The inventors ~~have~~ has now determined that Dsg2 has an important role in regulating epithelial-mesenchymal transition (EMT) in cells. EMT is a program of development of cells characterized by loss of cell adhesion and increased cell mobility. EMT is essential for numerous developmental processes including mesoderm formation and neural tube formation. EMT also plays a central role during tumour progression, invasion and metastasis. Cancer cells undergo EMT to lose cell adhesion and acquire mesenchymal characteristics, some of which are necessary for invasion and metastasis.

A first aspect of the invention provides an antagonist of Dsg2 for use as a medicament for preventing or treating cancer, wherein said antagonist modulates the function of the amino acid sequence: TQDVFVGSVEELSAAHTLVMKINATDADEPNTLNSKISYR (SEQ ID NO:1), or a fragment or variant thereof, of the EC2 domain of Dsg2.

As mentioned above, the inventors ~~have~~ has determined that Dsg2 has an important role in regulating EMT. They have shown that: (1) Triggering EMT using hepatocyte growth factor/scattering factor (HGF/SF) shows that most of the desmosomal adhesion components are down-regulated, except Dsg2. (2) Epithelial cells transfected with Dsg2 exhibit a mesenchymal-like morphology and showed greater migration and invasion abilities under treatment by HGF/SF. (3) Antibodies against EC2 domain of Dsg2 significantly block HGF/SF-induced EMT *in vitro*. Furthermore, the inventors has~~ve~~ determined that antibodies to the EC2 domain of Dsg2 inhibit invasion of cancer cells, including MCF7 human breast cancer cells, LNCaP human prostate cancer cells, and KM12 human colon cancer cells. While not wishing to be bound to any particular theory, they propose that Dsg2 can function in the cell to promote EMT.

The authors of international patent publication WO 99/57149 suggest that cell adhesion recognition (CAR) sites derived from the EC2 domain of Dsg2 can be used as modulating agents for treating cancer and/or inhibiting metastasis. The authors state that such modulating agents should inhibit cadherin-mediated cell adhesion. Surprisingly, the inventors ~~have~~ has determined that the CAR site within the EC2 domain of Dsg2 does *not* have a function in mediating the EMT promoting activity of Dsg2. In contrast,

non-CAR sequences of the EC2 domain of Dsg2 regulate the EMT promoting activity of Dsg2. Peptide fragments derived from non-CAR sequences of the EC2 domain of Dsg2 block EMT and cell invasion *in vitro*, as do antibodies raised to an EC2 domain of Dsg2 without the CAR sequence. Thus, the inventors ~~have~~ has demonstrated that non-CAR sequences of the EC2 domain of Dsg2 regulate the EMT promoting function of Dsg2. Until the present invention, no role has been ascribed to non-CAR sequences present in the EC2 domain of Dsg2. The non-CAR sequences have no homology to the CAR sequences.

Peptide fragments derived from non-CAR sequences of the EC2 domain of Dsg2, and antibodies raised to an EC2 domain of Dsg2 without the CAR sequence, antagonise the EMT promoting function of Dsg2. Therefore, antagonists of the function of the non-CAR sequences of the EC2 domain of Dsg2 clearly have much utility as agents for preventing or treating cancer, particularly by reducing EMT and associated invasion and metastatic potential of cancerous cells. This was not known, and could not have been predicated from, any information previously known concerning Dsg2 and its role in EMT.

Dsg2 (desmoglein2) is a human transmembrane cell adhesion protein. A schematic overview of the protein structure is provided in Figure 1. An example of an amino acid sequence of Dsg2 is provided at the end of the examples in SEQ ID NO:2; further examples can be located from protein databases, for example NCBI accession NP_001934.2.

The EC2 domain of Dsg2 (extracellular domain 2) is located from amino acid positions 161 to 273 of the sequence provided in SEQ ID NO:2, and is provided below:

QDVFVGSVEELSAAHTLVMKINATDADEPNTLNSKISYRIVSLEPAYPPVFYLNK
DTGEIYTTSVTLDREEHSSYTLTVEARDGNGEVTDKPVKQAQVQIRILDVNDNIP
VVE (SEQ ID NO:3).

The proposed CAR sequence in the EC2 domain of Dsg2 is located from amino acid positions 210 to 218 of the sequence provided in SEQ ID NO:2, and is provided below:

VFYLNKDTG (SEQ ID NO:4)

The inventors ~~have~~ has identified non-CAR sequences within the EC2 domain of Dsg2 that have a role in regulating the EMT promoting activity of Dsg2. This region is located from amino acid positions 160 to 199 of the sequence provided in SEQ ID NO:2, and is provided below:

TQDVFVGSVEELSAAHTLVMKINATDADEPNTLNSKISYR (SEQ ID NO:1)

The first aspect of the invention concerns antagonists of Dsg2, in which the antagonist modulates the function of the amino acid sequence: TQDVFVGSVEELSAAHTLVMKINATDADEPNTLNSKISYR (SEQ ID NO:1), or a fragment or variant thereof, of the EC2 domain of Dsg2.

