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

In one aspect, there is provided a method of identifying a subject undergoing epithelial-to-mesenchymal transition (EMT), the method comprising determining the level of Akt3 expression or activity in a sample derived from the subject relative to a control sample, an increase in expression or activity of Akt3 indicating the occurrence of EMT.

In another aspect, there is provided a prognostic method for either one of: (i) determining whether a subject will be susceptible to treatment with an Ax1 inhibitor; or (ii) determining whether a subject is particularly likely to suffer from metastatic cancer; said method comprising detecting the occurrence of epithelial-to-mesenchymal transition (EMT) in said subject by assessing Akt3 activity or expression in a sample derived from the subject relative to a control sample, wherein an increase in Akt3 activity or expression relative to a control sample is indicative of susceptibility to treatment with an Axl inhibitor or whether a subject is particularly likely to suffer from metastatic cancer.

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, 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 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 an *in vitro* cell or group of cells, and monitoring the activation and/or the expression of Akt3, optionally wherein the method further comprises: (i) administering the agent to a cell, or group of cells; and (ii)

measuring Akt3 expression and/or Akt3 activation in samples derived from the treated and the untreated cells; and (iii) detecting a decrease 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 undergoing epithelial-to-mesenchymal transition (EMT), the method comprising determining the level of Akt3 expression or activity in a sample derived from the subject relative to a control sample, an increase in expression or activity of Akt3 indicating the occurrence of EMT.

2. A method according to claim 1, wherein the occurrence of EMT indicated by an increased level of Akt3 expression or activity indicates an increased risk of the subject of developing metastatic or drug-resistant cancer.

3. A method according to claim 1, wherein the occurrence of EMT indicated by an increased expression or activity of Akt3 indicates the existence of a Cancer Stem Cell (CSC).

4. A prognostic method for either one of:

(i) determining whether a subject will be susceptible to treatment with an Axl inhibitor; or

(ii) determining whether a subject is particularly likely to suffer from metastatic cancer;

said method comprising detecting the occurrence of epithelial-to-mesenchymal transition (EMT) in said subject by assessing Akt3 activity or expression in a sample derived from the subject relative to a control sample, wherein an increase in Akt3 activity or expression relative to a control sample is indicative of susceptibility to treatment with an Axl inhibitor or whether a subject is particularly likely to suffer from metastatic cancer.5. A method according to any one of claims 1 to 4 in which the subject is mammalian, optionally wherein the subject is human.

6. A method according to any one of claims 1 to 5 in which expression of Akt3 and not Akt1 and/or Akt2 is determined.

7. A method according to any one of claims 1 to 6, 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

(ii) the level of Akt3 protein or mRNA.

8. A method according to any one of claims 1 to 7, wherein Akt3 activity is assessed by determining:

(i) phosphorylation of Akt3, wherein phosphorylation of Akt3 indicates active Akt3, optionally wherein Akt3 phosphorylation is determined at Serine 472; and/or

(ii) the intracellular localisation of Akt3 protein, wherein localisation in the nucleus indicates active Akt3.

9. Use of Akt3 as a biomarker for detecting the occurrence of epithelial-to-mesenchymal transition (EMT) in a subject, wherein an increase in the expression and/or activation of Akt3 is indicative of the occurrence of epithelial-to-mesenchymal transition (EMT).

10. 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).

11. A method for identifying an agent capable of inhibiting or reversing epithelial-to mesenchymal transition (EMT) , said method comprising administering said agent to an *in vitro* cell or group of cells, and monitoring the activation and/or the expression of Akt3, optionally wherein the method further comprises:

(i) administering the agent to a cell, or group of cells; and

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

(iii) detecting a decrease 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).

12. A use or method according to any one of claims 9 to 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

(ii) the level of Akt3 protein or mRNA.

13. A use or method according to any one of claims 9 to 12, wherein Akt3 activity is assessed by determining:

(i) phosphorylation of Akt3, wherein phosphorylation of Akt3 indicates Akt3 activity, optionally wherein Akt3 phosphorylation is determined at Serine 472; and/or

(ii) the intracellular localisation of Akt3 protein, wherein localisation in the nucleus indicates active Akt3.