CSCs are thought to be particularly resistant to existing anti-cancer drugs. Even though drug treatment may kill the vast majority of cells in the tumour, a single surviving CSC can therefore lead to a relapse of the disease. Recent evidence suggests an overlap between EMT and CSC phenotypes, suggesting that EMT may also play a role in recurrence of cancer after chemotherapy and the development of drug-resistant tumours.

Robust biomarkers for the EMT phenotype would be useful in identifying patients at particular risk of developing metastatic or drug-resistant cancer, while novel drugs that target cells that have undergone EMT will reduce metastasis and relapse following conventional therapy.

EMT activators (e.g. the transcription factor Slug) increase Akt1 activity/expression. It is also known that Akt1 activation (for example of the myristylated variant MyrAkt1) induces EMT activators (e.g. the transcriptional repressor, Snail; Oncogene. 2007 Nov 22;**26**(53):7445-56. Epub 2007 Jun 1) and also causes biomarker switching from epithelial to mesenchymal.

Summary of the Invention

In one aspect, there is provided a method of identifying a subject having an Akt3-related condition, the method comprising assessing the level of expression or activity of Akt3 in the subject, or in a sample derived from the subject.

In another aspect, there is provided a method of prognosing a cancer-related outcome in a subject, the method comprising assessing Akt3 activity or expression in the subject, or in a sample derived from the subject.

In another aspect, there is provided a method of identifying Axl activity, the method comprising determining the level of Akt3 expression or activity in the subject, or in a sample derived from the subject, increased expression or activity of Akt3 correlating with Axl activity.

In another aspect, there is provided a method of selecting patients, preferably human patients, for treatment of an Akt3-related condition, the method comprising identifying patients having

elevated Akt3 activity or expression and selecting thus identified patients for treatment, optionally wherein the Akt3-related condition is cancer.

In another aspect, there is provided the use of an Akt3 inhibitor for the manufacture of a medicament in the treatment of an Akt3-related condition, optionally wherein the condition is cancer.

In another aspect, there is provided the use of an Akt3 inhibitor for the manufacture of a medicament in the inhibition of EMT.

In another aspect, there is provided the use of a compound capable of inhibiting Akt3 activity for the manufacture of a medicament in the prevention, inhibition, or treatment of drug resistance in a subject having cancer, the method comprising contacting the subject with a compound capable of inhibiting Akt3 activity.

In another aspect, there is provided the use of an Akt3 inhibitor or pharmaceutical compound selected as, or derived from, a candidate compound obtained by a method disclosed herein, for the manufacture of a medicament for a method of treating a subject having an Akt3-related condition, the method comprising contacting the subject with an Akt3 inhibitor or pharmaceutical compound.

In another aspect, there is provided the use of a compound capable of inhibiting Akt3 activity for the manufacture of a medicament for a method of inhibiting EMT in a subject, the method comprising contacting the subject with the compound capable of inhibiting Akt3 activity.

In another aspect, there is provided the use of a compound capable of inhibiting Akt3 activity for the manufacture of a medicament for a method of inhibiting Cancer Stem cells in a subject, the method comprising contacting the subject with the compound capable of inhibiting Akt3 activity.

In another aspect, there is provided the use of a compound capable of inhibiting Akt3 activity for the manufacture of a medicament for a method of preventing or inhibiting drug resistance in a

subject having cancer, the method comprising contacting the subject with the compound capable of inhibiting Akt3 activity.

In another aspect, there is provided a method of selecting a candidate pharmaceutical compound useful: (i) for the prevention, inhibition or treatment of an Akt3-related condition; (ii) in the treatment of metastatic or drug resistant cancer; or (iii) in the prevention or inhibition of EMT; the method comprising providing a group of candidate pharmaceutical compounds for testing, testing the effect of candidate pharmaceutical compounds on Akt3 activity in a test system, and selecting a candidate pharmaceutical compound on the basis of inhibiting Akt3 activity.

