limited. Therefore, the construction and expression of recombinant hemoglobin protein or recombinant hemoglobin tetramer or dimer or subunits are important to solve this issue.

[0004] Hypoxia is common in cancers. Hypoxia and anemia (which contributes to tumor hypoxia) can lead to ionizing radiation and chemotherapy resistance by depriving tumor cells of the oxygen essential for the cytotoxic activities of these agents. Hypoxia may also reduce tumor sensitivity to radiation therapy and chemotherapy through one or more indirect mechanisms that include proteomic and genomic changes. Therefore, there is a need for improved cancer-treatment compositions, particularly, improved cancer-treatment compositions that facilitate targeting cancer cells and enhance the efficacy of cytotoxic agents.

Summary

[0005] In one aspect, the present invention refers to a recombinant hemoglobin protein, with or without heme, comprising recombinant hemoglobin tetramer as an oxygen carrier and supply replacing natural or native hemoglobin molecule isolated and/or purified from an animal or human source, said recombinant hemoglobin tetramer being $2\alpha\gamma1_2$.

[0005a] In another aspect, the present invention refers to a recombinant hemoglobin protein, with or without heme, comprising recombinant hemoglobin tetramer as an oxygen carrier and supply replacing natural or native hemoglobin molecule isolated and/or purified from an animal or human source, said recombinant hemoglobin tetramer being $2\alpha\beta2$, wherein said recombinant hemoglobin tetramer is originated from Homo sapiens, wherein each recombinant hemoglobin subunit of said recombinant hemoglobin tetramer is tagged with or without a His-sumo or a Poly-His tag

[0005aa] In another aspect , the present invention refers to a recombinant hemoglobin protein based therapeutic agent for treating cancer comprising the recombinant hemoglobin protein of the present invention and an active agent, the active agent selected from one or more of a chemotherapeutic agent, a radiotherapeutic agent, a cell labeling agent, and a fluorescent labeling agent, the recombinant hemoglobin protein being either directly conjugated to the active agent or indirectly conjugated to the active agent through a cleavable or non-cleavable linker.

[0005b] In yet another aspect, the present invention refers to a pharmaceutical composition comprising a therapeutically effective amount of the recombinant hemoglobin protein based

therapeutic agent of the present invention, said composition being formulated for administration to a subject in need thereof who may suffer from cancer.

[0005c] In yet another aspect, the present invention refers to a method for preparing the therapeutic agent of the present invention, said method comprising: a) purifying the recombinant hemoglobin protein of the present disclosure using different column chromatography methods; and b) chemically modifying the recombinant hemoglobin protein comprising reacting with one or more functional groups followed by conjugating said recombinant hemoglobin protein with an active agent, or conjugating an active agent with a cleavable or non-cleavable linker to form a linker-active agent conjugate prior to linking said conjugate to said recombinant hemoglobin protein.

[0005d] In yet another aspect, the present invention refers to the use of the recombinant hemoglobin protein of the present invention in the manufacture of a medicament for oxygenating tissue from or in an animal or human subject, wherein the tissue is hypoxic.

[0005e] In yet another aspect, the present invention refers to the use of the recombinant hemoglobin protein of the present invention in the manufacture of a medicament for treating oxygen-deprivation disorder in an animal or human subject, wherein the oxygen-deprivation disorder is hemorrhagic shock.

[0005f] In yet another aspect, the present invention refers to the use of a composition of the present invention in the manufacture of a medicament for targeting and inducing apoptosis of cancer cells.

CLAIMS

1. A recombinant hemoglobin protein, with or without heme, comprising recombinant hemoglobin tetramer as an oxygen carrier and supply replacing natural or native hemoglobin molecule isolated and/or purified from an animal or human source, said recombinant hemoglobin tetramer being $2\alpha\gamma1_2$.

2. The recombinant hemoglobin protein of claim 1, wherein said recombinant hemoglobin tetramer is originated from *Homo sapiens*.

3. The recombinant hemoglobin protein of claim 1, wherein each recombinant hemoglobin subunit of said recombinant hemoglobin tetramer is tagged with or without a His-sumo or a Poly-His tag.

4. A recombinant hemoglobin protein, with or without heme, comprising recombinant hemoglobin tetramer as an oxygen carrier and supply replacing natural or native hemoglobin molecule isolated and/or purified from an animal or human source, said recombinant hemoglobin tetramer being $2\alpha\beta_2$, wherein said recombinant hemoglobin tetramer is originated from *Homo sapiens*, wherein each recombinant hemoglobin subunit of said recombinant hemoglobin tetramer is tagged with or without a His-sumo or a Poly-His tag.

