

262-271; Gang et al., 2007 Blood 109:1743-1751; Battula et al., 2009 Haematologica-the Hematology Journal 94:173-184; Tormin et al., 2011 Blood 117:5067-5077). While these approaches are helpful in isolating hMSCs from the haematopoietic cells, they do not fully distinguish naïve cells from functionally mature populations. Another strategy for the isolation and preparation of uniform populations of hMSCs include size-based sorting to obtain cells that are highly clonogenic and multipotential (Colter et al., 2001 Proceedings of the National Academy of Sciences of the United States of America 98:7841-7845; Smith et al., 2004 Stem Cells 22:823-831); yet another that has emerged is the secretome of these cells. MSCs secrete a broad array of bioactive paracrine factors that play critical roles in engraftment and wound healing (Caplan and Dennis 2006 Journal of Cellular Biochemistry 98:1076-1084; Caplan and Correa, 2011 Cell Stem Cell 9:11-15). Such secretory profiling has also been hypothesised as a means of predicting of hMSC potency (Caplan 2009 Journal of Pathology 217:318-324; Prockop et al., 2010 14: 2190-2199).

Summary

The present inventors have unexpectedly found that GSTT1 expression is inversely correlated with growth rate i.e. cell doubling time and/or tissue forming potential. This discovery enables the identification, separation, enrichment and/or selection of cells having relatively fast growth rates. In the context of stem cells this allows for the provision of cell populations useful in the generation of tissues *in vitro* and *in vivo*. As such, tissues may be generated *in vitro* which are suitable for implantation into patients or cells identified or selected by the methods of the invention may be administered or implanted into patients in order to provide a medical treatment, e.g. the regeneration of tissue.

Accordingly, in one aspect, the present invention refers to a method for identifying a stem cell or plurality of stem cells, the method comprising determining the level of GSTT1 expression in said stem cell or plurality of stem cells; wherein the method further comprises comparing the level of GSTT1 expression determined to a reference value for the level of GSTT1 expression for that stem cell type; and wherein the method further comprises the step of identifying the stem cell or plurality of stem cells having decreased GSTT1 expression relative to the reference value; wherein decreased GSTT1 expression correlates with increased cell proliferation. In one example, there is disclosed a method for identifying a stem cell or plurality of stem cells, the method comprising determining the level of GSTT1 expression in said stem cell or plurality of stem cells.

Advantageously, the method can be used to identify stem cells having improved/enhanced growth rate, and/or tissue forming potential. Accordingly, in some examples, the method comprises: (i) determining the level of GSTT1 expression by a stem cell or plurality of stem cells, and; (ii) comparing the level of GSTT1 expression determined in step (i) to a reference

value for the level of GSTT1 expression for that stem cell type. In some examples, the method further comprises the step of identifying a stem cell or plurality of stem cells having decreased GSTT1 expression relative to the reference value. In some examples the method further comprises the step of separating and/or isolating a stem cell or plurality of stem cells having decreased GSTT1 expression relative to the reference value from other cells or stem cells.

The present inventors have also discovered that certain genotypes of GSTT1 are correlated with cell proliferation (i.e. growth rate) and/or tissue forming potential. Accordingly, in another aspect, the present invention refers to a method for identifying a stem cell or plurality of stem cells, the method comprising determining the GSTT1 genotype of the stem cell or plurality of stem cells; wherein the method further comprises the step of identifying the stem cell or plurality of stem cells having a non-homozygous wildtype GSTT1 genotype; wherein decreased GSTT1 expression correlates with increased cell proliferation. In one example, there is disclosed a method for identifying a stem cell or plurality of stem cells, the method comprising determining the GSTT1 genotype of the stem cell or plurality of stem cells. In some examples, the method further comprises the step of identifying a stem cell or plurality of stem cells having a non-homozygous wildtype GSTT1 genotype. In some examples, the method further comprises the step of separating and/or isolating a stem cell or plurality of stem cells having a non-homozygous wildtype GSTT1 genotype from other cells or stem cells.

