hypersensitivity response indicates a cellular response (see, e.g., Roychowdhury, et al. (2005) PS J. E834-E846).

[0007] Autophagy is a homeostatic process by which cytosolic components and organelles are delivered to the lysosome for degradation. Autophagy is also associated with innate and adaptive immune responses to intracellular pathogens whereby cytosolic antigens are loaded onto major histocompatibility complex (MHC) class II molecules for $CD4^+$ T-cell recognition.

Description

[0008] As used herein, including the appended claims, the singular forms of words such as "a," "an," and "the" include their corresponding plural references unless the context clearly dictates otherwise. All references cited herein are incorporated by reference to the same extent as if each individual publication, patent, published patent application, and sequence listing, as well as figures and drawings in said publications and patent documents, was specifically and individually indicated to be incorporated by reference.

Summary of the Disclosure

[0009] In one aspect there is provided a melanoma vaccine comprising:

at least one dendritic cell from a subject that has melanoma;

wherein the at least one dendritic cell had been contacted *in vitro* with at least one melanoma tumor cell from the same subject, wherein the at least one melanoma tumor cell is non-dividing and not treated *in vitro* with interferon-gamma (IFN-gamma) or a IFN-gamma mimetic, and at least 60% of the melanoma tumor cells are autophagic and non-apoptotic.

[0009a] In one aspect there is provided a method for preparing a melanoma vaccine comprising melanoma cells and dendritic cells from the same subject, the method comprising:

treating one or more melanoma cells with an agent that prevents cell division;

wherein the one or more melanoma cells are not treated *in vitro* with interferon-gamma (IFN-gamma) or with an IFN-gamma mimetic;

selecting melanoma cells that are autophagic and non-apoptotic, wherein melanoma cells that are non-autophagic and apoptotic are rejected; contacting the melanoma cells that are autophagic and non-apoptotic, or peptides derived from the

melanoma cells, and one or more autologous dendritic cells to produce a melanoma vaccine.

[0009b] In one aspect there is provided a composition comprising:

at least one melanoma cell from a subject with melanoma, wherein the at least one melanoma cell is not treated *in vitro* with interferon-gamma (IFN-gamma), and

at least one antigen presenting cell (APC) from the same subject, wherein the melanoma cell is autophagic; and non-apoptotic.

[0009c] In one aspect there is provided use of an immunologically effective amount of the composition described herein in the manufacture of a medicament for stimulating an immune response against a melanoma-associated antigen in a subject having melanoma.

[0009d] In one aspect there is provided a method for preparing a melanoma vaccine, comprising:

treating melanoma cells obtained from a subject with melanoma with an agent that prevents cell division, wherein the melanoma cells are not treated *in vitro* with IFN-gamma or an IFN-gamma mimetic;

selecting melanoma cells that are autophagic and non-apoptotic; and,

selecting melanoma cells that are autophagic and non-apoptotic; and, same subject to form a melanoma vaccine.

[0009e] In one aspect there is provided a composition comprising a melanoma vaccine, prepared by the method as described herein.

[0009f] In one aspect there is provided use of the melanoma vaccine as described herein in the manufacture of a medicament for stimulating an immune response against a melanoma-specific antigen in a subject with melanoma.

[0009g] In one aspect there is provided use of the composition as described herein in the manufacture of a medicament for stimulating an immune response against a melanoma-associated antigen in a subject with melanoma.

[0009h] In one aspect there is provided use of the composition as described herein for the treatment of melanoma.

[0009i] In one aspect there is provided use of the melanoma vaccine as described herein for the treatment of melanoma.

[0009j] In one aspect there is provided use of an immune-stimulatory amount of the melanoma vaccine as described herein in the manufacture of a medicament for treating melanoma in a subject in need thereof.

[0009k] In one aspect there is provided use of an immune-stimulatory amount of the composition as described herein in the manufacture of a medicament for treating melanoma in a subject in need thereof.