5. A recombinant hemoglobin protein based therapeutic agent for treating cancer comprising the recombinant hemoglobin protein of claim 1 or 4 and an active agent, the active agent selected from one or more of a chemotherapeutic agent, a radiotherapeutic agent, a cell labeling agent, and a fluorescent labeling agent, the recombinant hemoglobin protein being either directly conjugated to the active agent or indirectly conjugated to the active agent through a cleavable or non-cleavable linker.

6. The therapeutic agent of claim 5, wherein said recombinant hemoglobin protein is linked with one or more of said linker molecule(s) at one or more subunits of said recombinant hemoglobin protein.

7. The therapeutic agent of claim 5, wherein said recombinant hemoglobin protein is chemically modified by one or more functional groups before being conjugated to said active agent, said chemical modification comprising reacting the recombinant hemoglobin molecule with one or more of the following compound(s) in respect of different chemical reactions for said chemical modification:

a) for amine reactions, the compounds involved comprising anhydride, ketene, NHS ester, isothiocyanates, isocyanates, activated esters comprising fluorophenyl esters and carbonyl azides, sulfonyl chlorides, carbonyls followed by reductive amination, epoxides, carbonates, fluorobenzenes, imidoesters, hydroxymethyl phosphine derivatives, mannich condensation, diazonium derivatives, 4-sulfo-2,3,5,6-tetrafluorophenol, carbonyl diimidazole, sulfo-NHS, and N-terminal modification by pyridoxal-5-phoshpate-based biomimetic transamination;

b) for thiol reactions, the compounds involved comprising maleimides, alkyl halides, haloacetamides, disulfides, thiosulfates, aziridine-containing reagents, acryloyl derivatives, arylating agents, and vinylsulfone derivatives;

c) for carboxylate reactions, the compounds involved comprising diazoalkanes and diazoacetyl compounds, carbonyldiimidazoles, and carbodiimides;

d) for hydroxyl reactions, the compounds involved comprising epoxides and oxiranes, carbonyldiimidazoles, carbonates, chloroformates, chemical and enzymatic oxidations, alkyl halogens, and isocyanates;

e) for native chemical ligations, the compounds involved comprising thioesters;

f) for N-terminal modification, the compounds involved comprising N-terminal serine or threonine using periodate oxidation thereof to generate aldehydes for coupling with hydroxylamines, hydrazines, or hydrazides, carbodiimides;

g) for incorporation of bioorthogonal functionalities, the compounds involved comprising alkynes and azides with subsequent bioorthogonal conjugation reactions comprising dipolar addition Huisgen 1,3-dipolar additions of alkynes and azides, Staudinger ligation of azides and triarylphosphines, Diels-alder reaction of alkenes and tetrazines, and coupling of alkenes and tetrazoles;

h) for photochemical reactions, the compounds involved comprising photoaffinity labeling agents, diazirine derivatives, benzophenones and anthraquinones; and

i) for metal-mediated reactions, the compounds involved comprising metal carbenoids and palladium-activated allyl reagents.

8. The therapeutic agent of claim 5, wherein said cleavable linker comprises carbinolamine, disulfide, carbamide, aminal, carbonate, ester, carbamate, phosphate, amide, acetal, imine, oxime, ether or sulfonamide group linker.

9. The therapeutic agent of claim 5, wherein said chemotherapeutic agent comprises 5-Fluorouracil, temozolomide, cisplatin or other anti-cancer drugs.

10. The therapeutic agent of claim 5, wherein said cancer comprises pancreatic cancer, leukemia, head and neck cancer, colorectal cancer, lung cancer, breast cancer, liver cancer, nasopharyngeal cancer, esophageal cancer, prostate cancer, stomach cancer or brain cancer.

11. A pharmaceutical composition comprising a therapeutically effective amount of the recombinant hemoglobin protein based therapeutic agent of claim 5, said composition being formulated for administration to a subject in need thereof who may suffer from cancer.

12. The composition of claim 11, wherein the therapeutically effective amount of said therapeutic agent is at ⩽0.0024 g/kg of said subject and said subject is human.

13. The composition of claim 11, wherein the therapeutically effective amount of said therapeutic agent is at ⩽0.03 g/kg of said subject and said subject is animal.

