ANTI-NME ANTIBODY

BACKGROUND OF THE INVENTION

[0001] 1. Field of the Invention:

[0002] The present application relates to NME proteins, peptides derived from NME proteins, and antibodies generated from the peptides thereof or antibody or antibody fragments selected by virtue of their ability to bind to said peptides. The present application also relates to treating or preventing diseases associated with the expression of NME in a patient.

[0003] 2. General Background and State of the Art:

[0004] NDPK (nucleoside diphosphate protein kinase) proteins are a family of proteins grouped together because they all contain an NDPK domain. The first NME proteins discovered, previously called NM23 proteins, were NM23-H1 and NM23-H2. For decades it was unclear whether they induced differentiation or prevented differentiation of hematopoietic cells. The inventors previously discovered that NM23-H1 prevents differentiation when it is a dimer, which binds to the MUC 1 * growth factor receptor, but at higher concentrations NM23-H1 becomes a hexamer, which does not bind to MUC1*, and it induces differentiation. NM23 used to be called a metastasis suppressor when it was found that it was under-expressed in some very aggressive cancers. The present inventors previously disclosed that NM23-H1 dimers bind to and dimerize the extracellular domain of the MUC1* growth factor receptor that is over expressed on the vast majority of cancers and such binding promotes the growth of cancer cells. Conversely, at higher concentrations, NM23 forms tetramers and hexamers that do not bind to MUC1* and do not promote tumorigenesis. Very recently more NME family proteins (NME 1-10) have been discovered although until now, their functions have not been elucidated. NME7 is a newly discovered NME family protein, but its NDPK domain has no enzymatic activity, unlike other NME family members. NME7 is either not expressed at all in adult tissues or is expressed at extremely low levels.

SUMMARY OF THE DISCLOSURE

[0004a] In one aspect, provided herein is a use of an antibody that is made against a member of the NME7 family in the manufacture of a medicament for treating a MUC1* positive cancer or preventing metastasis of a MUC1* positive cancer in a subject in need thereof, wherein the medicament is to be administered to the subject, wherein the antibody is selected for its ability to bind to NME7-AB or NME7-X1 but not to NME1.

[0005] The present application is directed to a method of treating or preventing cancer in a subject, comprising administering to the subject an antibody made against a member of the NME family. The NME family may be NME7 family. The antibody may bind to NME7. The

antibody may bind to NME7-AB or NME-AB-like protein. The antibody may bind to NME7-XI. The antibody may inhibit binding between NME7 and its cognate binding partner. The cognate binding partner may be MUC1*. The cognate binding partner may be PSMGFR portion of the MUC1* extracellular domain. In one example, the antibody may be generated or selected for its ability to bind to a peptide selected from those listed in Figures 16-19 (SEQ ID NOS:88 to 145). Preferably, the peptide may be selected from those listed in Figure 19 (SEQ ID NOS:141 to 145).

[0006] The peptide may be highly homologous to, or to which is added or subtracted up to 7, up to 6, up to 5, up to 4, up to 3, up to 2, or up to 1 amino acid residues at the N-terminus or C-terminus, of the peptides listed in Figures 16-19 (SEQ ID NOS:88 to 145). In one example, the antibody may be selected for its ability to bind to NME7-AB or NME7-X1 but not to NME1. The antibody may be polyclonal, monoclonal, bivalent, monovalent, bispecific, an antibody fragment containing the variable region, or an antibody mimic. The antibody may be human or humanized. The antibody may be a single chain scFv.

[0007] In another example, the disclosure is directed to a method of treating or preventing cancer in a subject, comprising administering to the subject a peptide that is highly homologous or identical to regions of NME7-AB. The peptide may be at least 80% homologous to one or more of the peptides listed in Figure 16. The peptide may be at least 80% homologous to one or more of the peptides listed in Figure 17. The peptide may be at least 80% homologous to one or more of the peptides listed in Figure 18. The peptide may be at least 80% homologous to one or more of the peptides listed in Figure 19. The peptide may be selected from peptides listed in Figures 16-19 (SEQ ID NOS:88 to 145). The peptide may be selected from those listed in Figure 19 (SEQ ID NOS:141 to 145). Or, the peptide may be highly homologous to, or to which is added or subtracted up to 7 , up to 6, up to 5, up to 4, up to 3, up to 2, or up to 1 amino acid residues at the N-terminus or C-terminus, of the peptides listed in Figures 16-19 (SEQ ID NOS:88 to 145). The peptide may be connected to another peptide via a spacer or linker