In another aspect, there is provided a method of selecting a candidate pharmaceutical compound useful in the prevention, inhibition or treatment of an Akt3-related condition, the method comprising: (i) selectively reducing expression of Akt3 in a test cell, contacting the test cell with the candidate pharmaceutical compound and determining the effect of the candidate pharmaceutical compound on inhibition of Akt3 activity; or (ii) selectively reducing expression of Akt3 in an in vitro test system to a low level contacting the test system with a candidate pharmaceutical compound, and selecting candidate pharmaceutical compounds which inhibit Akt3 activity.

In another aspect, there is provided a cell line which is sensitive to inhibitors of EMT, the cell line having a level of Akt3 expression that is just insufficient to prevent EMT, optionally wherein the cell line is a human cell line.

In another aspect, there is provided a method of identifying a compound which inhibits Akt3 activity, the method comprising contacting a cell from a cell line as disclosed above with a test compound and determining inhibition of Akt3 activity in the cell, optionally wherein inhibition of Akt3 activity is identified by inhibition of EMT.

In another aspect, there is provided the use of Akt3 as a biomarker for detecting the occurrence of epithelial-to- mesenchymal transition (EMT) in a subject, optionally wherein an increase in

the expression and/or activation of Akt3 is indicative of the occurrence of epithelial-to-mesenchymal transition (EMT).

In another aspect, there is provided the use of Akt3 as a biomarker for detecting the expression and/or activation of Axl, wherein an increase in the expression and/or activation of Akt3 is indicative of an increase in the expression and/or activation of Axl.

In another aspect, there is provided a method for detecting the occurrence of epithelial-to-mesenchymal transition (EMT) in a sample, said method comprising determining the expression level or activation of Akt3 in a sample isolated from a cell, group of cells, an animal model or human as compared to a control sample, wherein an increase in the expression level or activation of Akt3 relative to the control sample is indicative of the occurrence of epithelial-to-mesenchymal transition (EMT).

In another aspect, there is provided a method for identifying an agent capable of inhibiting or reversing epithelial-to- mesenchymal transition (EMT) , said method comprising administering said agent to a cell, group of cells or animal model, and monitoring the activation and/or the expression of Akt3, optionally wherein the method further comprises: (i) administering the agent to a cell, group of cells or an animal model, not a human; and (ii) measuring Akt3 expression and/or Akt3 activation in samples derived from the treated and the untreated cells or animal model; and (iii) detecting an increase in the expression and/or activation of Akt3 in the treated sample as compared to the untreated sample as an indication of the ability to inhibit or reverse epithelial-to-mesenchymal transition (EMT).

Detailed Description

Unexpectedly it has now been found that Akt3 plays a central role in the induction of EMT and cancer stem cell traits in human cells. In particular, it has been found that constitutively active Akt3 significantly increases the ability of cells to form tumours *in vivo* and mammospheres, compared to control cells or cells expressing constitutively active Akt1. Further, inhibition of Akt3 was able to reverse EMT and CSC traits.

This was unexpected in view of the focus in the field on Akt1 and Akt2.

It has also been found that Akt3 is a biomarker for Axl receptor tyrosine kinase signalling. More specifically Akt3 has been shown to be a biomarker for Axl signalling in epithelial cells. Akt3 has also been found to participate in a feedback loop leading to maintenance of EMT. Further applications of Akt3, such as a biomarker of cancer stem cells, metastasis will be apparent from this disclosure.

Claims

1. A method of identifying a subject having an Akt3-related condition, the method comprising assessing the level of expression or activity of Akt3 in the subject, or in a sample derived from the subject.

2. A method according to claim 1 of identifying a subject having a particular risk of developing metastatic or drug-resistant cancer, the method comprising assessing the level of expression or activity of Akt3 in the subject, or in a sample derived from the subject, an increased level of Akt3 expression or activity indicating an increased risk of the subject of developing metastatic or drug-resistant cancer.

3. A method according to claim 1 of identifying the presence of a Cancer Stem Cell in a subject, the method comprising determining the level of Akt3 expression or activity in the subject, or in a sample derived from the subject, increased expression or activity of Akt3 indicating the existence of a Cancer Stem Cell (CSC).

4. A method according to claim 1 of identifying a subject undergoing epithelial-to-mesenchymal transition (EMT), the method comprising determining the level of Akt3 expression or activity in the subject, or in a sample derived from the subject, an increase in expression or activity of Akt3 indicating the occurrence of EMT.