[0009l] Disclosed is a population of mammalian dendritic cells comprising melanoma-specific peptides from a given subject that has melanoma and comprises melanoma cells; wherein said melanoma-specific peptides are acquired in vitro by dendritic cells from said melanoma cells that are not treated in vitro with IFN-gamma or IFN-gamma mimetic, wherein greater than 60 percent (%) of said melanoma cells that are not treated in vitro with IFN-gamma or or IFN-gamma mimetic are autophagic and non-apoptotic, and wherein the dendritic cells and melanoma cells are from the same subject.

[0010]Also disclosed is the above population of mammalian dendritic cells, wherein: greater than 80% of said melanoma cells are autophagic and non-apoptotic.

[0011] What is disclosed are the above dendritic cells, wherein essentially all of the melanoma cells that are not treated with IFN-gamma or IFN-gamma mimetic are incapable of cell division; as well as the above dendritic cells wherein essentially all of the melanoma cells that are not treated with IFN-gamma or IFN-gamma mimetic are irradiated and incapable of cell division; as well as the above dendritic cells, wherein at least 80% of the melanoma cells that are not treated with IFN-gamma or

What is claimed is:

Claim 1. A population of mammalian dendritic cells comprising:

melanoma-specific peptides from melanoma cells taken from a subject that has melanoma;

wherein the melanoma-specific peptides are acquired in vitro by dendritic cells from said melanoma cells;

wherein the melanoma cells are not treated in vitro with interferon-gamma (IFN-gamma) or with IFN-gamma mimetic;

wherein greater than 60 percent (%) of said melanoma cells that are not treated in vitro with IFN-gamma or IFN-gamma mimetic, are autophagic and non-apoptotic, and

wherein the dendritic cells and melanoma cells are from the same subject.

Claim 2. The population of dendritic cells of claim 1, wherein greater than 80% of said melanoma cells that are not treated in vitro with IFN-gamma or IFN-gamma mimetic, are autophagic and non-apoptotic.

Claim 3. A vaccine for the subject of claim 1 comprising the population of dendritic cells of claim 1.

Claim 4. The dendritic cells of claim 1, wherein essentially all of the melanoma cells that are not treated with IFN-gamma or IFN-gamma mimetic are incapable of cell division.

Claim 5. The dendritic cells of claim 1, wherein essentially all of the melanoma cells that are not treated with IFN-gamma or IFN-gamma mimetic are irradiated and incapable of cell division.

Claim 6. The dendritic cells of claim 1, wherein at least 80% of the melanoma cells that are not treated with IFN-gamma or IFN-gamma mimetic are irradiated and incapable of cell division.

Claim 7. The dendritic cells of claim 1, wherein at least 80% of the melanoma cells that are not treated with IFN-gamma or IFN-gamma mimetic are treated with a nucleic acid cross-linker and are incapable of cell division.

Claim 8. The dendritic cells of claim 1 that comprise one or more peptides derived from a melanoma-specific antigen that is S-100, HMB-45, Mel-2, Melan-A, Mel-5, MAGE-1, MART-1, or Tyrosinase.

Claim 9. The dendritic cells of claim 1, wherein the given subject is a human subject.

Claim 10. The dendritic cells of claim 1, wherein the given subject is a mammal that is not human.

Claim 11. A melanoma vaccine comprising:
at least one mature dendritic cell from a subject that has melanoma;
wherein the at least one mature dendritic cell had been contacted with at least one melanoma tumor cell from the same subject, wherein the at least melanoma tumor cell that is contacted with the at least one mature dendritic cell is non-dividing, autophagic, and non-apoptotic.

Claim 12. A method for stimulating immune response against a melanoma-specific antigen comprising administering an immune-stimulatory amount of the dendritic cells of claim 1 to the subject.

Claim 13. The method of claim 12, wherein the immune response that is stimulated comprises one or more of $CD4^+$ T cell response, $CD8^+$ T cell response, and B cell response.

Claim 14. The method of claim 13, wherein the $CD4^+$ T cell response, $CD8^+$ T cell response, or B cell response, can be measured by ELISPOT assays, by intracellular cytokine staining assays, by tetramer assays, or by detecting antigen-specific antibody production.

Claim 15. The method of claim 12, wherein the immune response comprises a survival time that comprises 2-year overall survival (OS), and where the 2-year overall survival is at least 60%.

