SUMMARY OF THE INVENTION

The present invention is based at least in part on the discovery of multiple somatic mutational events in the ERBB3 receptor of the human epidermal growth factor receptor (HER) family of receptor tyrosine kinases (RTK), that are associated with various human tumors including, without limitation, gastric and colon tumors. It is believed that these mutations predispose and/or directly contribute to human tumorigenesis. Indeed, as described herein, there is evidence that some of the mutations promote oncogenesis *in vivo*.

In one aspect, the present invention provides ErbB3 cancer detecting agents. In one embodiment, the ErbB3 cancer detecting agent is an ErbB3 gastrointestinal cancer detecting agent. In another embodiment, the detecting agent comprises a reagent capable of specifically binding to an ErbB3 mutation in an ErbB3 nucleic acid sequence. In one other embodiment, the ErbB3 nucleic acid sequence comprises ~~SEQ ID NO:3~~ or 1.

In some embodiments, the reagent comprises a polynucleotide of formula

5' X_a-Y-Z_b 3' Formula I,

wherein

X is any nucleic acid and a is between about 0 and about 250;

Y is an ErbB3 mutation codon; and

Z is any nucleic acid and b is between about 0 and about 250.

In one other embodiment, the mutation codon encodes (i) an amino acid at a position of SEQ ID NO:2 selected from the group consisting of 104, 809, 232, 262, 284, 325, 846, 928, 60, 111, 135, 295, 406, 453, 498, 1089, and 1164; or (ii) a stop codon at position 193. In one other embodiment, the gastrointestinal cancer is gastric cancer or colon cancer.

In another aspect, the present invention provides a method of determining the presence of ErbB3 gastrointestinal cancer in a subject. In one embodiment, the method comprises detecting in a biological sample obtained from the subject a mutation in a nucleic acid sequence encoding ErbB3, wherein the mutation results in an amino acid change at at least one position of the ErbB3 amino acid sequence and wherein the mutation is indicative of an ErbB3 gastrointestinal cancer in the subject. In another embodiment, the mutation resulting in an amino acid change is at a position of SEQ ID NO:2 selected from the group consisting of 104, 809, 232, 262, 284, 325, 846, 928, 60, 111, 135, 295, 406, 453, 498, 1089, 1164, and 193. In other embodiments, the gastrointestinal cancer is gastric cancer or colon cancer.

In another aspect, the present invention provides a method of determining the presence of ErbB3 cancer in a subject. In one embodiment, the method comprises detecting in a biological sample obtained from the subject the presence or absence of an amino acid mutation in a nucleic

KD. (D-F) Western blot showing pERBB3, pERBB2, pAKT and pERK status of BaF3 cells expressing ERBB3 mutants either alone (D) or in combination with ERBB2 (E) or ERBB2-KD (F). (G) Anti-NRG1, a NRG1 neutralizing antibody, does not affect IL-3-independent survival of BaF3 cells promoted by ERBB3 mutants co-expressed with ERBB2. (H) ERBB3 ECD mutants show increased IL-3 independent BaF3 survival in response to increasing dose of exogenous NRG1. BaF3 studies were performed in the absence of IL-3 (A-H) and NRG1 (A-F). EV = empty vector; M = monomer & D = dimer.

Figure 38A-J. shRNA-mediated ERBB3 knockdown delays tumor growth. (A-J) CW-2 and DV-90 stably expressing inducible ERBB3 targeting shRNA upon dox-induction showed lower levels of ERBB3 and pERK (A, B), anchorage independent growth (C-F) and reduced in vivo growth (H, J) compared to uninduced cells (A-F) or cells expressing luciferase targeting shRNA (A-F, G & I). Data in (E, F) represent the number of anchorage independent colonies formed quantitated from multiple filed of images like the one show in (C, D). Data are shown as Mean ± SEM.

Figure 39 provides a nucleic acid sequence ~~(SEQ ID NO: 3)~~ and amino acid sequence (SEQ ID NO: 2) for ErbB3. The mutations of the present invention are indicated by the boxed amino acids and boxed/underlined codons.