By "the amino acid sequence: TQDVFVGSVEELSAAHTLVMKINATDADEPNTLNSKISYR (SEQ ID NO:1), or a fragment or variant thereof, of the EC2 domain of Dsg2" we include that the antagonist modulates the function of the amino acid sequence of SEQ ID NO:1 to regulate the EMT promoting function of Dsg2. By "a fragment or variant thereof" of SEQ ID NO:1, we include that the antagonist affect part of the EC2 domain defined in SEQ ID NO:1, or variants of that sequence.

A "fragment" of said peptide will preferably comprise less than the total amino acid sequence of the full native peptide; preferably the fragment retains its biological activity: in this case, its ability to regulate the EMT promoting function of Dsg2.

A "variant" of the peptide also refers to a peptide wherein at one or more positions there have been amino acid insertions, deletions, or substitutions, either conservative or non-

conservative, provided that such changes result in a protein whose basic properties, for example ability to regulate the EMT promoting function of Dsg2; protein interaction; thermostability; activity in a certain pH-range (pH-stability), have not significantly been changed. "Significantly" in this context means that one skilled in the art would say that the properties of the variant may still be different but would not be unobvious over the ones of the original protein.

By "conservative substitutions" is intended combinations such as Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr.

Such variants may be made using the methods of protein engineering and site-directed mutagenesis as would be well known to those skilled in the art.

The accompanying examples provide assays that can be readily used by the skilled person to measure the effect of Dsg2 on EMT and cell invasion: the "Blocking EMT assay" and the "Cell invasion assay". As shown by the inventors, these assays can be used to measure the antagonistic potential of agents on Dsg2-mediated EMT and cell invasion.

By "antagonist" we include any substance that interferes with the physiological action of Dsg2 polypeptide; preferably by affecting the EMT promoting function. Preferably the aspects of the invention provide a therapeutically effective amount of the said antagonist. For the purposes of the present specification a "therapeutically effective amount" of an antagonist is an amount of such an antagonist that is sufficient to prevent or treat cancer in a subject to whom the antagonist is administered.

An embodiment of the first aspect of the invention is wherein the antagonist reduces the EMT promoting function of Dsg2; preferably by reducing the function of the amino acid sequence: TQDVFVGSVEELSAAHTLVMKINATDADEPNTLNSKISYR (SEQ ID NO:1), or a fragment or variant thereof, of the EC2 domain of Dsg2. This can be measured as a reduction in the EMT promoting function of Dsg2; preferably the

cannot effect its normal function. The antagonist may also alter the sub-cellular localisation of polypeptide. In this way, the amount of functional polypeptide is reduced.

Preferably, the antagonist of the first aspect of the invention is an antibody or antibody fragment or derivative; a peptide; a peptidomimetic; or a small molecule.

Preferably the antibody is a monoclonal or polyclonal antibody.

The use of antibodies as agents to modulate polypeptide activity is well known. Indeed, therapeutic agents based on antibodies are increasingly being used in medicine, including several different types of cancer. In the accompanying examples, the inventors provides exemplification of polyclonal antibody that can bind to a Dsg2 polypeptide lacking a CAR sequence in EC2; hence this antibody is an antagonist according to the first aspect of the invention.

Antibodies may be produced as polyclonal sera by injecting antigen into animals. Preferred polyclonal antibodies may be raised by inoculating an animal (e.g. a rabbit) with antigen using techniques known to the art.

Polyclonal antibodies, for use in treating human subjects, may be raised against a number of peptides derived from the amino acid sequence of SEQ ID NO:1. For instance, as shown in the accompanying examples, a polyclonal antibody was raised to the sequence: TQDVFVGSVEELSAAHTLVMKINATDADEPNTLNSKISYRIVSLEPAYPPVFYLN KDC (SEQ ID NO:5) which corresponds to part of the Dsg2 EC2 domain without a CAR region.

Alternatively the antibody may be monoclonal. Conventional hybridoma techniques may be used to raise such antibodies. The antigen used to generate monoclonal antibodies for use as antagonists in the present invention may be the same as would be used to generate polyclonal sera; i.e. all or a fragment or variant of

transferring the heavy and light chain variable regions from a single-chain Fv library into a Fab display vector. This library may yield 2.1 x 10^{10} different antibody fragments. The peptide may then be used as "bait" to identify antibody fragments from then library that have the desired binding properties.