Claim 16. The method of claim 12, wherein the administration comprises subcutaneous injections of the vaccine.

Claim 17. The method of claim 12, wherein the administration comprises injections of the vaccine given weekly for three months and then monthly for five months.

Claim 18. A method for preparing a dendritic cell vaccine, involving melanoma cells and dendritic cells from the same subject, the method comprising:

one or more melanoma cells is treated with an agent that prevents cell division;

the one or more melanoma cells are not treated in vitro with interferon-gamma (IFN-gamma) or with an IFN-gamma mimetic;

melanoma cells that are autophagic and non-apoptotic are selected;

melanoma cells that are non-autophagic and apoptotic are rejected; and,

wherein the melanoma cells that are autophagic and non-apoptotic are provided to one or more autologous dendritic cells, or, wherein peptides derived from the melanoma cells that are autophagic and non-apoptotic are provided to one or more autologous dendritic cells.

Claim 19. A composition comprising:

at least one melanoma cell that is not treated with interferon-gamma (IFN-gamma) from a first subject, and at least one antigen presenting cell (APC) from the same first subject, wherein the melanoma cell is:

autophagic; and

non-apoptotic.

Claim 20. The composition of claim 19, wherein the melanoma cell is MHC class II-expressing.

Claim 21. The composition of claim 19, wherein the APC is a dendritic cell, a macrophage, or a B cell.

Claim 22. The composition of claim 19, wherein the at least one melanoma cell comprises melanoma-specific peptides, and wherein the melanoma specific-peptides are substantially not contained in said APCs and are substantially not processed by said APCs.

Claim 23. The composition of claim 19, wherein the melanoma cells comprise melanoma-specific peptides, and wherein the melanoma specific-peptides are substantially contained in said APCs and are partially or substantially processed in said APCs.

Claim 24. The composition of claim 19, wherein the melanoma cell is loaded into the APC.

Claim 25. The composition of claim 19, wherein the melanoma cells is not loaded into the APC.

Claim 26. The composition of claim 19, wherein autophagy is demonstrated by a test that assays microtubule-associated protein light chain 3 (LC3).

Claim 27. The composition of claim 19, wherein the cells are demonstrated to be non-apoptotic using at least one of the reagent, 7-aminoactinomycin D (7-ADD), or the reagent, annexin.

Claim 28. A method of stimulating immune response in a subject having melanoma and comprising melanoma cells, wherein the subject is the same subject as the first subject, comprising administering an immunology effective amount of the composition of claim 19.

Claim 29. The composition of claim 19, wherein at least 90% of the melanoma cells are not treated in vitro with IFN-gamma, and less than 10% of the melanoma cells are treated in vitro with IFN-gamma.

Claim 30. A method for manufacturing the vaccine of claim 1 or the composition of claim 19, comprising contacting at least one melanoma tumor cell to at least one antigen presenting cell (APC), wherein the at least one melanoma tumor cell is from a first human subject, and wherein the at least one APC is from the same first human subject.

Claim 31. A method for preparing a dendritic cell vaccine, comprising: treating melanoma cells acquired from a first subject with an agent that prevents cell division; wherein the melanoma cells are not treated in vitro with IFN-gamma or an IFN-gamma mimetic; selecting melanoma cells that are autophagic and non-apoptotic; and, contacting the selected melanoma cells with autologous dendritic cells from the same first subject.

Claim 32. A composition that comprises a dendritic cell vaccine, as prepared by the method of claim 31.

Claim 33. A method for stimulating immune response against a melanoma-specific antigen, comprising administering the composition of claim 31 to a subject that has melanoma.

What is claimed is:

Claim 1. A melanoma vaccine comprising:

at least one dendritic cell from a subject that has melanoma;

wherein the at least one dendritic cell had been contacted *in vitro* with at least one melanoma tumor cell from the same subject, wherein the at least one melanoma tumor cell is non-dividing and not treated *in vitro* with interferon-gamma (IFN-gamma) or a IFN-gamma mimetic, and at least 60% of the melanoma tumor cells are autophagic and non-apoptotic.

Claim 2. Use of an immune-stimulatory amount of the melanoma vaccine of claim 1 in the manufacture of a medicament for stimulating an immune response against a melanoma-specific antigen in a subject with melanoma.