DETAILED DESCRIPTION OF THE INVENTION

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

Definitions

Unless otherwise defined, all terms of art, notations and other scientific terminology used herein are intended to have the meanings commonly understood by those of skill in the art to

antibody that has been identified and separated and/or recovered from a cell or cell culture from which it was expressed. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and can include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes antibodies in situ within recombinant cells, because at least one component of the polypeptide natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by at least one purification step.

An "ErbB3 cancer detecting agent" refers to an agent that is capable of detecting a mutation associated with an ErbB3 cancer within an ERBB3 nucleic acid sequence or amino acid sequence. Typically, the detecting agent comprises a reagent capable of specifically binding to an ERBB3 sequence. In a preferred embodiment, the reagent is capable of specifically binding to an ErbB3 mutation in an ERBB3 nucleic acid sequence. In one embodiment, the detecting agent comprises a polynucleotide capable of specifically hybridizing to an ERBB3 nucleic acid sequence (e.g., SEQ ID NO:1 or ~~3~~). In some embodiments, the polynucleotide is a probe comprising a nucleic acid sequence that specifically hybridizes to an ErbB3 sequence comprising a mutation. In another embodiment, the detecting agent comprises a reagent capable of specifically binding to an ERBB3 amino acid sequence. In another embodiment, the amino acid sequence comprises a mutation as described herein. The detecting agents may further comprise a label. In a preferred embodiment, the ErbB3 cancer detecting agent is an ErbB3 gastro-intestinal cancer detecting agent.

ErbB3 Somatic Mutations

In one aspect, the invention provides methods of detecting the presence or absence of ErbB3 somatic mutations associated with cancer in a sample from a subject, as well as methods of diagnosing and prognosing cancer by detecting the presence or absence of one or more of these somatic mutations in a sample from a subject, wherein the presence of the somatic mutation indicates that the subject has cancer. ErbB3 somatic mutations associated with cancer risk were identified using strategies including genome-wide association studies, modifier screens, and family-based screening.

Somatic mutations or variations for use in the methods of the invention include variations in ErbB3, or the genes encoding this protein. In some embodiments, the somatic mutation is in

nucleotide polymorphisms (SNPs) in the germline and any additional SNPs that are in linkage disequilibrium. Indeed, any additional SNP in linkage disequilibrium with a first SNP associated with cancer will be associated with cancer. Once the association has been demonstrated between a given SNP and cancer, the discovery of additional SNPs associated with cancer can be of great interest in order to increase the density of SNPs in this particular region.

Methods for identifying additional SNPs and conducting linkage disequilibrium analysis are well known in the art. For example, identification of additional SNPs in linkage disequilibrium with the SNPs disclosed herein can involve the steps of: (a) amplifying a fragment from the genomic region comprising or surrounding a first SNP from a plurality of individuals; (b) identifying of second SNPs in the genomic region harboring or surrounding said first SNP; (c) conducting a linkage disequilibrium analysis between said first SNP and second SNPs; and (d) selecting said second SNPs as being in linkage disequilibrium with said first marker. This method may be modified to include certain steps preceding step (a), such as amplifying a fragment from the genomic region comprising or surrounding a somatic mutation from a plurality of individuals, and identifying SNPs in the genomic region harboring or surrounding said somatic mutation.

ErbB3 Cancer Detecting Agents

In one aspect, the present invention provides ErbB3 cancer detecting agents. In one embodiment, the detecting agent comprises a reagent capable of specifically binding to an ErbB3 sequence shown in Figure 39 (amino acid sequence of SEQ ID NO: 2 or nucleic acid sequence of SEQ ID NO:~~3~~). In another embodiment, the detecting agent comprises a polynucleotide capable of specifically hybridizing to an ERBB3 nucleic acid sequence shown in Figure 2 (SEQ ID NO: 1) or Figure 39 (SEQ ID NO:~~3~~). In a preferred embodiment, the polynucleotide comprises a nucleic acid sequence that specifically hybridizes to an ErbB3 nucleic acid sequence comprising a mutation shown in Figure 39 (SEQ ID NO:~~3~~).