5. A method of prognosing a cancer-related outcome in a subject, the method comprising assessing Akt3 activity or expression in the subject, or in a sample derived from the subject.

6. A method according to claim 5, wherein an increase in Akt3 activity or expression relative to a control sample is indicative of susceptibility to treatment with an agent capable of inhibiting or reversing EMT, optionally ~~,~~

~~7. A method according to claim 6,~~ wherein the agent is an Akt3 inhibitor or an Axl inhibitor.

~~8~~7. A method of identifying Axl activity, the method comprising determining the level of Akt3 expression or activity in the subject, or in a sample derived from the subject, increased expression or activity of Akt3 correlating with Axl activity.

~~9~~8. A method according to any one of claims 1 to ~~8~~7 in which the subject is mammalian, optionally wherein ~~:~~

~~10. A method according to claim 9 in which~~ the subject is human.

~~11~~9. A method according to any one of claims 1 to ~~10~~8, wherein the level of expression or activity in the subject or sample derived from the subject is determined relative to a control sample.

~~12~~10. A method according to any one of claims 1 to ~~11~~9, further comprising assessing the level of expression or activity of Akt2 in the subject, or in a sample derived from the subject, wherein a decreased level of Akt2 expression or activity indicates:

(i) the subject has an Akt3-related condition;

(ii) an increased risk of the developing metastic or drug-resistant cancer;

(iii) the existence of a cancer stem cell; and/or

(iv) the occurrence of EMT.

~~13~~11. A method according to any one of claims 1 to ~~12~~10 in which expression of Akt3 and not Akt1 and/or Akt2 is determined.

~~14~~12. A method according to any one of claims 1 to ~~13~~11, wherein the level of expression of Akt3 is assessed by determining:

(i) the copy number of the gene encoding Akt3 relative to a control sample, wherein an increase in the copy number indicates an increased level of expression of Akt3; and/or~~:~~

~~15. A method according to any one of claims 1 to 14, wherein the level of expression of Akt3 is assessed by determining~~ (ii) the level of Akt3 protein or mRNA.

~~16~~13. A method according to any one of claims 1 to ~~15~~12, wherein Akt3 activity is assessed by determining:

(i) phosphorylation of Akt3, wherein phosphorylation of Akt3 indicates active Akt3, optionally -

~~17. A method according to claim 16,~~ wherein Akt3 phosphorylation is determined at Serine 472; and/or-

~~18. A method according to any one of claims 1 to 17, wherein Akt3 activity is assessed by determining~~ (ii) the intracellular localisation of Akt3 protein, wherein localisation in the nucleus indicates active Akt3.

~~19~~14. A method of selecting patients, preferably human patients, for treatment of an Akt3-related condition, the method comprising identifying patients having elevated Akt3 activity or expression and selecting thus identified patients for treatment, optionally wherein -

~~20. A method of selecting patients according to claim 19 in which~~ the Akt3-related condition is cancer.

~~21~~15. A method according to ~~any one of~~ claims ~~19~~ 14, ~~or 20,~~ wherein the patient is identified according to a method of any one of claims 1 to ~~18~~13.

~~22~~16. A method according to any one of claims ~~19~~ 14 to ~~21~~15, wherein the treatment comprises administering an agent capable of inhibiting or reversing EMT, optionally -

~~23. A method according to claim 22,~~ wherein the agent comprises an Akt3 inhibitor or an Axl inhibitor.

~~24~~17. A method according to any one of claims 1 to ~~23~~16, wherein the cancer or Akt3-related condition is a cancer selected from breast, melanoma, prostate, ovarian, colorectal, lung or glioma cancer.

~~25~~18. Use of ~~A~~an Akt3 inhibitor for the manufacture of a medicament ~~use~~ in the treatment of an Akt3-related condition, optionally wherein -

~~26. An Akt3 inhibitor according to claim 25 in which~~ the condition is cancer.

~~27~~19. Use of ~~A~~an Akt3 inhibitor for the manufacture of a medicament ~~use~~ in the inhibition of EMT.

~~28~~20. Use of a~~A~~ compound capable of inhibiting Akt3 activity for the manufacture of a medicament ~~use~~ in the prevention, inhibition, or treatment of drug resistance in a subject having cancer, the method comprising contacting the subject with a compound capable of inhibiting Akt3 activity.

