INHIBITION OF THE GLYCINE CLEAVAGE SYSTEM FOR TREATMENT OF CANCER

Cross-Reference to Related Application

[0001] This application claims the benefit of U.S. Provisional Application No. 61/597,550, filed February 10, 2012. The entire teachings of the above application(s) are incorporated herein by reference.

Background of the Invention

[0002] Cancer is a major cause of death and was responsible for approximately 7.6 million deaths worldwide (around 13% of all deaths) in 2008 according to the World Health Organization. Although notable successes have been achieved in the pharmacological therapy of a number of tumor types, many tumors remain difficult to treat. For example, the median life expectancy of people with glioblastoma multiforme (GBM), the most common primary brain tumor in adults, is about 12-18 months post-diagnosis, even when treated with current state of the art therapies. There is a need for new targets and therapeutic approaches for the treatment of cancer.

Summary

[0003] In one aspect, the present disclosure refers to an *in vitro* method of inhibiting proliferation or survival of a tumor cell, the method comprising contacting the cell with an inhibitor of the glycine cleavage system (GCS). In another aspect, the present disclosure refers to an *in vitro* method of inhibiting proliferation or survival of a cell that overexpresses SHMT2, the method comprising contacting the cell with an inhibitor of the GCS. In a further aspect, the present disclosure refers to a method of identifying a candidate anti-tumor agent, the method comprising determining whether a test agent binds to or inhibits a GCS component or inhibits the GCS, wherein if the test agent binds to or inhibits a GCS component or inhibits the GCS, the test agent is identified as a candidate anti-tumor agent. In yet another aspect, the present disclosure refers to a method of identifying an anti-tumor agent, the method comprising (a) contacting a tumor cell, tumor cell line, or tumor with a GCS inhibitor; and (b) determining whether the survival, proliferation, growth, or metastasis of the tumor cell or tumor is inhibited, wherein if the survival, proliferation, growth, or metastasis of the tumor cell, tumor cell line, or tumor is inhibited, the GCS inhibitor is an anti-tumor agent. In one aspect, the present disclosure refers to a method of predicting the likelihood that a tumor, tumor cell, or tumor cell line is sensitive to GCS inhibition, the method comprising: (a) determining whether the tumor, tumor cell, or tumor cell line overexpresses SHMT2 or belongs to a tumor type that has a tendency to overexpress SHMT2, wherein if the tumor,

tumor cell, or tumor cell line overexpresses SHMT2 or belongs to a tumor type that has a tendency to overexpress SHMT2, the tumor, tumor cell, or tumor cell line has increased likelihood of being sensitive to GCS inhibition than if tumor, tumor cell, or tumor cell line does not overexpress SHMT2 or does not belong to a tumor type that has a tendency to overexpress SHMT2. In another aspect, the present disclosure refers to a method of identifying a tumor cell or tumor that has an increased likelihood of being sensitive to GCS inhibition, the method comprising assessing expression of SHMT2 in the tumor cell or tumor, wherein if the tumor cell or tumor overexpresses SHMT2, the tumor cell or tumor has an increased likelihood of being sensitive to GCS inhibition than if SHMT2 is not overexpressed. In yet another aspect, the present disclosure refers to a method of determining whether a subject in need of treatment for a tumor is a suitable candidate for treatment with a GCS inhibitor, the method comprising determining whether the tumor overexpresses SHMT2 or is of a tumor type that has a tendency to overexpress SHMT2, wherein if the tumor overexpresses SHMT2 or is of a tumor type that has a tendency to overexpress SHMT2, the subject is a suitable candidate for treatment with a GCS inhibitor. In some ~~aspects~~examples, the present disclosure provides a method of inhibiting proliferation or survival of a tumor cell, the method comprising inhibiting the glycine cleavage system (GCS) of the cell. In some ~~embodiments~~examples the method comprises contacting the tumor cell with a GCS inhibitor. In some ~~embodiments~~examples, the tumor cell overexpresses serine hydroxymethyltransferase 2 (SHMT2).

[0004] In some ~~aspects~~examples, the disclosure provides a method of inhibiting tumor maintenance, growth, or metastasis, the method comprising contacting a tumor with a GCS inhibitor. In some ~~embodiments~~examples the tumor overexpresses SHMT2. In some ~~embodiments~~examples the method comprises determining that the tumor overexpresses SHMT2.

[0005] In some ~~aspects~~examples, the disclosure provides method of treating a subject in need of treatment for a tumor the method comprising treating the subject with a GCS inhibitor. In

some ~~embodiments~~examples a method of treating a subject comprises (a) providing a subject in need of treatment for a tumor, and (b) administering a GCS inhibitor to the subject. In some ~~embodiments~~examples the tumor overexpresses SHMT2. In some ~~embodiments~~examples a method comprises (a) diagnosing a subject as having a tumor; and (b) treating the subject with a GCS inhibitor. In some ~~embodiments~~examples a method comprises (a) diagnosing a subject as having a tumor; and (b) administering a GCS inhibitor to the subject. In some ~~embodiments~~examples a method comprises determining that the subject has a tumor that overexpresses SHMT2.