The stem cells having comparatively reduced or lower GSTT1 expression, and/or having GSTT1 genotypes correlated with enhanced or improved cell proliferation (i.e. growth rate) and/or tissue forming potential have various other characteristics which may help to distinguish them from stem cells having comparatively higher GSTT1 expression, and/or having a GSTT1 genotype not correlated with enhanced or improved cell proliferation (i.e. growth rate) and/or tissue forming potential. Accordingly, in some examples of the methods of the invention, the stem cells possess one or more of the following characteristics as compared to a reference population of stem cells: (i) enhanced colony forming capacity; (ii) reduced cell size; (iii) increased telomere length and/or reduced rate of telomere shortening; (iv) increased expression of STRO-1, SSEA-4, CD146 and/or PDGFR β ; (v) increased secretion of FGF-2, VEGF, SDF-1 α , fractalkine, PDGF-BB and/or MIP-1 α ; (vi) enhanced suppression of T cells; (vii) decreased expression of ALP, RUNX2 and/or BSP-II; (viii) increased expression of TWIST-1 and/or DERMO-1.

The present inventors have discovered that the growth rate and/or tissue forming potential of stem cells can be modified by changing the level of GSTT1 gene or protein expression, or GSTT1 function.

In another example, there is disclosed a method for modifying a stem cell or plurality of stem cells, the method comprising contacting a stem cell or plurality of stem cells with an agent capable of modifying a stem cell to reduce GSTT1 expression and/or function. In some examples the method comprises: (i) optionally isolating a stem cell or plurality of stem cells from an individual, and; (ii) contacting an isolated stem cell or plurality of stem cells *in vitro* with an agent capable of modifying a stem cell to reduce GSTT1 expression and/or function.

The present disclosure also describes a stem cell or plurality of stem cells which have been modified to reduce endogenous GSTT1 expression and/or function.

Also disclosed is a stem cell or plurality of stem cells, which contain an agent capable of reducing GSTT1 expression and/or function.

Also disclosed is a stem cell or plurality of stem cells, which have been modified to have decreased GSTT1 expression and/or function relative to stem cells which are homozygous for wildtype GSTT1 and/or stem cells having the average (i.e. mean) level of GSTT1 expression and/or function for that stem cell type.

Furthermore, there is disclosed an isolated stem cell or plurality of stem cells, which have decreased GSTT1 expression and/or function relative to stem cells which are homozygous for wildtype GSTT1.

In a further example, a stem cell or plurality of stem cells according to the disclosure are described for use in a method of medical treatment. In one aspect, the present invention refers to use of a stem cell or plurality of stem cells as disclosed herein in the manufacture of a medicament for treating a bone fracture in a patient in need thereof.

In example, the use of a stem cell or plurality of stem cells according to the disclosure in the manufacture of a medicament for use in a method of medical treatment is disclosed.

In example, there is disclosed a method of regenerating tissue in a patient in need of such treatment, the method comprising administering to the patient a therapeutic number of stem cells as disclosed herein.

The present disclosure also describes the stem cell or plurality of stem cells for use in a method of treating a bone fracture, or in the repair of cartilage tissue, wherein the method comprises administering the stem cells to tissue surrounding the fracture or to the site of the injury.

Also disclosed is the use of a stem cell or plurality of stem cells for the generation of bone or cartilage tissue *in vitro* or *in vivo*.

In one aspect, the present invention refers to a method for selecting a stem cell donor, the method comprising determining the genotype for GSTT1 in a DNA-containing sample isolated from an individual, wherein an individual determined to have a GSTT1 genotype which is known, and/or which would be predicted, to result in decreased expression of GSTT1 relative to individuals homozygous for wildtype GSTT1 is selected as a stem cell donor; wherein the method further comprises the step of identifying the DNA-containing sample having a non-homozygous wildtype GSTT1 genotype. In yet another aspect, the present invention refers to a method for selecting a stem cell donor, the method comprising (i) determining the level of GSTT1 expression by a stem cell or plurality of stem cells in a sample isolated from an individual, and; (ii) comparing the level of GSTT1 expression determined in step (i) to a reference value for the level of GSTT1 expression for that stem cell type; wherein an individual determined to have a stem cell or plurality of stem cells having decreased GSTT1 expression relative to the reference value is selected as a stem cell donor. In a further example, there is disclosed a method for selecting a stem cell donor, the method comprising determining the genotype for GSTT1 in a nucleic acid containing sample isolated from an individual, or determining GSTT1 expression in a sample isolated from an individual.

