

In mesangial cells, Gas6 was found to have a mitogenic effect, indicative of a possible role in the progression of glomerulosclerosis. Evidence has suggested that the Gas6/Axl pathway also plays a role in glomerulonephritis (Yanagita M. *et al*, The Journal of Clinical Investigation, 2002, 110 (2) 239-246). Further studies have shown that Gas6 promotes the survival of endothelial cells in a model for arterial injury. Angiotensin II, via its AT1 receptor, was shown to increase Axl mRNA and protein receptor in vascular smooth muscle cells (Melaragno M. G. *et al*, Circ Res., 1998, 83 (7): 697- 704).

Axl has also been shown to be involved in cellular adhesion, cell proliferation and regulation of homeostasis in the immune system (Lu Q., 2001) Science 293 (5528): 306-311). Following Axl activation, the following phenomena have been observed: inhibition of apoptosis, increase in "normal" cell (non-transformed) survival of fibroblasts and endothelial cells, migration of Vascular Smooth Muscle Cell (VSMC) (inactivation of the Axl kinase blocks migration), enhancement of neointima formation in blood vessel wall (Melaragno M.G. *et al*, Trends Cardiovasc Med., 1999, (Review) 9 (8): 250-253) and involvement in lesion formation and the progression of atherosclerosis.

The present invention seeks to provide new diagnostic, prognostic and therapeutic applications involving Axl. In particular, the invention seeks to provide methods for detecting the occurrence of epithelial-to-mesenchymal transition (EMT), which have therapeutic implications in the treatment of cancer, more specifically, metastatic and drug resistant cancers.

STATEMENT OF INVENTION

A first aspect of the ~~present disclosure~~invention relates to the use of Axl as a biomarker for detecting the occurrence of epithelial-to-mesenchymal transition (EMT) in a subject.

The present ~~disclosure~~invention is based on the finding that Axl expression is correlated to the occurrence of epithelial-to-mesenchymal transition (EMT). To our knowledge, the present ~~disclosure~~invention represents the first demonstration of such a correlation.

Advantageously, this finding opens up exciting new opportunities for providing diagnostic, prognostic and therapeutic methods in the field of cancer, more particularly, metastatic and drug resistant cancers.

The present inventors have demonstrated a previously unrecognized role for the receptor tyrosine kinase Axl as an essential EMT-induced effector in the invasion-metastasis cascade. The results show that EMT program activation leads to Axl upregulation that is essential for invasiveness and spontaneous metastasis of malignant breast carcinoma cells and drug resistance phenotype. Axl expression correlates strongly with breast cancer patient mortality from interval mammography-detected tumors and clinically identified breast tumours, suggesting a link between Axl activation and the development of metastatic disease.

A second aspect of the ~~present disclosure~~^{invention} relates to a method for detecting the occurrence of epithelial-to-mesenchymal transition (EMT) in a sample, said method comprising the steps of:

- (i) isolating a sample from a cell, group of cells, an animal model or human;
- (ii) determining the expression of Axl in said sample as compared to a control sample, wherein upregulation of Axl expression relative to the control sample is indicative of the occurrence of epithelial-to-mesenchymal transition (EMT).

Advantageously, determining the expression of Axl provides a "permanent" marker for detecting the occurrence of an EMT event, which in itself is transient in nature. Thus, the detection of Axl expression provides a unique and permanent indication of whether an EMT event has taken place.

Studies by the Applicant have shown that this is due to the fact that EMT-related activation establishes an autocrine Axl-Gas6 signalling loop that is advantageous to malignant cells. Axl may also be activated via paracrine mechanisms.

Detection of tumor cells that have undergone EMT is complicated by the fact that current markers (eg. vimentin, N-Cadherin, lack of E-cadherin) are based on mesenchymal cytoskeletal and junctional proteins that are present in normal stromal cells. Distinguishing tumor cells from surrounding stroma cells is difficult. Axl expression is more likely to be restricted to tumor cells in solid tumors providing a distinguishing characteristic to malignant tumor cells.

Reversibility of EMT is important for metastasis formation at distant sites. Axl expression can be used to detect metastasis.

~~An embodiment third aspect of the present disclosure invention~~ relates to a method of diagnosing metastatic cancer in a subject by detecting the occurrence of epithelial-to-mesenchymal transition (EMT), said method comprising determining the level of an Axl receptor polypeptide in a sample from the subject, wherein a higher level of the polypeptide compared to the level in a subject free of metastatic cancer is indicative of the occurrence of epithelial-to-mesenchymal transition (EMT).

~~Another embodiment fourth aspect of the present disclosure invention~~ relates to the use of Axl or a gene encoding Axl in monitoring the activity of an agent capable of inhibiting or reversing epithelial-to-mesenchymal transition (EMT).

~~An fifth aspect of the present disclosure invention~~ relates to 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, animal model or human and monitoring the activity and/or or expression of Axl.

~~An embodiment sixth aspect of the present disclosure invention~~ relates to a method for detecting the ability of an agent to inhibit or reverse epithelial-to-mesenchymal transition (EMT), said method comprising:

- (i) administering the agent to a cell, group of cells, an animal model or human;
- (ii) measuring Axl expression in samples derived from the treated and the untreated cells, animal or human; and
- (iii) detecting an increase or a decrease in the expression or activity of Axl 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).