[0006] In some ~~aspects~~examples, the disclosure provides a method of classifying a tumor cell, tumor cell line, or tumor, the method comprising assessing SHMT2 expression in the tumor cell, tumor cell line, or tumor, and (b) classifying the tumor cell, tumor cell line, or tumor based on the result of step (a). In some ~~embodiments~~examples step (b) comprises classifying the tumor cell, tumor cell line, or tumor as having increased likelihood of being sensitive to GCS inhibition if the tumor cell, tumor cell line, or tumor overexpresses SHMT2.

[0007] In some ~~aspects~~examples, the disclosure provides a method of identifying a subject who is a suitable candidate for treatment with a GCS inhibitor, the method comprising assessing SHMT2 expression in a tumor sample obtained from the subject. In one aspect, the present disclosure refers to an *in vitro* method of selecting a therapeutic agent for a subject in need of treatment for a tumor, the method comprising: (a) determining whether the tumor overexpresses SHMT2 or is of a tumor type that has a tendency to overexpress SHMT2; and (b) selecting a therapeutic agent for the subject based at least in part of the results of step (a). In another aspect, the present disclosure refers to use of a GCS inhibitor in the manufacture of a medicament for treating a subject having a tumor. In yet another aspect, the present disclosure refers to use of a GCS inhibitor in the manufacture of a medicament for treating a subject having a tumor that overexpresses SHMT2. In a further aspect, the present disclosure refers to a method of monitoring a subject in need of treatment for a tumor, the method comprising monitoring the subject at one or more time points after administration of a GCS inhibitor. In one aspect, the present disclosure refers to a pharmaceutical composition for anti-tumor therapy, comprising: (a) an effective amount of a GCS inhibitor; and (b) a pharmaceutically acceptable carrier. In another aspect, the present disclosure refers to a unit dosage form comprising a GCS inhibitor in an amount appropriate for administration to a subject for treatment of a tumor. In yet another aspect, the present disclosure refers to a composition comprising: (a) a GCS inhibitor; and (b) a second anti-tumor agent. In a further aspect, the present disclosure refers to a kit comprising: (a) a reagent that binds to an SHMT2 gene product; and (b) at least one item selected from the group consisting of: (i) instructions for using the reagent to determine whether a tumor overexpresses SHMT2; (ii) a detection reagent; and (iii) a control reagent.

Brief Description of the Drawings

[0008] Figures 1A-1C. shRNAs which knock down GLDC are toxic to GBMSCs. BT145 cells were either infected with a lentivirus expressing hairpins targeting GLDC (G1 to G5) or a control hairpin targeting GFP. (A) Western blotting for GLDC demonstrates strong suppression of GLDC expression for hairpins G1, G4, and G5. (B) When cell viability was measured using a ATP assay , we found that shRNAs G1, G4, and G5 significantly impaired cell viability relative to GFP control hairpin, but not G2 or G3 shRNAs. Differences between G1, G4, and G5 to the GFP control were each statistically significant at 5 days and 7.5 days ($p<0.05$) (error bars not shown for clarity). (C) Morphology of BT145 neurospheres following GLDC knockdown indicates toxicity. As shown, G2, G3, and GFP infected neurospheres form large, round, and regular spheres, indicating viability. On the other hand, G1, G4, or G5 infected spheres are small, irregular, and are in the process of disintegrating, indicating cell death and impaired growth.

Figure 2. Different cell lines are either sensitive or insensitive to shRNA-mediated knockdown of GLDC. As shown on the left table in red font, cell lines of varying

[00293] shRNA that target the following sequences in GCSH ~~shRNA~~ mRNA were used:

[00294] GS1: CGTTGGGAGATGTTGTTTATT (SEQ ID NO: 6)

[00295] GS2: GTGCGTAAATTCACAGAGAAA (SEQ ID NO: 7)

[00296] GS3: GTGAACTCTATTCTCCTTTAT (SEQ ID NO: 8)

[00297] GS4: GATGAACTTATGAGTGAAGAA (SEQ ID NO: 9)

Example 6: Mitochondrial serine hydroxylmethyltransferase expression is tightly correlated with sensitivity to GLDC

[00298] To uncover what determines sensitivity to GLDC knockdown, we examined the expression levels of GLDC and enzymes directly relevant to glycine metabolism in these cell types. Unexpectedly, we observed that expression levels of GLDC did not correlate with whether cells are sensitive to GLDC knockdown (Figure 4). No correlation was observed for GCSH or cytoplasmic serine hydroxymethyltransferase (SHMT1), which is involved in the interconversion of glycine and serine. On the other hand, we observed that mitochondrial serine hydroxymethyltransferase (SHMT2), which is involved in the conversion of serine to glycine in the mitochondria (8), was tightly correlated with sensitivity to GLDC knockdown. Cells that were sensitive to GLDC knockdown had a markedly higher expression of SHMT2 compared to insensitive cells.