In another aspect, the ErbB3 cancer detecting agents comprise a polynucleotide having a particular formula. In one embodiment, the polynucleotide formula is

$$5' \quad X_a\text{-}Y\text{-}Z_b \quad 3' \qquad \text{Formula I}$$

, wherein

X is any nucleic acid and a is between about 0 and about 250 (i.e., in the 5' direction);

Y represents an ErbB3 mutation codon; and

WHAT IS CLAIMED IS:

1. An ErbB3 gastrointestinal cancer detecting agent comprising a reagent capable of specifically binding to an ErbB3 mutation in an ErbB3 nucleic acid sequence.

2. The cancer detecting agent of claim 1, wherein the ErbB3 nucleic acid sequence comprises SEQ ID NO:3 or 1.

3. The cancer detecting agent of claim 1, wherein the reagent comprises a polynucleotide of formula

5' X_a-Y-Z_b 3' Formula I,

wherein

X is any nucleic acid and a is between about 0 and about 250;

Y is an ErbB3 mutation codon; and

Z is any nucleic acid and b is between about 0 and about 250.

4. The cancer detecting agent of claim 3, wherein the mutation codon encodes (i) an amino acid at a position of SEQ ID NO:2 selected from the group consisting of 104, 809, 232, 262, 284, 325, 846, 928, 60, 111, 135, 295, 406, 453, 498, 1089, and 1164; or (ii) a stop codon at position 193.

5. A method of determining the presence of ErbB3 gastrointestinal cancer in a subject comprising detecting in a biological sample obtained from the subject a mutation in a nucleic acid sequence encoding ErbB3, wherein the mutation results in an amino acid change at at least one position of the ErbB3 amino acid sequence and wherein the mutation is indicative of an ErbB3 gastrointestinal cancer in the subject.

6. The method of claim 5, wherein the mutation resulting in an amino acid change is at a position of SEQ ID NO:2 selected from the group consisting of 104, 809, 232, 262, 284, 325, 846, 928, 60, 111, 135, 295, 406, 453, 498, 1089, 1164, and 193.

7. A method of determining the presence of ErbB3 cancer in a subject comprising detecting in a biological sample obtained from the subject the presence or absence of an amino

acid mutation in a nucleic acid sequence encoding ErbB3, wherein the mutation results in an amino acid change at at least one position in SEQ ID NO: 2 selected from the group consisting of 104, 809, 232, 262, 284, 325, 846, 928, 60, 111, 135, 295, 406, 453, 498, 1089, 1164, 193, 492, and 714, and wherein the presence of the mutation is indicative of an ErbB3 cancer in the subject.

8. The method of claim 5 or 7, further comprising administering a therapeutic agent to said subject.

9. The method of claim 8, wherein the therapeutic agent is an ErbB inhibitor.

10. The method of claim 9, wherein the ErbB inhibitor is selected from the group consisting of an EGFR antagonist, an ErbB2 antagonist, an ErbB3 antagonist, an ErbB4 antagonist, and an EGFR/ErbB3 antagonist.

11. The method of claim 10, wherein the inhibitor is a small molecule inhibitor.

12. The method of claim 10, wherein the antagonist is an antagonist antibody.

13. The method of claim 12, wherein the antibody is selected from the group consisting of a monoclonal antibody, a bispecific antibody, a chimeric antibody, a human antibody, a humanized antibody and an antibody fragment.

14. The detecting agent of claim 1 or the method of claim 5, wherein the gastrointestinal cancer is gastric cancer or colon cancer.

15. The method of claim 7, wherein the ErbB3 cancer is selected from the group consisting of gastric, colon, esophageal, rectal, cecum, non-small-cell lung (NSCLC) adenocarinoma, NSCLC (Squamous carcinoma), renal carcinoma, melanoma, ovarian, lung large cell, small-cell lung cancer (SCLC), hepatocellular (HCC), lung, and pancreatic.

16 The method of claim 5 or 7, further comprising (i) identifying the subject in need and/or (ii) obtaining the sample from a subject in need.

17. The method of claim 5 or 7, wherein the detecting comprises amplifying or

sequencing the mutation and detecting the mutation or sequence thereof.