[0008] In another example, the disclosure is directed to a chimeric antigen receptor (CAR), for the treatment or prevention of cancer wherein the targeting extracellular portion of the CAR comprises at least a peptide fragment of a member of the NME family. NME family may be NME7 family. The member of the NME7 family may be NME7. Or, the member of the NME7

family may be NME7-AB or NME-AB-like protein. The member of the NME7 family may be also NME7-X1. The targeting extracellular portion of the CAR may include a peptide of the peptides listed in Figures 16-19 (SEQ ID NOS:88 to 145). The peptide may be selected from those listed in Figure 19 (SEQ ID NOS:141 to 145). The peptide may include a peptide, which is highly homologous to, or to which is added or subtracted up to 7, up to 6, up to 5, up to 4, up to 3, up to 2, or up to 1 amino acid residues at the N-terminus or C-terminus, of the peptides listed in Figures 16-19 (SEQ ID NOS:88 to 145). The peptide may be connected to another peptide via a spacer or linker.

[0009] In yet another example, the disclosure is directed to a method of treating or preventing cancer or cancer metastasis, comprising engineering the chimeric antigen receptor according to claim 3, into an immune system cell and administering the cell to a subject in need thereof.

[0010] In another example, the disclosure is directed to a chimeric antigen receptor (CAR), for the treatment or prevention of cancer, wherein the targeting extracellular portion of the chimeric antigen receptor comprises a portion of an antibody that binds to NME7-AB, NME-AB-like protein or NME7-X1. The portion of the antibody may be a single chain scFv or may be human or humanized.

[0011] In yet another example, the disclosure is directed to a method of vaccinating a person against cancer or metastatic cancer comprising immunizing the person with a peptide fragment of a member of the NME family. The NME family may be NME7 family. The member of the NME7 family may be NME7 or NME7b. The member of the NME7 family may be NME7-AB or NME7-AB-like protein. The NME7 family may be NME7-X1. The immunizing peptide may be a peptide from the peptides listed in Figures 16-19 (SEQ ID NOS:88 to 145). Preferably, the peptide may be selected from those listed in Figure 19 (SEQ ID NOS:141 to 145). The immunizing peptide may include a peptide, which is highly homologous to, or to which is added or subtracted up to 7, up to 6, up to 5, up to 4, up to 3, up to 2, or up to 1 amino acid residues at the N-terminus or C-terminus, of the peptides listed in Figures 16-19 (SEQ ID NOS:88 to 145). The immunizing peptide may be connected to another peptide via a spacer or linker.

[0012] In yet another example, the disclosure is directed to a method of treating or preventing cancer in a subject, comprising administering to the subject a nucleic acid that inhibits the expression of NME7, NME7b, NME7-AB-like protein or NME7-X1. The nucleic acid may be an anti-sense nucleic acid that suppresses expression of NME7, NME7-AB-like protein or NME7-X1. The nucleic acid may be an inhibitory RNA, siRNA, RNAi, or shRNA that inhibits expression of NME7, NME7-AB-like protein or NME7-X1.

[0013] In another example, the disclosure is directed to a method of treating or preventing cancer in a subject, comprising administering to the subject genetically edited nucleic acids that inhibit expression of NME7, NME7b, NME7-AB-like protein or NME7-X1. The genetically edited nucleic acids that inhibit expression of NME7, NME7b, NME7-AB-like protein or NME7-X1 may be inserted into cells that may be then administered to the patient. The genetically edited nucleic acids that inhibit expression of NME7, NME7b, NME7-AB-like protein or NME7-X1 may be inserted into cells using a viral vector. The viral vector may be a lentiviral system.

[0014] In another example, the disclosure is directed to a method of growing cancer cells comprising contacting the cells with NME7-AB, NME7b, NME7-AB-like protein or NME7-XI, 2i or 5i. The method may include culturing the cells in a medium that contains NME7-AB, NME7b, NME7-AB-like protein or NME7-X1, 2i or 5i, or growing cells in an animal that expresses human NME7-AB, NME7b, NME7-AB-like protein or NME7-X1, or to which NME7-AB, NME7b, NME7-AB-like protein or NME7-X1 is administered. The cancer cells may be breast, prostate, ovarian, colorectal, pancreatic, liver, melanoma or brain cancer cells. Drug candidates may be tested on the cells. Th efficacy of the drugs may be assessed by comparing cancer growth to a no drug control or comparing expression levels of metastatic markers or stem cell markers to a no drug control or comparing the ability of the resultant cells to form tumors in animals from low cell copy number compared to a no drug control and determining the efficacy of a candidate drug for the treatment of cancer or metastasis. The cells may be obtained from a patient being assessed for treatment for cancer and drugs that would be effective for that patient are selected based on results using methods described above. The cells may not be obtained from a patient being assessed for treatment for cancer but drugs that would be effective for that patient are selected based on results using the methods described above.