Domain antibodies (dAbs) represent another preferred agent that may be used according to this embodiment of the invention. dAbs are the smallest functional binding unit of antibodies and correspond to the variable regions of either the heavy or light chains of human antibodies. Such dAbs may have a molecule weight of around 13kDa (corresponding to about 1/10 (or less) the size of a full antibody).

Further preferred agents that may be used according to this embodiment of the invention include bispecific Fab-scFv (a "bibody") and trispecific Fab- (scFv)(2) (a "tribody"). For bibodies or tribodies, a scFv molecule is fused to one or both of the VL-CL (L) and VH-CH_1 (Fd) chains, e.g., to produce a tribody two scFvs are fused to C-term of Fab while in a bibody one scFv is fused to C-term of Fab. The preparation of such molecules can be routinely performed by the skilled person from information available in the field.

The antagonist can also be a peptide or peptiomimetic, comprising some or all of the amino acid sequence of SEQ ID NO:1, or amino acid derivative or analogues thereof.

In the accompanying examples, the inventors provides exemplification of peptides containing some of the amino acid sequence of SEQ ID NO:1: KINATDADEPNTLNSKISYR (SEQ ID NO:6) and EELSAAHTLV (SEQ ID NO:7). The inventors ~~have~~ has demonstrated that the peptides can antagonise the EMT promoting function of Dsg2, and hence they are preferred embodiments of the antagonist of the invention.

In addition to the peptides of SEQ ID NO:6 and SEQ ID NO:7, further peptides can be prepared comprising some of the amino acid sequence of SEQ ID NO:1. The utility of such further peptides as antagonists of the EMT promoting function of Dsg2 can be

measured using the "Blocking EMT assay" and the "Cell invasion assay" described in the accompanying examples.

The preparation of peptides is a routine process. For example, the peptides used in the accompanying experiments were synthesized by Peptide 2.0 Inc (Chantilly, VA, USA); many other companies offer the commercial synthesis of peptides. Laboratory techniques are also well known for the preparation of peptides, such methods being readily performed by a skilled person. Hence the skilled person can prepare peptides containing some or all of the amino acid sequence of SEQ ID NO:1, and test their utility as antagonists of the EMT promoting function of Dsg2 using the information provided herein and from common general knowledge.

The peptide antagonists according to the invention may comprise all 40 amino acid residues of SEQ ID NO:1, with or without any amino acid substitutions. However, as mentioned above the inventors ~~have~~ has determined that peptides having 10 amino acids (EELSAAHTLV (SEQ ID NO:7)) and 20 amino acids (KINATDADEPNTLNSKISYR (SEQ ID NO:6)) can antagonise the EMT promoting function of Dsg2. Accordingly preferred peptides according to the invention can range from about 10 amino acids (for example, 7, 8, 9, 10, 11 or 12) to around 20 amino acids (for example, 18, 19, 20, 21, 22).

The peptide antagonists can also contain further amino acid sequences which are not derived from the amino acid sequence of SEQ ID NO:1: for example, other amino acid sequences which provide a separate function of the peptide (such as a tag, or a catalytic domain). Such peptides are also included in the antagonists of the invention.

Also, it will be appreciated that the invention may be put into effect using derivatives or analogues of these preferred peptides that still lie within the definition of the amino acid sequence provided by SEQ ID NO:1.

By "cancer" we include all types of cancer; for example, bladder; breast (female and male); colon; rectal; endometrial; kidney (renal cell); leukemias; lung; melanoma; Non-Hodgkin lymphoma; pancreatic; prostate; skin; and thyroid.

In the accompanying examples the inventors ~~have~~ has demonstrated the EMT promoting function of Dsg2 in prostate cancer, breast cancer and skin cancer cell lines. Hence a preferred embodiment of the invention is wherein said cancer is prostate cancer, breast cancer or skin cancer.

The antagonists of the first, second and third aspects of the invention are used to prevent or treat cancer.

Methods of diagnosing cancer are well known in the art; see, for example, http://www.cancer.gov/ which provides details of different types of cancer; how they are diagnosed; and potential treatments.

The antagonists of the first, second and third aspects of the invention are used as medicaments. Various means by which the medicaments can be formulated are provided below.

A further aspect of the invention provide the use of an antagonist according to the first, second or third aspect of the invention in the manufacture of a medicament for preventing or treating cancer.

A still further aspect of the invention provides a method of treating cancer comprising administering to a subject a therapeutically effective quantity of an antagonist according to the first, second or third aspect of the invention.