18 The method of claim 17, wherein the amplifying comprises admixing an amplification primer or amplification primer pair with a nucleic acid template isolated from the sample.

19. The method of claim 18, wherein the primer or primer pair is complementary or partially complementary to a region proximal to or including said mutation, and is capable of initiating nucleic acid polymerization by a polymerase on the nucleic acid template.

20 The method of claim 18, further comprising extending the primer or primer pair in a DNA polymerization reaction comprising a polymerase and the template nucleic acid to generate an amplicon.

21. The method of claim 17, wherein the mutation is detected by a process that includes one or more of: sequencing the mutation in a genomic DNA isolated from the biological sample, hybridizing the mutation or an amplicon thereof to an array, digesting the mutation or an amplicon thereof with a restriction enzyme, or real-time PCR amplification of the mutation.

22. The method of claim 17, comprising partially or fully sequencing the mutation in a nucleic acid isolated from the biological sample.

23. The method of claim 17, wherein the amplifying comprises performing a polymerase chain reaction (PCR), reverse transcriptase PCR (RT-PCR), or ligase chain reaction (LCR) using a nucleic acid isolated from the biological sample as a template in the PCR, RT-PCR, or LCR.

24. A method of treating gastrointestinal cancer in a subject in need comprising

a) detecting in a biological sample obtained from the subject a mutation in a nucleic acid sequence encoding ErbB3, wherein the mutation results in an amino acid change at at least one position of the ErbB3 amino acid sequence and wherein the mutation is indicative of an ErbB3 gastrointestinal cancer in the subject; and

b) administering a therapeutic agent to said subject.

25. The method of claim 24, wherein the mutation resulting in an amino acid change

is at a position of SEQ ID NO:2 selected from the group consisting of 104, 809, 232, 262, 284, 325, 846, 928, 60, 111, 135, 295, 406, 453, 498, 1089, 1164, and 193.

26. A method of treating an ErbB3 cancer in a subject comprising:

a) detecting in a biological sample obtained from the subject the presence or absence of an amino acid mutation in a nucleic acid sequence encoding ErbB3, wherein the mutation results in an amino acid change at at least one position in SEQ ID NO: 2 selected from the group consisting of 104, 809, 232, 262, 284, 325, 846, 928, 60, 111, 135, 295, 406, 453, 498, 1089, 1164, 193, 492, and 714, and wherein the presence of the mutation is indicative of an ErbB3 cancer in the subject; and

b) administering a therapeutic agent to said subject.

27. The method of claim 24 or 26, wherein the therapeutic agent is an ErbB inhibitor.

28. The method of claim 27, wherein the ErbB inhibitor is selected from the group consisting of an EGFR antagonist, an ErbB2 antagonist, an ErbB3 antagonist, an ErbB4 antagonist, and an EGFR/ErbB3 antagonist.

29. The method of claim 28, wherein the antagonist is a small molecule inhibitor.

30. The method of claim 28, wherein the antagonist is an antagonist antibody.

31. The method of claim 30, wherein the antibody is selected from the group consisting of a monoclonal antibody, a bispecific antibody, a chimeric antibody, a human antibody, a humanized antibody and an antibody fragment.

32. The method of claim 24, wherein the gastrointestinal cancer is gastric cancer or colon cancer.

33. The method of claim 26, wherein the ErbB3 cancer is selected from the group consisting of gastric, colon, esophageal, rectal, cecum, colorectal, non-small-cell lung (NSCLC) adenocarinoma, NSCLC (Squamous carcinoma), renal carcinoma, melanoma, ovarian, lung large cell, small-cell lung cancer (SCLC), hepatocellular (HCC), lung, and pancreatic.

SUMMARY OF THE INVENTION

The present invention is based at least in part on the discovery of multiple somatic mutational events in the ERBB3 receptor of the human epidermal growth factor receptor (HER) family of receptor tyrosine kinases (RTK), that are associated with various human tumors including, without limitation, gastric and colon tumors. It is believed that these mutations predispose and/or directly contribute to human tumorigenesis. Indeed, as described herein, there is evidence that some of the mutations promote oncogenesis *in vivo*.