~~29~~21. The use ~~An Akt3 inhibitor~~ according to any one of claims ~~25~~ 18 to ~~28~~ 20, wherein the medicament is in combination with another therapeutic agent.

~~30~~22. Use of an Akt3 inhibitor or pharmaceutical compound selected as, or derived from, a candidate compound obtained by a method according to any one of claims 32 to 33, for the manufacture of a medicament for a method of treating a subject having an Akt3-related condition, the method comprising contacting the subject with an Akt3 inhibitor or pharmaceutical compound ~~selected as, or derived from, a candidate compound obtained by a method according to any one of claims 42 to 46~~.

~~31~~23. Use of an Akt3 inhibitor or pharmaceutical compound for the manufacture of a medicament for a ~~A~~ method of treatment of a subject according to claim ~~30~~ 22 having an Akt3-related condition, the method comprising periodically assessing Akt3 activity in the subject~~.~~

~~32. A method according to claim 30 or 31 in which~~, optionally wherein the Akt3-related condition is cancer.

~~33~~24. Use of an Akt3 inhibitor or pharmaceutical compound for the manufacture of a medicament for a ~~A~~ method according to claim ~~30, 31~~ 22 or ~~32~~ 23 in which treatment of the subject is adjusted according to detected levels of Akt3 activity.

~~34~~25. Use of an Akt3 inhibitor or pharmaceutical compound for the manufacture of a medicament for a ~~A~~ method according to any one of claims 22 to 24 ~~30 to 33~~ in which the subject is being treated with an Axl inhibitor.

~~35~~26. Use of a compound capable of inhibiting Akt3 activity for the manufacture of a medicament for a ~~A~~ method of inhibiting EMT in a subject, the method comprising contacting the subject with the ~~a~~ compound capable of inhibiting Akt3 activity.

~~36~~27. Use of a compound capable of inhibiting Akt3 activity for the manufacture of a medicament for a ~~A~~ method of inhibiting Cancer Stem cells in a subject, the method comprising contacting the subject with the ~~a~~ compound capable of inhibiting Akt3 activity.

~~37~~28. Use of an Akt3 inhibitor, pharmaceutical compound, or compound capable of inhibiting Akt3 activity for the manufacture of a medicament for a ~~A~~ method according to any one of claims ~~30~~ 22 to ~~36~~ 27 in which the subject is also contacted with another cancer therapeutic.

~~38~~29. Use of a compound capable of inhibiting Akt3 activity for the manufacture of a medicament for a ~~A~~ method of preventing or inhibiting drug resistance in a subject having cancer, the method comprising contacting the subject with the ~~a~~ compound capable of inhibiting Akt3 activity.

~~39~~30. Use of an Akt3 inhibitor, pharmaceutical compound, or compound capable of inhibiting Akt3 activity for the manufacture of a medicament for a ~~A~~ method according to any one of claims ~~30~~ 22 to ~~38~~ 29 in which the subject is mammalian, optionally wherein ~~:~~

~~40. A method according to claim 39 in which~~ the subject is human.

~~41~~31. Use of ~~A~~an Akt3 inhibitor, pharmaceutical compound, or compound capable of inhibiting Akt3 activity according to any one of claims ~~25~~ 18 to ~~29~~21, or ~~a method of treatment according to~~ any one of claims ~~37~~ 28 to ~~40~~ 30 in which the other therapeutic agent is a cancer treatment selected from alkylating agents, including alkyl sulfonates such as busulfan, nitrogen mustards such as chlorambucil, cyclophosphamide, estramustine, ifosfamide, mechlorethamine, melphalan, and uramustine, ethyleneimine derivatives such as thiotepa, nitrosoureas such as carmustine, lomustine, and streptozocin, triazenes such as dacarbazine, procarbazine, and temozolamide, platinum compounds such as cisplatin, carboplatin, oxaliplatin, satraplatin, and picoplatin onnaplatin, tetraplatin, sprioplatin, iproplatin, chloro(diethylenediamino)-platinum (II) chloride, dichloro(ethylenediamino)-platinum (II), diamino(2-ethylmalonato)platinum (II), (1 ,2-diaminocyclohexane)malonatoplatinum (II), (4-carboxyphthalo)-(1,2-diaminocyclohexane)platinum (II), (1 ,2-diaminocyclohexane)-(isocitrato)platinum (II), and (1 ,2-diaminocyclohexane)-cis-(pyruvato)platinum (II); antimetabolites, including antifolates such as methotrexate, permetrexed, raltitrexed, and trimetrexate,pyrimidine analogues such as azacitidine, capecitabine, cytarabine, edatrexate, floxuridine, fluorouracil, gemcitabine, and troxacitabine, and purine analogues such as cladribine, chlorodeoxyadenosine, clofarabine, fludarabine, mercaptopurine, pentostatin, and thioguanine; natural products, including antitumor