A fourth aspect of the invention provides a peptide comprising the amino acid sequence TQDVFVGSVEELSAAHTLVMKINATDADEPNTLNSKISYR (SEQ ID NO:1),

Desmosomes are one of the principal types of cell-cell adhesion junction between epithelial, myocardial and other tissues. Such desmosomes contain transmembrane glycoproteins called desmosomal cadherin, desmocollin (Dsc) and desmoglein (Dsg). Each occurs as at least three distinct genetic isoforms that show tissue-specific expression patterns.

Dsg2 is ubiquitously expressed in all tissues that form desmosomes. The extracellular domains of Dsg2 contain four cadherin repeat domains (EC1-4) about 110 amino acids each in length. The extracellular repeat domain EC1 contains cell adhesion recognition (CAR) sites, which provide cell-cell adhesion. Therefore, Dsg2 has been identified to be a transmembrane cell adhesion molecule. However, very recent studies show that Dsg2 is not just a simple cell-cell adhesion molecule. Dsg2 is involved in promotion of angiogenesis, signalling of apoptosis, and is a substrate for MMPs. Moreover, it has previously been determined that Dsg2 can play a role in EMT. EMT is a very different physiological action to cell-cell adhesion.

Against this background, the inventors investigated the role of Dsg2 and specific regions within that protein in regulating EMT, and whether Dsg2 could be modulated to inhibit cancer cell invasion.

Results and Discussion

It has previously been suggested that Dsg2 has a role in EMT. However it is not known how this activity of Dsg2 is mediated.

Figure 1 presents a schematic diagram of Dsg2, along with known and proposed functions of domains within that protein. The extracellular repeat domain EC1 and EC2 contains cell adhesion recognition (CAR) sites, which provide cell-cell adhesion. The cell adhesion function of Dsg2 CAR sequence (LTGYALDARG; SEQ ID NO: 10) in

EC1 domain is known, and it has been proposed that a CAR sequence (VFYLNKDTGE; SEQ ID NO: 4) in EC2 domain has a similar function in cell adhesion.

The inventors studied the effect of antibodies that specifically bind to the EC1, EC2 and EC4 domains of Dsg2 on EMT. As can be seen in Figures 2 and 3, Anti-EC2 antibodies inhibit EMT and inhibit cell invasion of human melanoma, breast cancer and prostate cancer cells, while anti-EC1 and anti-EC4 antibodies do not have such an effect. Hence it seems that the EMT regulating activity of Dsg2 is mediated by sequences within the EC2 domain of that protein.

The EC2 domain of Dsg2 has been proposed to contain a CAR domain. CAR domains in Dsg2 and other desmocollin (Dsc) and desmoglein proteins (Dsc1, Dsc2, Dsc3, Dsg1, Dsg2, and Dsg3) have previously been demonstrated to have function in cell adhesion. However, it is known that blocking desmosomal adhesion via CAR *promotes* cell migration, cell invasion, and metastasis. In contrast anti-EC2 antibodies inhibit EMT, resulting in maintaining cell adhesion and impeding cell migration, cell invasion, and metastasis. Hence it seems unlikely that a CAR sequence in the EC2 domain of Dsg2 is responsible for any EMT-promoting activity of that protein.

Accordingly, the inventors decided to locate the region of the EC2 domain of Dsg2 which regulates the EMT promoting activity of that protein. To do so, the inventors generated a rabbit polyclonal antibody to peptide derived from Dsg2 EC2, but lacking the full CAR domain:

TQDVFVGSVEELSAAHTLVMKINATDADEPNTLNSKISYRIVSLEPAYPPVFYLN KDC (SEQ ID NO:5).

The antibodies were then used in a "blocking EMT assay" and "cell invasion assay" (details below) to determine their effect on cells. The results are shown in Figure 4. It can be seen that EMT is significantly inhibited by the antibodies generated (panel A), and also that the antibodies generated inhibit the invasion abilities of human breast

cancer MCF7 cells (panel B). Hence this data clearly shows that the EMT promoting function of Dsg2 is mediated by a non-CAR region in EC2.

The inventors then prepared a series of peptides derived from the EC2 domain of Dsg2:

Peptide 1: Control Peptide (YTRLGANLAG) (SEQ ID NO:11).

Peptide 2: CAR Sequence of EC1 (LTGYALDARG) (SEQ ID NO:10).

Peptide 3: CAR Sequence of EC2 (VFYLNKDTGE) (SEQ ID NO:4).

Peptide 4: non-CAR Sequence of EC2 (KINATDADEPNTLNSKISYR) (SEQ ID NO:6).

Peptide 5: non-CAR Sequence of EC2 (YTTSVTLDREEHSSY) (SEQ ID NO:12).

Peptide 6: non-CAR Sequence of EC2 (ARDGNGEVTDKPVKQ) (SEQ ID NO:13).