In one aspect, the present invention provides ErbB3 cancer detecting agents. In one embodiment, the ErbB3 cancer detecting agent is an ErbB3 gastrointestinal cancer detecting agent. In another embodiment, the detecting agent comprises a reagent capable of specifically binding to an ErbB3 mutation in an ErbB3 nucleic acid sequence. In one other embodiment, the ErbB3 nucleic acid sequence comprises SEQ ID N0:230 or 1.

In some embodiments, the reagent comprises a polynucleotide of formula

$$5' \; X_a\text{-}Y\text{-}Z_b \; 3' \qquad \text{Formula I,}$$

wherein

X is any nucleic acid and a is between about 0 and about 250;

Y is an ErbB3 mutation codon; and

Z is any nucleic acid and b is between about 0 and about 250.

In one other embodiment, the mutation codon encodes (i) an amino acid at a position of SEQ ID N0:2 selected from the group consisting of 104, 809, 232, 262, 284, 325, 846, 928, 60, 111, 135, 295, 406, 453, 498, 1089, and 1164; or (ii) a stop codon at position 193. In one other embodiment, the gastrointestinal cancer is gastric cancer or colon cancer.

In another aspect, the present invention provides a method of determining the presence of ErbB3 gastrointestinal cancer in a subject. In one embodiment, the method comprises detecting in a biological sample obtained from the subject a mutation in a nucleic acid sequence encoding ErbB3, wherein the mutation results in an amino acid change at at least one position of the ErbB3 amino acid sequence and wherein the mutation is indicative of an ErbB3 gastrointestinal cancer in the subject. In another embodiment, the mutation resulting in an amino acid change is at a position of SEQ ID NO:2 selected from the group consisting of 104, 809, 232, 262, 284, 325, 846, 928, 60, 111, 135, 295, 406, 453, 498, 1089, 1164, and 193. In other embodiments, the gastrointestinal cancer is gastric cancer or colon cancer.

In another aspect, the present invention provides a method of determining the presence of ErbB3 cancer in a subject. In one embodiment, the method comprises detecting in a biological sample obtained from the subject the presence or absence of an amino acid mutation in a nucleic

KD. (D-F) Western blot showing pERBB3, pERBB2, pAKT and pERK status of BaF3 cells expressing ERBB3 mutants either alone (D) or in combination with ERBB2 (E) or ERBB2-KD (F). (G) Anti-NRG1, a NRG1 neutralizing antibody, does not affect IL-3-independent survival of BaF3 cells promoted by ERBB3 mutants co-expressed with ERBB2. (H) ERBB3 ECD mutants show increased IL-3 independent BaF3 survival in response to increasing dose of exogenous NRG1. BaF3 studies were performed in the absence of IL-3 (A-H) and NRG1 (A-F). EV = empty vector; M = monomer & D = dimer.

Figure 38A-J. shRNA-mediated ERBB3 knockdown delays tumor growth. (A-J) CW-2 and DV-90 stably expressing inducible ERBB3 targeting shRNA upon dox-induction showed lower levels of ERBB3 and pERK (A, B), anchorage independent growth (C-F) and reduced in vivo growth (H, J) compared to uninduced cells (A-F) or cells expressing luciferase targeting shRNA (A-F, G & **I**). Data in (E, F) represent the number of anchorage independent colonies formed quantitated from multiple filed of images like the one show in (C, D). Data are shown as Mean ± SEM.

Figure 39 provides a nucleic acid sequence (SEQ ID NO: 230) and amino acid sequence (SEQ ID NO: 231) for ErbB3. The mutations of the present invention are indicated by the boxed amino acids and boxed/underlined codons.

DETAILED DESCRIPTION OF THE INVENTION

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, and biochemistry, which are within the skill of the art. Such techniques are explained fully in the literature, such as, "Molecular Cloning: A Laboratory Manual", 2nd edition (Sambrook et al., 1989); "Oligonucleotide Synthesis" (M.J. Gait, ed., 1984); "Animal Cell Culture" (R.I. Freshney, ed., 1987); "Methods in Enzymology" (Academic Press, Inc.); "Handbook of Experimental Immunology", 4th edition (D.M. Weir & C.C. Blackwell, eds., Blackwell Science Inc., 1987); "Gene Transfer Vectors for Mammalian Cells" (J.M. Miller & M.P. Calos, eds., 1987); "Current Protocols in Molecular Biology" (F.M. Ausubel et al., eds., 1987); and "PCR: The Polymerase Chain Reaction", (Mullis et al., eds., 30 1994).

