detection reagent which binds to (a) TOM34, or (b) polypeptides encoded by TOM34 and a positive control reagent for colon cancer.

7. An isolated decapeptide comprising the amino acid sequence of SEQ ID NO: 7.

8. An isolated peptide having cytotoxic T cell inducibility, wherein the peptide consists of the amino acid sequence of SEQ ID NO: 7, in which 1 or 2 amino acids are substituted or added.

9. The isolated peptide of claim 8, wherein the second amino acid from the N terminus of the amino acid sequence of SEQ ID NO: 7 is phenylalanine, tyrosine, methionine, or tryptophan.

10. The isolated peptide of claim 8, wherein the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 7 is phenylalanine, leucine, isoleucine, tryptophan, or methionine.

11. A pharmaceutical composition for treating or preventing colon cancer, wherein said composition comprises a pharmaceutically effective amount of the peptide of claim 7 or 8 as active ingredient.