Peptide 7: non-CAR Sequence of EC2 (EELSAAHTLV) (SEQ ID NO:7).

Peptide 8: non-CAR Sequence of EC2 (IVSLEPAYPP) (SEQ ID NO:14).

The peptides were then used in a "blocking EMT assay" and "cell invasion assay" (details below) to determine their effect on cells. The results are shown in Figure 5. It can be seen that EMT and cell invasion is significantly inhibited by peptides 4 and 7. Again this data clearly shows that the EMT promoting function of Dsg2 is mediated by a non-CAR region in EC2.

Hence the inventors ~~have~~ has demonstrated that inhibition and antagonists of the non-CAR sequences present in EC2 of Dsg2 can block EMT and invasion *in vitro*, and have the potential to block cancer invasion and metastasis *in vivo*.

Experimental protocols

Generation of the antibodies: The polyclonal antibodies were generated by Abgent (San Diego, CA, USA). In brief, the immunizing peptide sequence (TQDVFVGSVEELSAAHTLVMKINATDADEPNTLNSKISYRIVSLEPAYPPVFYLN KDC; SEQ ID NO:5) was synthesized, conjugated with a carrier protein KLH (Keyhole Limpet Hemocyanin) and used in immunization of rabbits. The rabbits were bled and the

Sequence listing

Fragment of ~~N~~non-CAR sequence of EC2 of Dsg2 (SEQ ID NO:1)

TQDVFVGSVEELSAAHTLVMKINATDADEPNTLNSKISYR

Human Dsg2 (SEQ ID NO:2):

marspgraya lllllicfnv gsglhlqvls trnenkllpk hphlvrqkra witapvalre
gedlskknpi akihsdlaee rglkitykyt gkgiteppfg ifvfnkdtge lnvtsildre
etpffllтgy aldargnnve kplelrikvl dindnepvft qdvfvgsvee lsaahtlvmk
inatdadepn tlnskisyri vslepayppv fylnkdtgei yttsvtldre ehssytltve
ardgngevtd kpvkqaqvqi rildvndnip vvenkvlegm veenqvnvev trikvfdade
igsdnwlanf tfasgneggy fhietdaqtn egivtlikev dyeemknldf svivankaaf
hksirskykp tpipikvkvk nvkegihfks svisiyvses mdrsskgqii gnfqafdedt
glpaharyvk ledrdnwisv dsvtseikla klpdfesryv qngtytvkiv aisedyprkt
itgtvlinve dindncptli epvqtichda eyvnvtaedl dghpnsgpfs fsvidkppgm
aekwkiarqe stsvllqqse kklgrseiqf lisdnqgfsc pekqvltltv ceclhgsgcr
eaqhdsyvgl gpaaialmil afllllvpl lllmchcgkg akgftpipgt iemlhpwnne
gappedkvvp sflpvdqggs lvgrngvggm akeatmkgss sasivkgqhe msemdgrwee
hrsllsgrat qftgatgaim ttettktara tgasrdmaga qaaavalnee flrnyftdka
asyteedenh takdcllvys qeeteslnas igccsfiege lddrflddlg lkfktlaevc
lgqkidinke ieqrqkpate tsmntashsl ceqtmvnsen tyssgssfpv pkslqeanae
kvtqeivter svssrqaqkv atplpdpmas rnviatetsy vtgstmpptt vilgpsqpqs
livtervyap astlvdqpya negtvvvter viqphgggsn plegtqhlqd vpyvmvrere
sflapssgvq ptlampniav gqnvtvterv lapastlqss yqiptensmt arnttvsgag
vpgplpdfgl eesghsnsti ttsstrvtkh stvqhsys

EC2 domain of Dsg2 (SEQ ID NO:3)

QDVFVGSVEELSAAHTLVMKINATDADEPNTLNSKISYRIVSLEPAYPPVFYLNKDTGEIYTTSVTLDREE
HSSYTLTVEARDGNGEVTDKPVKQAQVQIRILDVNDNIPVVE

CAR sequence of EC2 of Dsg2 (SEQ ID NO:4)

VFYLNKDTGE

Fragment of non-CAR sequence and part of the CAR sequence of the Dsg2 EC2 domain ~~without a CAR sequence~~ (SEQ ID NO:5)

TQDVFVGSVEELSAAHTLVMKINATDADEPNTLNSKISYRIVSLEPAYPPVFYLNKDC

Fragment of non-CAR sequence of EC2 of Dsg2 (SEQ ID NO:6)

KINATDADEPNTLNSKISYR

Fragment of non-CAR sequence of EC2 of Dsg2 (SEQ ID NO:7)