In some examples, the method comprises detecting the presence of a GSTT1 allele in the nucleic acid containing sample. In some examples, the GSTT1 allele is one or more of the wildtype GSTT1 allele, a GSTT1 genotype which is known and/or which would be predicted to result in decreased expression of GSTT1 relative to wildtype GSTT1, and the GSTT1 null (i.e. deletion) allele.

In a further example, there is disclosed a method for selecting a stem cell donor, the method comprising determining the genotype for GSTT1 in a DNA-containing sample isolated from an individual, wherein an individual determined to have a GSTT1 genotype which is known and/or which would be predicted to result in decreased expression of GSTT1 relative to individuals homozygous for wildtype GSTT1 is selected as a stem cell donor.

In another example, there is disclosed a method for selecting a stem cell donor, the method comprising: (i) determining the level of GSTT1 expression by a stem cell or plurality of stem cells in a sample isolated from an individual, and; (ii) comparing the level of GSTT1 expression determined in step (i) to a reference value for the level of GSTT1 expression for that stem cell type; wherein an individual determined to have a stem cell or plurality of stem cells having decreased GSTT1 expression relative to the reference value is selected as a stem cell donor.

In accordance with methods for selecting stem cell donors, in some examples the stem cell or plurality of stem cells additionally possess one or more of the following characteristics as compared to a reference population of stem cells: (i) enhanced colony forming capacity; (ii)

reduced cell size; (iii) increased telomere length and/or reduced rate of telomere shortening; (iv) increased expression of STRO-1, SSEA-4, CD146 and/or PDGFR β ; (v) increased secretion of FGF-2, VEGF, SDF-1 α , fractalkine, PDGF-BB and/or MIP-1 α ; (vi) enhanced suppression of T cells; (vii) decreased expression of ALP, RUNX2 and/or BSP-II; (viii) increased expression of TWIST-1 and/or DERMO-1.

In another aspect, the present invention refers to a method of enriching for colony forming units (CFU-F) in a culture of stem cells, preferably mesenchymal stem cells (MSC), the method comprising partitioning stem cells not having a homozygous positive GSTT1 genotype. In one example, there is disclosed a method of enriching for colony forming units (CFU-F) in a culture of stem cells, preferably mesenchymal stem cells (MSC), the method comprising partitioning stem cells having a decreased level of GSTT1 expression.

In another example, there is disclosed a method of enriching for colony forming units (CFU-F) in a culture of stem cells, preferably mesenchymal stem cells (MSC), the method comprising partitioning stem cells not having a homozygous wildtype GSTT1 genotype.

Partitioning of cells may involve separating and/or isolating cells having, or not having, a selected characteristic from cells that do not have, or have, the characteristic respectively. As such, a population of cells enriched for cells having a desired characteristic may be provided. The characteristic may be a decreased level of GSTT1 expression or none homozygous wildtype GSTT1 genotype.

In another example, there is disclosed a kit for determining GSTT1 expression, the kit comprising reagents for determining the level of expression of GSTT1 by a stem cell or plurality of stem cells in a sample.

In another example, there is disclosed a kit for determining GSTT1 genotype, the kit comprising reagents for determining the GSTT1 genotype in a DNA containing sample.

Kits in accordance with the present disclosure are useful in methods for selecting a stem cell donor as disclosed herein. In particular, the kits are useful for the identifying suitable donors of bone marrow for obtaining stem cells.