Definitions

Unless otherwise defined, all terms of art, notations and other scientific terminology used herein are intended to have the meanings commonly understood by those of skill in the art to

antibody that has been identified and separated and/or recovered from a cell or cell culture from which it was expressed. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and can include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes antibodies in situ within recombinant cells, because at least one component of the polypeptide natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by at least one purification step.

An "ErbB3 cancer detecting agent" refers to an agent that is capable of detecting a mutation associated with an ErbB3 cancer within an ERBB3 nucleic acid sequence or amino acid sequence. Typically, the detecting agent comprises a reagent capable of specifically binding to an ERBB3 sequence. In a preferred embodiment, the reagent is capable of specifically binding to an ErbB3 mutation in an ERBB3 nucleic acid sequence. In one embodiment, the detecting agent comprises a polynucleotide capable of specifically hybridizing to an ERBB3 nucleic acid sequence (e.g., SEQ ID NO:1 or 230). In some embodiments, the polynucleotide is a probe comprising a nucleic acid sequence that specifically hybridizes to an ErbB3 sequence comprising a mutation. In another embodiment, the detecting agent comprises a reagent capable of specifically binding to an ERBB3 amino acid sequence. In another embodiment, the amino acid sequence comprises a mutation as described herein. The detecting agents may further comprise a label. In a preferred embodiment, the ErbB3 cancer detecting agent is an ErbB3 gastro-intestinal cancer detecting agent.

ErbB3 Somatic Mutations

In one aspect, the invention provides methods of detecting the presence or absence of ErbB3 somatic mutations associated with cancer in a sample from a subject, as well as methods of diagnosing and prognosing cancer by detecting the presence or absence of one or more of these somatic mutations in a sample from a subject, wherein the presence of the somatic mutation indicates that the subject has cancer. ErbB3 somatic mutations associated with cancer risk were identified using strategies including genome-wide association studies, modifier screens, and family-based screening.

Somatic mutations or variations for use in the methods of the invention include variations in ErbB3, or the genes encoding this protein. In some embodiments, the somatic mutation is in

nucleotide polymorphisms (SNPs) in the germline and any additional SNPs that are in linkage disequilibrium. Indeed, any additional SNP in linkage disequilibrium with a first SNP associated with cancer will be associated with cancer. Once the association has been demonstrated between a given SNP and cancer, the discovery of additional SNPs associated with cancer can be of great interest in order to increase the density of SNPs in this particular region.

Methods for identifying additional SNPs and conducting linkage disequilibrium analysis are well known in the art. For example, identification of additional SNPs in linkage disequilibrium with the SNPs disclosed herein can involve the steps of: (a) amplifying a fragment from the genomic region comprising or surrounding a first SNP from a plurality of individuals; (b) identifying of second SNPs in the genomic region harboring or surrounding said first SNP; (c) conducting a linkage disequilibrium analysis between said first SNP and second SNPs; and (d) selecting said second SNPs as being in linkage disequilibrium with said first marker. This method may be modified to include certain steps preceding step (a), such as amplifying a fragment from the genomic region comprising or surrounding a somatic mutation from a plurality of individuals, and identifying SNPs in the genomic region harboring or surrounding said somatic mutation.

ErbB3 Cancer Detecting Agents

In one aspect, the present invention provides ErbB3 cancer detecting agents. In one embodiment, the detecting agent comprises a reagent capable of specifically binding to an ErbB3 sequence shown in Figure 39 (amino acid sequence of SEQ ID NO: 231 or nucleic acid sequence of SEQ ID NO:230). In another embodiment, the detecting agent comprises a polynucleotide capable of specifically hybridizing to an ERBB3 nucleic acid sequence shown in Figure 2 (SEQ ID NO: 1) or Figure 39 (SEQ ID NO:230). In a preferred embodiment, the polynucleotide comprises a nucleic acid sequence that specifically hybridizes to an ErbB3 nucleic acid sequence comprising a mutation shown in Figure 39 (SEQ ID NO:230).