EELSAAHTLV

Human Dsg2 without a fragment of the non-CAR region of EC2 (SEQ ID NO:8):

marspgraya llllicfnv gsglhlqvls trnenkllpk hphlvrqkra witapvalre
gedlskknpi akihsdlaee rglkitykyt gkgiteppfg ifvfnkdtge lnvtsildre
etpffltgy aldargnnve kplelrikvl dindnepvfi vslepayppv fylnkdtgei
yttsvtldre ehssytltve ardgngevtd kpvkqaqvqi rildvndnip vvenkvlegm
veenqvnvev trikvfdade igsdnwlanf tfasgneggy fhietdaqtn egivtlikev
dyeemknldf svivankaaf hksirskykp tpipikvkvk nvkegihfks svisiyvses
mdrsskgqii gnfqafdedt glpaharyvk ledrdnwisv dsvtseikla klpdfesryv
qngtytvkiv aisedyprkt itgtvlinve dindncptli epvqtichda eyvnvtaedl
dghpnsgpfs fsvidkppgm aekwkiarqe stsvllqqse kklgrseiqf lisdnqgfsc
pekqvltltv ceclhgsgcr eaqhdsyvgl gpaaialmil afllllvpl lllmchcgkg
akgftpipgt iemlhpwnne gappedkvvp sflpvdqggs lvgrngvggm akeatmkgss
sasivkgqhe msemdgrwee hrsllsgrat qftgatgaim ttettktara tgasrdmaga
qaaavalnee flrnyftdka asyteedenh takdcllvys qeeteslnas igccsfiege
lddrflddlg lkfktlaevc lgqkidinke ieqrqkpate tsmntashsl ceqtmvnsen
tyssgssfpv pkslqeanae kvtqeivter svssrqaqkv atplpdpmas rnviatetsy
vtgstmpptt vilgpsqpqs livtervyap astlvdqpya negtvvvter viqphgggsn
plegtqhlqd vpyvmvrere sflapssgvq ptlampniav gqnvtvterv lapastlqss
yqiptensmt arnttvsgag vpgplpdfgl eesghsnsti ttsstrvtkh stvqhsys

Dsg2 without EC1 and EC2 CAR sequence (SEQ ID NO:9)

marspgraya llllicfnv gsglhlqvls trnenkllpk hphlvrqkra witapvalre
gedlskknpi akihsdlaee rglkitykyt gkgiteppfg ifvfnkdtge lnvtsildre
etpffl nnve kplelrikvl dindnepvft qdvfvgsvee lsaahtlvmk
inatdadepn tlnskisyri vslepayppi yttsvtldre ehssytltve ardgngevtd

kpvkqaqvqi rildvndnip vvenkvlegm veenqvnvev trikvfdade igsdnwlanf
tfasgneggy fhietdaqtn egivtlikev dyeemknldf svivankaaf hksirskykp
tpipikvkvk nvkegihfks svisiyvses mdrsskgqii gnfqafdedt glpaharyvk
inatdadepn tlnskisyri vslepayppi yttsvtldre ehssytltve ardgngevtd
kpvkqaqvqi rildvndnip vvenkvlegm veenqvnvev trikvfdade igsdnwlanf
tfasgneggy fhietdaqtn egivtlikev dyeemknldf svivankaaf hksirskykp
tpipikvkvk nvkegihfks svisiyvses mdrsskgqii gnfqafdedt glpaharyvk
ledrdnwisv dsvtseikla klpdfesryv qngtytvkiv aisedyprkt itgtvlinve
dindncptli epvqtichda eyvnvtaedl dghpnsgpfs fsvidkppgm aekwkiarqe
stsvllqqse kklgrseiqf lisdnqgfsc pekqvltltv ceclhgsgcr eaqhdsyvgl
gpaaialmil afllllvpl lllmchcgkg akgftpipgt iemlhpwnne gappedkvvp
sflpvdqggs lvgrngvggm akeatmkgss sasivkgqhe msemdgrwee hrsllsgrat
qftgatgaim ttettktara tgasrdmaga qaaavalnee flrnyftdka asyteedenh
takdcllvys qeeteslnas igccsfiege lddrflddlg lkfktlaevc lgqkidinke
ieqrqkpate tsmntashsl ceqtmvnsen tyssgssfpv pkslqeanae kvtqeivter
svssrqaqkv atplpdpmas rnviatetsy vtgstmpptt vilgpsqpqs livtervyap
astlvdqpya negtvvvter viqphgggsn plegtqhlqd vpyvmvrere sflapssgvq
ptlampniav gqnvtvterv lapastlqss yqiptensmt arnttvsgag vpgplpdfgl
eesghsnsti ttsstrvtkh stvqhsys

CAR sequence of EC1 of Dsg2 (SEQ ID NO:10)