Description

GSTT1

Claims:

1. A method for identifying a stem cell or plurality of stem cells, the method comprising determining the level of GSTT1 expression in said stem cell or plurality of stem cells; wherein the method further comprises comparing the level of GSTT1 expression determined to a reference value for the level of GSTT1 expression for that stem cell type; and wherein the method further comprises the step of identifying the stem cell or plurality of stem cells having decreased GSTT1 expression relative to the reference value; wherein decreased GSTT1 expression correlates with increased cell proliferation.

2. The method according to claim 1, wherein the method further comprises the step of separating and/or isolating the stem cell or plurality of stem cells having decreased GSTT1 expression relative to the reference value.

3. A method for identifying a stem cell or plurality of stem cells, the method comprising determining the GSTT1 genotype of the stem cell or plurality of stem cells; wherein the method further comprises the step of identifying the stem cell or plurality of stem cells having a non-homozygous wildtype GSTT1 genotype; wherein decreased GSTT1 expression correlates with increased cell proliferation.

4. The method according to claim 3, wherein the method further comprises the step of separating and/or isolating a stem cell or plurality of stem cells having a non-homozygous wildtype GSTT1 genotype from other cells or stem cells.

5. The method according to any one of claims 1 to 4, wherein the stem cell or plurality of stem cells additionally possess one or more of the following characteristics as compared to a reference population of stem cells from which the reference value for the level of GSTT1 expression is derived:

- (i) enhanced colony forming capacity;
- (ii) reduced cell size;
- (iii) increased telomere length and/or a reduced rate of telomere shortening;
- (iv) increased expression of STRO-1, SSEA-4, CD146 and/or PDGFR β ;
- (v) increased secretion of FGF-2, VEGF, SDF-1 α , fractalkine, PDGF-BB and/or MIP-1 α ;
- (vi) enhanced suppression of T cells;
- (vii) decreased expression of ALP, RUNX2 and/or BSP-II
- (viii) increased expression of TWIST-1 and DERMO-1.

6. Use of a stem cell or plurality of stem cells according to any one of claims 1 to 5 in the manufacture of a medicament for treating a bone fracture in a patient in need thereof.

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7. A method for selecting a stem cell donor, the method comprising determining the genotype for GSTT1 in a DNA-containing sample isolated from an individual, wherein an individual determined to have a GSTT1 genotype which is known, and/or which would be predicted, to result in decreased expression of GSTT1 relative to individuals homozygous for wildtype GSTT1 is selected as a stem cell donor; wherein the method further comprises the step of identifying the DNA-containing sample having a non-homozygous wildtype GSTT1 genotype.

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8. A method for selecting a stem cell donor, the method comprising:

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(i) determining the level of GSTT1 expression by a stem cell or plurality of stem cells in a sample isolated from an individual, and;

(ii) comparing the level of GSTT1 expression determined in step (i) to a reference value for the level of GSTT1 expression for that stem cell type;

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wherein an individual determined to have a stem cell or plurality of stem cells having decreased GSTT1 expression relative to the reference value is selected as a stem cell donor.

9. The method according to claim 8, wherein the stem cell or plurality of stem cells additionally possess one or more of the following characteristics as compared to a reference population of stem cells from which the reference value for the level of GSTT1 expression is derived:

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(i) enhanced colony forming capacity;

(ii) reduced cell size;

(iii) increased telomere length and/or a reduced rate of telomere shortening;

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(iv) increased expression of STRO-1, SSEA-4, CD146 and/or PDGFR β ;

(v) increased secretion of FGF-2, VEGF, SDF-1 α , fractalkine, PDGF-BB and/or MIP-1 α ;

(vi) enhanced suppression of T cells;

(vii) decreased expression of ALP, RUNX2 and/or BSP-II

(viii) increased expression of TWIST-1 and DERMO-1.

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10. A method of enriching for colony forming units (CFU-F) in a culture of stem cells, preferably mesenchymal stem cells (MSC), the method comprising partitioning stem cells not having a homozygous positive GSTT1 genotype.