[00299] As shown in Figure 4, protein expression levels of GLDC, GCSH, or SHMT1 did not correlate with sensitivity to GLDC knockdown/inhibition. On the other hand, protein expression levels of SHMT2 were tightly correlated with sensitivity to GLDC knockdown/inhibition; sensitive cells expressed high levels of SHMT2, while insensitive cells had low levels.

Example 7: Knockdown of SHMT2 greatly reduces sensitivity to GLDC knockdown.

To examine whether the high expression of SHMT2 in GLDC-knockdown-sensitive cells is functionally relevant or only correlative, we examined the effect of knocking down SHMT2 using shRNAs. First, we examined whether knocking down SHMT2 affected cell viability in either the sensitive or insensitive group of cells (Figure 5). We observed that SHMT2 knockdown did not significantly affect cell viability in either the sensitive or insensitive cells (Figure 5B). To examine whether SHMT2 expression directly affects sensitivity to GLDC knockdown, we first generated cell lines (GBM-derived tumor initiating cells and GBM-derived cell lines) stably expressing either control shRNA or shRNAs against

We claim:

1. An *in vitro* method of inhibiting proliferation or survival of a tumor cell, the method comprising contacting the cell with an inhibitor of the glycine cleavage system (GCS).

2. The method of claim 1, wherein the tumor cell overexpresses serine hydroxymethyltransferase 2 (SHMT2) as compared with a control cell.

3. The method of claim 1, wherein the tumor cell is a brain tumor cell, bladder tumor cell, breast tumor cell, cervical tumor cell, colorectal tumor cell, embryonal tumor cell, gastric tumor cell, germ cell tumor cell, head and neck tumor cell, hematologic tumor cell, kidney tumor cell, melanoma cell, mesothelial tumor cell, ovarian tumor cell, yolk sac tumor cell, or sarcoma cell.

4. The method of claim 1, wherein the tumor cell is a glioblastoma multiforme (GBM) cell.

5. The method of claim 1, wherein the tumor cell is a tumor stem cell.

6. The method of claim 1, wherein the tumor cell is a human tumor cell.

7. The method of claim 1, wherein the GCS inhibitor comprises a small molecule.

8. The method of claim 1, wherein the GCS inhibitor comprises an RNAi agent.

9. The method of claim 1, wherein the GCS inhibitor comprises cysteamine or a cysteamine salt, analog, or prodrug.

10. The method of claim 1, wherein the GCS inhibitor is a glycine dehydrogenase (GLDC) inhibitor.

11. The method of claim 1, wherein the GCS inhibitor is a glycine cleavage system protein H (GCSH) inhibitor.

12. The method of claim 1, wherein the tumor cell is in a subject.

13. An *in vitro* method of inhibiting proliferation or survival of a cell that overexpresses SHMT2, the method comprising contacting the cell with an inhibitor of the GCS.

49. The method of claim 46, wherein the SHMT2 gene product is a SHMT2 RNA or a SHMT2 polypeptide.

50. The method of claim 46, wherein the method comprises performing immunohistochemistry (IHC) on a sample obtained from the tumor, wherein IHC is performed using an antibody that binds to SHMT2 polypeptide.

51. The method of claim 45, wherein determining whether the tumor is of a tumor type prone to overexpress SHMT2 comprises determining whether the tumor is a brain tumor, bladder tumor, breast tumor, cervical tumor, colorectal tumor, embryonal tumor, gastric tumor, germ cell tumor, head and neck tumor, hematologic tumor, kidney tumor, melanoma, mesothelial tumor, ovarian tumor, yolk sac tumor, or sarcoma.

52. An *in vitro* method of selecting a therapeutic agent for a subject in need of treatment for a tumor, the method comprising: (a) determining whether the tumor overexpresses SHMT2 or is of a tumor type that has a tendency to overexpress SHMT2; and (b) selecting a therapeutic agent for the subject based at least in part of the results of step (a).

53. The method of claim 52, wherein step (b) comprises selecting a GCS inhibitor as a therapeutic agent if the tumor overexpresses SHMT2 or is of a tumor type that has a tendency to overexpress SHMT2.