cetuximab, ibritumomab tiuxetan, rituximab, and trastuzumab, immunotoxins such as gemtuzumab ozogamicin, radioimmunoconjugates such as I-tositumobab, and cancer vaccines; Biologic therapy agents including: interferons such as interferon-[alpha]2a and interferon-[alpha]2b, and interleukins such as aldesleukin, denileukin diftitox, and oprelvekin anticancer therapies involving the use of protective or adjunctive agents, including:cytoprotective agents such as amifostine, and dexrazoxane, phosphonates such as pamidronate and zoledronic acid, and stimulating factors such as epoetin, darbeopetin, filgrastim, PEG-filgrastim, and sargramostim; and Axl inhibitor such as 1-(6,7-dihydro-5*H*-benzo[6,7]cyclohepta[1,2-*c*]pyridazin-3-yl)-N^3-((7-(*S*)-pyrrolidin-1-yl)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulene-2-yl)-1*H*-1,2,4-triazole-3,5-diamine; or further combination chemotherapeutic regimens, such as combinations of carboplatin/paclitaxel, capecitabine/docetaxel, fluorauracil/levamisole, fluorauracil/leucovorin, methotrexate/leucovorin, and trastuzumab/paclitaxel, alone or in further combination with carboplatin, and the like.

~~42~~32. A method of selecting a candidate pharmaceutical compound useful:

(i) for the prevention, inhibition or treatment of an Akt3-related condition;

(ii) in the treatment of metastatic or drug resistant cancer; or

(iii) in the prevention or inhibition of EMT;~~,~~

the method comprising providing a group of candidate pharmaceutical compounds for testing, testing the effect of candidate pharmaceutical compounds on Akt3 activity in a test system, and selecting a candidate pharmaceutical compound on the basis of inhibiting Akt3 activity.

~~43. A method of selecting a candidate pharmaceutical compound useful in the treatment of metastatic or drug resistant cancer, the method comprising providing a group of candidate pharmaceutical compounds for testing, testing the effect of candidate pharmaceutical compounds on Akt3 activity in a test system, and selecting a candidate pharmaceutical compound on the basis of its inhibition of Akt3 activity.~~

~~44. A method of selecting a candidate pharmaceutical compound useful in the prevention or inhibition of EMT, the method comprising providing a group of candidate pharmaceutical compounds for testing, testing the effect of candidate pharmaceutical compounds on Akt3 activity in a test system, and selecting a candidate pharmaceutical compound on the basis of inhibiting Akt3 activity.~~

~~45~~33. A method of selecting a candidate pharmaceutical compound useful in the prevention, inhibition or treatment of an Akt3-related condition, the method comprising:

(i) selectively reducing expression of Akt3 in a test cell, contacting the test cell with the candidate pharmaceutical compound and determining the effect of the candidate pharmaceutical compound on inhibition of Akt3 activity; or.

~~46. A method of selecting a candidate pharmaceutical compound useful in the prevention, inhibition or treatment of an Akt3-related condition, the method comprising~~ (ii) selectively reducing expression of Akt3 in an in vitro test system to a low level contacting the test system with a candidate pharmaceutical compound, and selecting candidate pharmaceutical compounds which inhibit Akt3 activity.