LTGYALDARG

Control peptide sequence (SEQ ID NO:11)

YTRLGANLAG

Fragment of non-CAR sequence of EC2 of Dsg2 (SEQ ID NO:12)

YTTSVTLDREEHSSY

Fragment of non-CAR sequence of EC2 of Dsg2 (SEQ ID NO:13)

ARDGNGEVTDKPVKQ

Fragment of non-CAR sequence of EC2 of Dsg2 (SEQ ID NO:14)

IVSLEPAYPP

Claims

1. ~~1.~~ A monoclonal antibody as an antagonist for use as a medicament for preventing or treating cancer, characterised in that the monoclonal antibody specifically binds to the amino acid sequence: ~~of Dsg2 for use as a medicament for preventing or treating cancer, wherein said antagonist modulates the function of the amino acid sequence:~~ TQDVFVGSVEELSAAHTLVMKINATDADEPNTLNSKISYR (SEQ ID NO:1), KINATDADEPNTLNSKISYR (SEQ ID NO:6) or EELSAAHTLV (SEQ ID NO:7),~~or a fragment or variant thereof,~~ of the non-cell adhesion recognition (non-CAR) sites region of the EC2 domain of Dsg2 and inhibits epithelial mesenchymal transition (EMT).

2. A monoclonal antibody according to claim 1 wherein the monoclonal antibody is raised against the amino acid sequence TQDVFVGSVEELSAAHTLVMKINATDADEPNTLNSKISYR (SEQ ID NO:1), the sequence of the EC2 domain of Dsg2 (SEQ ID NO:3), KINATDADEPNTLNSKISYR (SEQ ID NO:6) or EELSAAHTLV (SEQ ID NO:7).

3. A polyclonal antibody as an antagonist for use as a medicament for preventing or treating cancer, characterised in that the polyclonal antibody is raised against the amino acid sequence TQDVFVGSVEELSAAHTLVMKINATDADEPNTLNSKISYR (SEQ ID NO:1) or the Dsg2 polypeptide lacking a CAR sequence in EC2 (SEQ ID NO:5) and inhibits epithelial mesenchymal transition (EMT).

~~2. The antagonist of Claim 1 wherein the antagonist reduces the EMT promoting function of Dsg2.~~

~~3. The antagonist of Claims 1 or 2 wherein the antagonist specifically binds to the amino acid sequence of SEQ ID NO:1.~~

~~4. The antagonist of Claims 1 or 2 wherein the antagonist includes some or all of the amino acid sequence of SEQ ID NO:1 or amino acid derivatives or analogues thereof.~~

~~5. The antagonist of any of the previous claims wherein the antagonist is an antibody or antibody fragment or derivative; a peptide; a peptidomimetic; or a small molecule.~~

~~6. The antagonist of Claim 5 wherein the antagonist is a polyclonal antibody.~~

~~7. The antagonist of Claim 5 wherein the antagonist is a peptide or peptiomimetic, including some or all of the amino acid sequence of SEQ ID NO:1, or amino acid derivatives or analogues thereof.~~

~~8. The antagonist of Claim 7 wherein the peptide comprises the amino acid sequence: TQDVFVGSVEELSAAHTLVMKINATDADEPNTLNSKISYR (SEQ ID NO:1), KINATDADEPNTLNSKISYR (SEQ ID NO:6) or EELSAAHTLV (SEQ ID NO:7), or amino acid derivatives or analogues thereof.~~

~~9. An antagonist of Dsg2 for use as a medicament for preventing or treating cancer, wherein said antagonist is a Dsg2 polypeptide or a fragment or variant thereof, wherein said polypeptide has one or more amino acid residue substitutions, inversion and/or deletions within the sequence TQDVFVGSVEELSAAHTLVMKINATDADEPNTLNSKISYR (SEQ ID NO:1) or a fragment or variant thereof, of the EC2 domain of Dsg2.~~

~~10. The antagonist of Claim 9 wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:8 or SEQ ID NO:9.~~

~~11. An antagonist of the EMT promoting function of Dsg2 for use as a medicament for preventing or treating cancer.~~

~~12. The antagonist of the EMT promoting function of Dsg2 according to Claim 11, wherein said antagonist is defined in any one of Claims 1 to 10.~~

~~13. The antagonist of any of the previous claims, wherein said antagonist reduces the cell metastasis and/or cell invasion promoting function of Dsg2.~~

~~14~~ 4. The monoclonal antibody or polyclonal antibody according to any one of claims 1 to 3 ~~antagonist of any of the previous claims~~ wherein said cancer is prostate cancer, breast cancer or skin cancer.