Claim 3. The use of claim 2, wherein the immune response comprises one or more of a $CD4^+$ T cell response, a $CD8^+$ T cell response, and a B cell response.

Claim 4. The use of claim 3, wherein the $CD4^+$ T cell response, $CD8^+$ T cell response, or B cell response is determined by ELISPOT assays, by intracellular cytokine staining assays, by tetramer assays, or by detecting antigen-specific antibody production.

Claim 5. The use of claim 2, wherein the immune response results in a 2-year overall survival of at least 60%.

Claim 6. The use of claim 2, wherein the administration comprises subcutaneous injections of the vaccine.

Claim 7. The use of claim 2, wherein the administration comprises injections of the vaccine given weekly for three months and then monthly for five months.

Claim 8. A method for preparing a melanoma vaccine comprising melanoma cells and dendritic cells from the same subject, the method comprising:

treating one or more melanoma cells with an agent that prevents cell division; wherein the one or more melanoma cells are not treated *in vitro* with interferon-gamma (IFN-gamma) or with an IFN-gamma mimetic;

selecting melanoma cells that are autophagic and non-apoptotic, wherein melanoma cells that are non-autophagic and apoptotic are rejected;

contacting the melanoma cells that are autophagic and non-apoptotic, or peptides derived from the melanoma cells, and one or more autologous dendritic cells to produce a melanoma vaccine.

Claim 9. A composition comprising:

at least one melanoma cell from a subject with melanoma, wherein the at least one melanoma cell is not treated *in vitro* with interferon-gamma (IFN-gamma), and

at least one antigen presenting cell (APC) from the same subject, wherein the melanoma cell is autophagic; and non-apoptotic.

Claim 10. The composition of claim 9, wherein the melanoma cell expresses MHC class II.

Claim 11. The composition of claim 9, wherein the APC is a dendritic cell, a macrophage, or a B cell.

Claim 12. The composition of claim 9, wherein the melanoma cells comprise melanoma-specific peptides and, as a result of the contacting step, the melanoma specific-peptides are substantially contained in said APCs and are partially or substantially processed in said APCs.

Claim 13. The composition of claim 9, wherein the melanoma cell is loaded into the APC.

Claim 14. Use of an immunologically effective amount of the composition of claim 9 in the manufacture of a medicament for stimulating an immune response against a melanoma-associated antigen in a subject having melanoma.

Claim 15. A method for preparing a melanoma vaccine, comprising:

treating melanoma cells obtained from a subject with melanoma with an agent that prevents cell division, wherein the melanoma cells are not treated *in vitro* with IFN-gamma or an IFN-gamma mimetic;

selecting melanoma cells that are autophagic and non-apoptotic; and,

contacting the selected melanoma cells with autologous dendritic cells from the same subject to form a melanoma vaccine.

Claim 16. A composition comprising a melanoma vaccine, prepared by the method of claim 15.

Claim 17. The melanoma vaccine of claim 1, wherein greater than 80% of the melanoma cells are autophagic and non-apoptotic.

Claim 18. The melanoma vaccine of claim 1, wherein essentially all of the melanoma cells are incapable of cell division.

Claim 19. The melanoma vaccine of claim 1, wherein the melanoma cells are rendered incapable of cell division by irradiation or by a nucleic acid cross-linker.

Claim 20. Use of the melanoma vaccine of claim 1 in the manufacture of a medicament for stimulating an immune response against a melanoma-specific antigen in a subject with melanoma.

Claim 21. Use of the composition of claim 9 in the manufacture of a medicament for stimulating an immune response against a melanoma-associated antigen in a subject with melanoma.

Claim 22. Use of the composition of claim 9 for the treatment of melanoma.

Claim 23. Use of the melanoma vaccine of claim 1 for the treatment of melanoma.

Claim 24. Use of an immune-stimulatory amount of the melanoma vaccine of claim 1 in the manufacture of a medicament for treating melanoma in a subject in need thereof.

Claim 25. Use of an immune-stimulatory amount of the composition of claim 9 in the manufacture of a medicament for treating melanoma in a subject in need thereof.