~~47~~34. A method according to claim 32 or ~~33~~5 in which candidate pharmaceutical compounds which substantially or completely inhibit Akt3 activity are selected

~~48~~35. A method of selecting candidate pharmaceutical compounds according to claim 33 or 34 ~~45, 46 or 47~~ in which inhibition of Akt3 activity is indicated by a reduction in EMT.

~~49~~36. A method according to any one of claims ~~44~~32 to ~~48~~35 in which the expression of Akt3 in cells in the test system is reduced by 90%, 80%, 70%, 60%, 50%, 40%, 30%, or 20%, optionally wherein .

~~50. A method according to claim 49 in which~~ the expression of Akt3 is reduced so as to not cause inhibition of EMT.

~~51~~37. A method according to any one of claims ~~44~~32 to ~~50~~36 in which the expression of Akt3 is selectively reduced by introducing into cells in the test system with a nucleotide which interferes with expression of Akt3.

~~52~~38. A cell line which is sensitive to inhibitors of EMT, the cell line having a level of Akt3 expression that is just insufficient to prevent EMT, optionally wherein the cell line .

~~53. A cell line according to claim 52 which~~ is a human cell line.

~~54~~39. A method of identifying a compound which inhibits Akt3 activity, the method comprising contacting a cell from a cell line according to claim 38 ~~52 or 53~~ with a test compound and determining inhibition of Akt3 activity in the cell, optionally wherein :

~~55. A method according to claim 54 in which~~ inhibition of Akt3 activity is identified by inhibition of EMT.

~~56~~40. Use of Akt3 as a biomarker for detecting the occurrence of epithelial-to-mesenchymal transition (EMT) in a subject, optionally :

~~57. Use according to claim 56~~ wherein an increase in the expression and/or activation of Akt3 is indicative of the occurrence of epithelial-to-mesenchymal transition (EMT).

~~58~~41. Use of Akt3 as a biomarker for detecting the expression and/or activation of Axl, wherein an increase in the expression and/or activation of Akt3 is indicative of an increase in the expression and/or activation of Axl.

~~59~~42. A method for detecting the occurrence of epithelial-to-mesenchymal transition (EMT) in a sample, said method comprising

determining the expression level or activation of Akt3 in a sample isolated from a cell, group of cells, an animal model or human as compared to a control sample, wherein an increase in the expression level or activation of Akt3 relative to the control sample is indicative of the occurrence of epithelial-to-mesenchymal transition (EMT).

~~60~~43. A method for identifying an agent capable of inhibiting or reversing epithelial-to- mesenchymal transition (EMT) , said method comprising administering said agent to a cell, group of cells or animal model, and monitoring the activation and/or the expression of Akt3, optionally wherein the method further :

~~61. A method according to claim 60 which~~ comprises:

(i) administering the agent to a cell, group of cells or an animal model, not a human; and

(ii) measuring Akt3 expression and/or Akt3 activation in samples derived from the treated and the untreated cells or animal model; and

(iii) detecting an increase in the expression and/or activation of Akt3 in the treated sample as compared to the untreated sample as an indication of the ability to inhibit or reverse epithelial-to-mesenchymal transition (EMT).

~~62~~44. A method according to claim 43 ~~60 or claim 61~~, wherein the animal model is not a human.

~~63~~45. A use or method according to any one of claims ~~57~~ 40 to ~~62~~ 44 wherein the level of expression of Akt3 is assessed by determining:

(i) the copy number of the gene encoding Akt3 relative to a control sample, wherein an increase in the copy number indicates an increased level of expression of Akt3; and/or~~:~~

~~64. A use or method according to any one of claims 56 to 63 wherein the level of expression of Akt3 is assessed by determining~~ (ii) the level of Akt3 protein or mRNA.

~~65~~46. A use or method according to any one of claims ~~56~~ 40 to ~~64~~45, wherein Akt3 activity is assessed by determining:

(i) phosphorylation of Akt3, wherein phosphorylation of Akt3 indicates Akt3 activity, optionally ~~:~~

~~66. A use or method according to claim 65,~~ wherein Akt3 phosphorylation is determined at Serine 472; and/or~~:~~

~~67. A use or method according to any one of claims 56 to 66, wherein Akt3 activity is assessed by determining~~ (ii) the intracellular localisation of Akt3 protein, wherein localisation in the nucleus indicates active Akt3.