In another aspect, the ErbB3 cancer detecting agents comprise a polynucleotide having a particular formula. In one embodiment, the polynucleotide formula is

$$5' X_a\text{-}Y\text{-}Z_b \quad 3' \qquad \text{Formula I}$$

, wherein

X is any nucleic acid and a is between about 0 and about 250 (i.e., in the 5' direction);

Y represents an ErbB3 mutation codon; and

to the kinase domain and the intracellular tail of ERBB3. Interestingly, among the ECD mutants were four positions, V104, A232, P262 and G284, that contained recurrent substitutions across multiple samples, indicating that these are mutational hotspots. Two of the four ECD hotspot positions identified in our analysis, V104 and G284, were previously reported mutated in an ovarian and a lung (adenocarinoma) sample respectively (Greenman *et al. Nature* **446,** 153-158 (2007); Ding *et aL Nature* **455,** 1069-1075 (2008)). Furthermore, most of the recurrent missense substitutions at each of the hotspot positions resulted in the same amino acid change indicative of a potential driver role for these mutations. We also identified a hotspot mutation, S846I, in the kinase domain when we combined our data with a single ERBB3 mutation previously published in colon cancer (Jeong *et al. International Journal of Cancer* **119,** 2986-2987 (2006)).

It is interesting to note that a majority of the mutated residues identified were conserved across ERBB3 orthologs (shown in Figure 6, as well as the C. *lupus* (X0_538226.2) sequence of SEQ ID NO:131) and some of the residues were conserved between ERBB family members, which further suggest that these mutations likely have a functional effect.

WHAT IS CLAIMED IS:

1. An ErbB3 gastrointestinal cancer detecting agent comprising a polynucleotide reagent capable of specifically binding to an ErbB3 mutation codon in an ErbB3 nucleic acid sequence, wherein the mutation codon encodes an amino acid mutation in SEQ ID NO:2 at a position selected from the group 104, 809, 232, 262, 284, 325, 846, 928, 60, 111, 135, 295, 406, 453, 1164, 193, 492, and 714.

2. The cancer detecting agent of claim 1, wherein the ErbB3 nucleic acid sequence comprises SEQ ID NO:230 or 1.

3. The cancer detecting agent of claim 1, wherein the reagent comprises a polynucleotide of formula

5' X_a-Y-Z_b 3' Formula I,

wherein

X is a nucleic acid sequence complementary to the ErbB3 nucleic acid sequence and a is between about 0 and about 250;

Y is the ErbB3 mutation codon; and

Z is a nucleic acid sequence complementary to the ErbB3 nucleic acid sequence and b is between about 0 and about 250.

4. The cancer detecting agent of any one of claims 1-3, further comprising a detectable label.

5. The cancer detecting agent of claim 4, wherein the detectable label is selected from the group radioisotope label, fluorescent label, bioluminescent label, and enzymatic label.

6. The cancer detecting agent of any one of claims 1-5, wherein the amino acid mutation is selected from V104M, V104L, Q809R, A232V, P262H, P262S, G284R, G325R, S846I, E928G, M60K, Y111C, R135L, V295A, M406K, M406T, R453H, T1164A, a stop codon at position 193, D492H, and V714M.

7. A method of determining the presence of ErbB3 gastrointestinal cancer in a subject comprising detecting in a biological sample obtained from the subject a mutation in a nucleic acid sequence encoding ErbB3, wherein the mutation results in an amino acid change at a position of SEQ ID NO:2 selected from the group 104, 809, 232, 262, 284, 325, 846, 928, 60, 111, 135, 295, 406, 453, 498, 1164, 193, 492, and 714 and wherein the mutation is indicative of an ErbB3 gastrointestinal cancer in the subject.

8. The method of claim 7, wherein the amino acid mutation is selected from V104M, V104L, Q809R, A232V, P262H, P262S, G284R, G325R, S846I, E928G, M60K, Y111C, R135L, V295A, M406K, M406T, R453H, K498I, T1164A, a stop codon at position 193, D492H, and V714M.