~~Another embodiment seventh aspect of the present disclosure invention~~ relates to a method of monitoring the activity of an Axl inhibitor, said method comprising detecting the occurrence of epithelial-to-mesenchymal transition (EMT) by:

- (i) administering said Axl inhibitor to a cell, group of cells, an animal model or human; and
- (ii) measuring Axl expression in samples derived from the treated and the untreated cells, animal or human; and

- (iii) detecting an increase or a decrease in the expression or activity of Axl in the treated sample as compared to the untreated sample as an indication of Axl inhibitory activity.

Another embodiment ~~eight aspect~~ of the ~~present disclosure~~invention relates to a method for identifying an agent capable of inhibiting or reversing epithelial-to-mesenchymal transition (EMT), said method comprising the steps of:

- (i) contacting the agent with Axl receptor or cells expressing the Axl receptor;
- (ii) measuring the Axl receptor activity in the presence of the agent; and
- (iii) comparing the activity measured in step (ii) to that measured under controlled conditions, wherein a decrease identifies the agent as being capable of inhibiting or reversing epithelial-to-mesenchymal transition (EMT).

Another embodiment ~~ninth aspect~~ of the ~~present disclosure~~invention relates to a method for identifying an agent capable of inhibiting or reversing epithelial-to-mesenchymal transition (EMT) by screening a plurality of agents, said method comprising the steps of:

- (i) contacting the plurality of agents with the Axl receptor or cells expressing the Axl receptor;
- (ii) measuring the Axl receptor activity in the presence of the plurality of agents;
- (iii) comparing the activity measured in step (ii) to that measured under controlled conditions, wherein a decrease identifies the plurality of agents as being capable of inhibiting or reversing epithelial-to-mesenchymal transition (EMT); and
- (iv) separately determining which agent or agents present in the plurality inhibit or reverse epithelial-to-mesenchymal transition (EMT).

Another embodiment ~~tenth aspect~~ relates to the use of an agent identified according to the method of the ~~present disclosure~~invention in the preparation of a medicament for the treatment of metastatic cancer.

Another embodiment ~~eleventh aspect~~ relates to a pharmaceutical composition comprising an agent identified according to the method of the ~~present disclosure~~invention admixed with a pharmaceutically acceptable diluent, excipient or carrier.

Another embodiment ~~twelfth aspect~~ relates to a process of preparing a composition which comprises:

- (i) identifying an agent capable of inhibiting or reversing epithelial-to-mesenchymal transition (EMT) using the method according to the invention; and
- (ii) admixing said agent with a pharmaceutically acceptable diluent, carrier or excipient.

~~Another embodiment-thirteenth aspect of the present disclosureinvention~~ relates to a method for inhibiting or reversing epithelial-to-mesenchymal transition (EMT) in a subject in need thereof, said method comprising administering an Axl inhibitor to said subject.

~~Another embodiment-fourteenth aspect of the present disclosureinvention~~ relates to a method for treating metastatic cancer in a subject in need thereof, said method comprising inhibiting or reversing epithelial-to-mesenchymal transition (EMT) by administering to said subject an Axl inhibitor.

~~Another embodiment-fifteenth aspect of the present disclosureinvention~~ relates to the use of an Axl inhibitor in the preparation of a medicament for inhibiting or reversing epithelial-to-mesenchymal transition (EMT).

~~Another embodiment-sixteenth aspect of the present disclosureinvention~~ relates to the use of an Axl inhibitor in the preparation of a medicament for treating metastatic cancer by inhibiting or reversing epithelial-to-mesenchymal transition (EMT).

~~Another embodiment-seventeenth aspect of the present disclosureinvention~~ relates to a kit for assessing the ability of an agent to inhibit or reverse epithelial-to-mesenchymal transition (EMT), said kit comprising anti-Axl antibodies, a nucleic acid probe for Axl or a QPCR primer for Axl.

~~An embodiment-eighteenth aspect~~ relates to the use of a kit as defined above in a method according to the invention.

An aspect relates to the use of a kit for assessing the ability of an agent to inhibit or reverse epithelial-to-mesenchymal transition (EMT), said kit comprising anti-Axl antibodies.

Another aspect relates to the use of a kit for assessing the ability of an agent to inhibit or reverse epithelial-to-mesenchymal transition (EMT), said kit comprising a nucleic acid probe for Axl.

Another aspect relates to the use of use of a kit for assessing the ability of an agent to inhibit or reverse epithelial-to-mesenchymal transition (EMT), said kit comprising at least one QPCR primer for Axl.

Another aspect relates to the use of a kit as defined herein in a method defined herein.

Another aspect relates to an ex vivo prognostic method for determining whether a subject will be susceptible to treatment with an Axl inhibitor, said method comprising detecting the occurrence of epithelial-to-mesenchymal transition (EMT) in said subject.

DETAILED DESCRIPTION

Metastasis underlies the majority of cancer-related deaths. Hence, furthering the understanding of the molecular mechanisms that enable tumour cell dissemination is a vital health issue. Epithelial-to-mesenchymal transitions (EMT) endow carcinoma cells with enhanced migratory and survival attributes that facilitate malignant progression. Characterization of EMT effectors is likely to yield new insights into metastasis and novel avenues for treatment. The Applicant has shown that the presence of the