5. The monoclonal antibody or polyclonal antibody according to any one of claims 1 to 4 wherein the monoclonal antibody or polyclonal antibody reduces the cell metastasis and/or cell invasion promoting function of Dsg2.

~~15~~6. A peptide comprising the amino acid sequence TQDVFVGSVEELSAAHTLVMKINATDADEPNTLNSKISYR (SEQ ID NO:1), KINATDADEPNTLNSKISYR (SEQ ID NO:6) or EELSAAHTLV (SEQ ID NO:7)~~, or a fragment or variant thereof.~~ of the non-cell adhesion recognition (non-CAR) sites region of the EC2 domain of Dsg2, or a biologically active fragment or variant thereof wherein at one or more positions there is an amino acid deletion or conservative substitution, and wherein said peptide, fragment or variant is capable of inhibiting the epithelial mesenchymal transition (EMT) promoting function of Dsg2 for use as a medicament for preventing or treating cancer.

~~16~~7. An isolated polypeptide comprising the amino acid sequence of SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:7 or SEQ ID NO:9 of the non-cell adhesion recognition (non-CAR) sites region of the EC2 domain of Dsg2, or a biologically active fragment or variant thereof, and wherein at one or more positions there is an amino acid deletion or

conservative substitution and wherein said polypeptide, fragment or variant thereof is capable of inhibiting the epithelial mesenchymal transition (EMT) promoting function of Dsg2 for use as a medicament for preventing or treating cancer.

~~SEQ ID NO:2 or a fragment or variant thereof, wherein said polypeptide has one or more amino acid residue replacements, inversions and/or deletions within the sequence TQDVFVGSVEELSAAHTLVMKINATDADEPNTLNSKISYR (SEQ ID NO:1) or a fragment or variant thereof, of the EC2 domain of Dsg2.~~

8. The polypeptide of claim 7, wherein the polypeptide comprises the amino acid sequence SEQ ID NO:1.

9. The polypeptide of claim 7, wherein the polypeptide comprises the amino acid sequence SEQ ID NO:6.

10. The polypeptide of claim 7, wherein the polypeptide comprises the amino acid sequence SEQ ID NO:7.

11. The polypeptide of claim 7, wherein the polypeptide comprises the amino acid sequence SEQ ID NO:9.

12. The peptide or polypeptide according to any one or claims 6 to 11 wherein the peptide or polypeptide reduces the cell metastasis and/or cell invasion promoting function of Dsg2.

13. The peptide or polypeptide according to any one of claims 6 to 12 wherein said cancer is prostate cancer, breast cancer or skin cancer.

~~17. The polypeptide of Claim 16, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:8 or SEQ ID NO:9.~~

~~18. An antibody capable of specifically binding to the amino acid sequence TQDVFVGSVEELSAAHTLVMKINATDADEPNTLNSKISYR (SEQ ID NO:1) KINATDADEPNTLNSKISYR (SEQ ID NO:6) or EELSAAHTLV (SEQ ID NO:7), or a fragment or variant thereof.~~

~~19. A peptide, polypeptide or antibody of any of Claims 10 to 13 for use in medicine.~~

~~20~~14. A pharmaceutical preparation comprising ~~an~~ a monoclonal antibody, polyclonal antibody, ~~antagonist~~ peptide or~~,~~ polypeptide ~~or antibody~~ according to ~~of~~ any ~~of~~ one of ~~Claims~~ claims 1 to ~~19~~ 13 and a pharmaceutically acceptable excipient.

~~21. A method of screening for antagonists of Dsg2, wherein said antagonist modulates the function of the amino acid sequence: TQDVFVGSVEELSAAHTLVMKINATDADEPNTLNSKISYR (SEQ ID NO:1), or a fragment or variant thereof, of the EC2 domain of Dsg2, comprising (i) exposing a cell having a Dsg2 polypeptide to a test compound; (ii) and the effect of the test compound on the function of the amino acid sequence of SEQ ID NO:1 of the EC2 domain of Dsg2 is determined.~~

~~22. The method of claim 21 further comprising the step of selecting a test compound that reduces the EMT promoting activity of Dsg2.~~

~~23. The method of any of claims 16 to 18 wherein the method has the additional step of mixing the selected agent (or a derivative or analogue thereof) with a pharmaceutically acceptable carrier.~~

15. Use of a monoclonal antibody according to any one of claims 1, 2, 4 or 5 in the preparation of a medicament for preventing or treating cancer.

16. Use of a polyclonal antibody according to any one of claims 3, 4 or 5 in the preparation of a medicament for preventing or treating cancer.

17. Use of a peptide according to claim 6 in the preparation of a medicament for preventing or treating cancer.

18. Use of a polypeptide according to any one of claims 7 to 11 in the preparation of a medicament for preventing or treating cancer.