54. The method of claim 52, wherein step (a) comprises performing immunohistochemistry (IHC) on a sample obtained from the tumor, wherein IHC is performed using an antibody that binds to SHMT2 polypeptide.

55. ~~A method of treating a subject in need of treatment for a tumor, the method comprising: administering~~ Use of a GCS inhibitor ~~to the subject~~ in the manufacture of a medicament for treating a subject having a tumor.

56. The ~~method~~ use of claim 55, wherein the tumor overexpresses SHMT2.

57. The ~~method~~ use of claim 55, wherein the tumor is of a tumor type that has a tendency to overexpress SHMT2.

58. The ~~method~~ use of claim 55, wherein the method comprises determining that the tumor overexpresses SHMT2.

59. The ~~method~~ use of claim 55, wherein the method comprises determining that the tumor overexpresses SHMT2 by performing IHC on a sample obtained from the tumor.

60. The ~~method~~ use of claim 55, wherein the method comprises determining that the tumor is sensitive to inhibition of the GCS.

61. The ~~method~~ use of claim 55, wherein the method comprises determining that the subject is a suitable candidate for treatment with a GCS inhibitor to treat the tumor.

62. The ~~method~~ use of claim 55, wherein the GCS inhibitor comprises an RNAi agent

63. The ~~method~~ use of claim 55, wherein the GCS inhibitor comprises cysteamine or a cysteamine salt, analog, or prodrug.

64. The ~~method~~ use of claim 55, wherein the GCS inhibitor is a GLDC inhibitor.

65. The ~~method~~ use of claim 55, wherein the GCS inhibitor is a GCSH inhibitor.

66. The ~~method~~ use of claim 55, wherein the tumor is a brain tumor, bladder tumor, breast tumor, cervical tumor, colorectal tumor, embryonal tumor, gastric tumor, germ cell tumor, head and neck tumor, hematologic tumor, kidney tumor, melanoma, mesothelial tumor, ovarian tumor, yolk sac tumor, or sarcoma.

67. The ~~method~~ use of claim 55, wherein the tumor is a GBM.

68. The ~~method~~ use of claim 55, further comprising administering radiation therapy to the subject.

69. The ~~method~~ use of claim 55, further comprising administering a second anti-tumor agent to the subject.

70. ~~A method of treating a subject in need of treatment for a tumor, the method comprising: (a) determining that a subject has a tumor that overexpresses SHMT2; and (b) treating the subject with~~ Use of a GCS inhibitor in the manufacture of a medicament for treating a subject having a tumor that overexpresses SHMT2.

71. The ~~method~~ use of claim 70, wherein determining that the tumor overexpresses SHMT2 comprises performing IHC on a sample obtained from the tumor.

72. The ~~method~~ use of claim 70, wherein the GCS inhibitor comprises an RNAi agent.

73. The ~~method~~ use of claim 70, wherein the GCS inhibitor comprises cysteamine or a cysteamine salt, prodrug, or analog.

74. The ~~method~~ use of claim 70, wherein the GCS inhibitor is a GLDC inhibitor.

75. The ~~method~~ use of claim 70, wherein the GCS inhibitor is a GCSH inhibitor.

76. The ~~method~~ use of claim 70, wherein the tumor is a brain tumor, bladder tumor, breast tumor, cervical tumor, colorectal tumor, embryonal tumor, gastric tumor, germ cell tumor, head and neck tumor, hematologic tumor, kidney tumor, melanoma, mesothelial tumor, ovarian tumor, yolk sac tumor, or sarcoma.

77. The ~~method~~ use of claim 70, wherein the tumor is a GBM.

78. The ~~method~~ use of claim 70, further comprising treating the subject using radiation therapy.

79. The ~~method~~ use of claim 70, further comprising treating the subject with a second anti-tumor agent.

80. A method of monitoring a subject in need of treatment for a tumor, the method comprising: ~~(a) administering a GCS inhibitor to the subject; and (b)~~ monitoring the subject at one or more time points after administration of a GCS inhibitor.

81. The method of claim 80, wherein the tumor overexpresses SHMT2.

82. The method of claim 80, wherein the GCS inhibitor comprises an RNAi agent

83. The method of claim 80, wherein the GCS inhibitor comprises cysteamine or a cysteamine salt, prodrug, or analog.

84. The method of claim 80, wherein the GCS inhibitor is a GLDC inhibitor.

85. The method of claim 80, wherein the GCS inhibitor is a GCSH inhibitor.

86. The method of claim 80, wherein the tumor is a brain tumor, bladder tumor, breast tumor, cervical tumor, colorectal tumor, embryonal tumor, gastric tumor, germ cell tumor, head and neck tumor, hematologic tumor, kidney tumor, melanoma, mesothelial tumor, ovarian tumor, yolk sac tumor, or sarcoma.