[0015] In another aspect, the disclosure is directed to a method of generating antibodies or antibody-like molecules from peptides or peptide mimics having a sequence derived from the sequence of NME. The NME may be NME7. The peptide may be used as an immunogen to generate antibodies or antibody-like molecules. The peptide may be administered to an animal to generate anti-NME7 antibodies. The peptide may be administered to a human to generate anti-NME7 antibodies. The peptide may have a sequence listed in Figures 16 to 19 (SEQ ID NOS:88 to 145). Preferably, the peptide may be selected from those listed in Figure 19 (SEQ ID NOS: 141 to 145). The peptide may include a peptide, which is highly homologous to, or to which is added or subtracted up to 7, up to 6, up to 5, up to 4, up to 3, up to 2, or up to 1 amino acid residues at the N-terminus or C-terminus, of the peptides listed in Figures 16-19 (SEQ ID NOS:88 to 145).

[0016] In another aspect, the disclosure is directed to a method of detecting presence of cancer or the progression of cancer, comprising the steps of:

[0017] 1) obtaining a sample from a patient having cancer or at risk of developing a cancer;

[0018] 2) subjecting that sample to an assay capable of detecting or measuring levels of a member of the NME7 family, or levels of nucleic acids encoding a member of the NME7 family;

[0019] 3) comparing levels of the measured member of the NME7 family or the member of the NME7 family-encoding nucleic acids in the test sample to levels in control patients or control cells;

[0020] 4) determining that the levels of the member of the NME7 family or nucleic acids encoding the member of the NME7 family are elevated compared to the controls; and

[0021] 5) concluding that the donor of the test sample has cancer or has had a progression of cancer if the control to which the test was compared came from a donor previously diagnosed with a cancer. In this method, the detection of the member of the NME7 family in circulation or in a tissue may be an indicator of cancer in a patient. The member of the NME7 family may be NME7, NME7b, NME7-X1 , or NME7-AB-like protein.**[0022]** In yet another aspect, the disclosure is directed to a method comprising:

[0023] detecting presence of a member of the NME7 family or MUC 1 * in a patient; and

[0024] administering anti-NME7 or anti-MUCl * antibody or antibodies to the patient exhibiting the member of the NME7 family or MUC1* expression. The member of the

NME7 family may be NME7, NME7b, NME7-X1, or NME7-AB-like protein.

[0025] In yet another example, the disclosure is directed to a method for treating or preventing cancer comprising:

[0026] 1) obtaining a sample from a patient suspected of having a cancer or at risk of developing a cancer or at risk of developing a metastatic cancer;

[0027] 2) measuring an amount of the member of an NME7 family or a member of the NME7 family encoding nucleic acid, wherein the measured levels are significantly above those measured in a control sample;

[0028] 3) determining that the patient has a cancer or has developed a more aggressive or a metastatic cancer;

[0029] 4) administering to the patient an effective amount of a therapeutic agent that suppresses expression of the member of the NME7 family, inhibits cleavage of NME7 or inhibits NME7 binding to its targets. The target of the member of the NME7 family may be

WHAT IS CLAIMED IS:

1. Use of an antibody that is made against a member of the NME7 family in the manufacture of a medicament for treating a MUC1* positive cancer or preventing metastasis of a MUC1* positive cancer in a subject in need thereof, wherein the medicament is to be administered to the subject, wherein the antibody is selected for its ability to bind to NME7-AB or NME7-X1 but not to NME1.

2. The use according to claim 1, wherein the antibody binds to NME7-AB.

3. The use according to claim 1, wherein the antibody binds to NME7-X1.

4. The use according to claim 1, wherein the antibody inhibits binding between NME7 and its cognate binding partner.

5. The use according to claim 4, wherein the cognate binding partner is MUC1*.

6. The use according to claim 4, wherein the cognate binding partner is PSMGFR portion of the MUC1* extracellular domain.

7. The use according to claim 1, wherein the antibody is generated or selected for its ability to bind to a peptide selected from those listed in Figures 16 or 19 (SEQ ID NOS:88 to 118 or 141 to 145).

8. The use according to claim 7, wherein the peptide is selected from those listed in Figure 19 (SEQ ID NOS:141 to 145).

9. The use according to claim 1, wherein the antibody is generated or selected for its ability to bind to a peptide having at least 90% amino acid sequence identity to, or to which is added or subtracted up to 7 amino acid residues at the N-terminus or C-terminus, of a peptide listed in Figure 19 (SEQ ID NOS: 141 to 145).

10. The use according to claim 1, wherein the antibody is an antibody fragment containing the variable region.

11. The use according to claim 1, wherein the antibody is human or humanized.

12. The use according to claim 1, wherein the antibody is a single chain scFv.