14. The composition of claim 11, wherein route of said administration comprises intravenous injection, intraperitoneal injection, or subcutaneous injection, and said administering of the composition can be in single dose or in multiple doses for once a week.

15. The composition of claim 14, wherein said cancer comprises pancreatic cancer, leukemia, head and neck cancer, colorectal cancer, lung cancer, breast cancer, liver cancer, nasopharyngeal cancer, esophageal cancer or brain cancer.

16. The composition of claim 14, wherein the composition has a pH in a range of 5.5 to 9.5.

17. A method for preparing the therapeutic agent of claim 5, said method comprising:

a) purifying the recombinant hemoglobin protein of claim 1 or 4 using different column chromatography methods; and

b) chemically modifying the recombinant hemoglobin protein comprising reacting with one or more functional groups followed by conjugating said recombinant hemoglobin protein with an active agent, or conjugating an active agent with a cleavable or non-cleavable linker to form a linker-active agent conjugate prior to linking said conjugate to said recombinant hemoglobin protein.

18. The method of claim 17, wherein said chemically modifying comprises one or more of the following reactions or modifications:

a) amine reactions, wherein the compounds involved comprises anhydride, ketene, NHS ester, isothiocyanates, isocyanates, activated esters comprising fluorophenyl esters and carbonyl azides, sulfonyl chlorides, carbonyls followed by reductive amination, epoxides, carbonates,

fluorobenzenes, imidoesters, hydroxymethyl phosphine derivatives, mannich condensation, diazonium derivatives, 4-sulfo-2,3,5,6-tetrafluorophenol, carbonyl diimidazole, sulfo-NHS, and N-terminal modification by pyridoxal-5-phoshpate-based biomimetic transamination;

b) thiol reactions, wherein the compounds involved comprises maleimides, alkyl halides, haloacetamides, disulfides, thiosulfates, aziridine-containing reagents, acryloyl derivatives, arylating agents, and vinylsulfone derivatives;

c) carboxylate reactions, wherein the compounds involved comprises diazoalkanes and diazoacetyl compounds, carbonyldiimidazoles, and carbodiimides;

d) hydroxyl reactions, wherein the compounds involved comprises epoxides and oxiranes, carbonyldiimidazoles, carbonates, chloroformates, chemical and enzymatic oxidations, alkyl halogens, and isocyanates;

e) native chemical ligations, wherein the compounds involved comprises thioesters;

f) N-terminal modification, wherein the compounds involved comprises N-terminal serine or threonine using periodate oxidation thereof to generate aldehydes for coupling with hydroxylamines, hydrazines, or hydrazides, carbodiimides;

g) incorporation of bioorthogonal functionalities, wherein the compounds involved comprises alkynes and azides with subsequent bioorthogonal conjugation reactions comprising dipolar addition Huisgen 1,3-dipolar additions of alkynes and azides, Staudinger ligation of azides and triarylphosphines, Diels-alder reaction of alkenes and tetrazines, and coupling of alkenes and tetrazoles;

h) photochemical reactions, wherein the compounds involved comprises photoaffinity labeling agents, diazirine derivatives, benzophenones and anthraquinones; and

i) metal-mediated reactions, wherein the compounds involved comprises metal carbenoids and palladium-activated allyl reagents.

19. The method of claim 17, wherein said cleavable linker comprises carbinolamine, disulfide, carbamide, aminal, carbonate, ester, carbamate, phosphate, amide, acetal, imine, oxime, ether or sulfonamide group linker.

20. The method of claim 17, wherein said active agent is a chemotherapeutic agent comprising 5-Fluorouracil, temozolomide, cisplatin or other anti-cancer drug.

21. Use of the recombinant hemoglobin protein of any one of claims 1 to 4 in the manufacture of a medicament for oxygenating tissue from or in an animal or human subject, wherein the tissue is hypoxic.

22. Use of the recombinant hemoglobin protein of any one of claims 1 to 4 in the manufacture of a medicament for treating oxygen-deprivation disorder in an animal or human subject, wherein the oxygen-deprivation disorder is hemorrhagic shock.

23. Use of a composition of any one of claims 11to 16 in the manufacture of a medicament for targeting and inducing apoptosis of cancer cells.

24. Use of a composition of any one of claims 11 to 16 in the manufacture of a medicament for inhibiting tumor metastasis and reducing recurrence of cancer.