9. The method of claim 7 or 8, wherein administration of a therapeutic agent to said subject is contemplated.

10. The method of claim 9, wherein the therapeutic agent is an ErbB inhibitor.

11. The method of claim 10, wherein the ErbB inhibitor is selected from the group consisting of an EGFR antagonist, an ErbB2 antagonist, an ErbB3 antagonist, an ErbB4 antagonist, and an EGFR/ErbB3 antagonist.

12. The method of claim 11, wherein the inhibitor is a small molecule inhibitor.

13. The method of claim 11, wherein the antagonist is an antagonist antibody.

14. The method of claim 13, wherein the antibody is selected from the group consisting of a monoclonal antibody, a bispecific antibody, a chimeric antibody, a human antibody, a humanized antibody and an antibody fragment.

15. The detecting agent of claim 1, wherein the gastrointestinal cancer is gastric cancer or colon cancer.

16. The method of claim 7, wherein the gastrointestinal cancer is gastric cancer

or colon cancer.

17. The method of claim 7, further comprising obtaining the sample from the subject.

18. The method of claim 7, wherein the detecting comprises amplifying or sequencing the mutation and detecting the mutation or sequence thereof.

19. The method of claim 18, wherein the amplifying comprises admixing an amplification primer or amplification primer pair with a nucleic acid template isolated from the sample.

20. The method of claim 19, wherein the primer or primer pair is complementary or partially complementary to a region proximal to or including said mutation, and is capable of initiating nucleic acid polymerization by a polymerase on the nucleic acid template.

21. The method of claim 19, further comprising extending the primer or primer pair in a DNA polymerization reaction comprising a polymerase and the template nucleic acid to generate an amplicon.

22. The method of claim 18, wherein the mutation is detected by a process that includes one or more of: sequencing the mutation in a genomic DNA isolated from the biological sample, hybridizing the mutation or an amplicon thereof to an array, digesting the mutation or an amplicon thereof with a restriction enzyme, or real-time PCR amplification of the mutation.

23. The method of claim 18, comprising partially or fully sequencing the mutation in a nucleic acid isolated from the biological sample.

24. The method of claim 18, wherein the amplifying comprises performing a polymerase chain reaction (PCR), reverse transcriptase PCR (RT-PCR), or ligase chain reaction (LCR) using a nucleic acid isolated from the biological sample as a template in the

PCR, RT-PCR, or LCR.

25. Use of an ErbB inhibitor in a method of treating gastrointestinal cancer in a subject in need comprising

a) detecting in a biological sample obtained from the subject a mutation in a nucleic acid sequence encoding ErbB3, wherein the mutation results in an amino acid change at a position of SEQ ID NO:2 selected from the group consisting of 104, 809, 232, 262, 284, 325, 846, 928, 60, 111, 135, 295, 406, 453, 498, 1164, 193, 491, and 714 and wherein the mutation is indicative of an ErbB3 gastrointestinal cancer in the subject; and

b) administering the ErbB inhibitor to said subject.

26. The use of claim 25, wherein the amino acid mutation is selected from V104M, V104L, Q809R, A232V, P262H, P262S, G284R, G325R, S846I, E928G, M60K, Y111C, R135L, V295A, M406K, M406T, R453H, K498I, T1164A, a stop codon at position 193, D492H, and V714M.

27. The use of claim 25 or 26, wherein the ErbB inhibitor is selected from the group consisting of an EGFR antagonist, an ErbB2 antagonist, an ErbB3 antagonist, an ErbB4 antagonist, and an EGFR/ErbB3 antagonist.

28. The use of claim 27, wherein the antagonist is a small molecule inhibitor.

29. The use of claim 27, wherein the antagonist is an antagonist antibody.

30. The use of claim 29, wherein the antibody is selected from the group consisting of a monoclonal antibody, a bispecific antibody, a chimeric antibody, a human antibody, a humanized antibody and an antibody fragment.

31. The use of claim 25, wherein the gastrointestinal cancer is gastric cancer or colon cancer.