It is therefore an object of the present invention to provide an improved cancer immunotherapy against colorectal cancer that overcomes, or at least ameliorates, one or more of the disadvantages described above.

It is also an object of the present invention to provide sufficient quantities of tumor cells bearing tumor-specific antigens (TSAs) and tumor-associated antigens (TAAs) to activate immune system cells against CRC cells for cancer immunotherapy.

It is yet another object of the present invention to provide sufficient quantities of antigens, such as tumor-specific antigens (TSAs) and tumor-associated antigens (TAAs), for activation of immune system cells against CRC cells for cancer immunotherapy.

Summary

Disclosed is a composition comprising at least one isolated immune system cell pulsed by contact to at least one colorectal cancer stem cell, or fragments thereof, wherein the colorectal cancer stem cell is obtained by reverting at least one differentiated colorectal tumour cell to have undifferentiated colorectal stem-cell state.

Disclosed is a composition for stimulating the immune system of a subject comprising at least one antigen presenting cell pulsed by contact to one or more antigens obtained from a colorectal cancer stem cell, wherein the colorectal cancer stem cell is obtained by reverting at least one differentiated colorectal tumour cell to have undifferentiated colorectal stem-cell state, wherein the reversion is obtained by reprogramming the differentiated colorectal tumour cell obtained from the subject with cell reprogramming factors or transcription factors selected from the group consisting of Oct4 and Sox2.

Disclosed is a composition for pulsing a dendritic cell such that the dendritic cell is capable of inducing specific immune response of cytotoxic T lymphocytes against an *in vitro* colorectal cancer stem cell, the composition comprising at least one *in vitro* colorectal cancer stem cell-like cell, or fragments thereof, which has been enriched from a re-programmed *in vitro* colorectal cancer cell.

Disclosed is a method of producing a stimulated immune system cell comprising: contacting a colorectal cancer stem cell, or fragments thereof, with at least one immune system cell, wherein the colorectal cancer stem cell is obtained by reverting at least one differentiated colorectal tumour cell to its undifferentiated stem-cell state.

Disclosed is a vaccine comprising at least one immune cell stimulated with the method as defined herein.

Disclosed is a stimulated immune cell obtained from the method as defined herein.

Disclosed is a method of treating a colorectal cancer in a subject, comprising: immunising the subject with a composition defined herein or a vaccine as defined herein or an immune cell stimulated by the methods as defined herein.

Disclosed is the use of a composition as defined herein or a vaccine as defined herein or an immune cell stimulated by the methods as defined herein, in the manufacture of a medicament for treating a colorectal cancer in a subject.

Disclosed is a method of producing an anti-colorectal cancer vaccine for a subject comprising autologous dendritic cells, comprising the steps of: (a) extracting and purifying peripheral blood mononuclear cells (monocytes) obtained from a sample from the subject; (b) cultivating the monocytes under conditions effective to induce monocytes to dendritic cell differentiation; (c) contacting the cultivated immature dendritic cells of (b) with a cancer stem cell, or fragment thereof, obtained by reverting at least one differentiated colorectal tumour cell to its undifferentiated colorectal stem-cell state; and (d) cultivating the colorectal cancer stem-cell loaded dendritic cells of (c) with a dendritic cell maturation-inducing agent; and (e) harvesting the colorectal cancer stem-cell loaded mature dendritic cells as the anti-cancer vaccine.

In one aspect, there is provided a composition comprising at least one isolated dendritic cell loaded with at least one colorectal cancer stem cell, or fragments thereof, wherein the colorectal cancer stem cell comprises a differentiated colorectal tumour cell that has been reprogrammed with a baculoviral vector into an undifferentiated colorectal stem-cell state, wherein the baculoviral vector comprises zinc-finger nuclease-coding sequences and a fusion gene comprising a cell reprogramming factor or transcription factor capable of reverting or reprogramming the differentiated colorectal tumour cell into the undifferentiated colorectal cancer stem-cell state.

In another aspect, there is provided a composition for stimulating the immune system of a subject comprising at least one dendritic cell loaded with one or more antigens of a colorectal cancer stem cell, wherein said colorectal cancer stem cell comprises a differentiated colorectal tumour cell that has been reprogrammed with a baculoviral vector into an undifferentiated colorectal stem-cell state, wherein the baculoviral vector comprises zinc-finger nuclease-coding sequences and a fusion gene comprising a cell reprogramming factor or transcription factor selected from the group consisting of Oct4, Sox2, Klf4 and c-myc.

In another aspect, there is provided a composition for pulsing a dendritic cell such that the dendritic cell is capable of inducing specific immune response of cytotoxic T lymphocytes against an in vitro colorectal cancer stem cell, said composition comprising at least one in vitro colorectal cancer stem cell-like cell, or fragments thereof, which has been enriched from an in vitro colorectal cancer cell that has been reprogrammed with a baculoviral vector into an undifferentiated colorectal stem-cell state, wherein the baculoviral vector comprises zinc-finger nuclease-coding sequences and a fusion gene comprising a cell reprogramming factor or transcription factor capable of reverting or reprogramming the differentiated colorectal tumour cell into the undifferentiated colorectal cancer stem-cell state.

In another aspect, there is provided a method of producing a stimulated dendritic cell comprising: contacting a colorectal cancer stem cell, or fragments thereof, with at least one dendritic cell, wherein said colorectal cancer stem cell comprises a differentiated colorectal tumour cell that has been reprogrammed with a baculoviral vector into an undifferentiated colorectal stem-cell state, wherein the baculoviral vector comprises zinc-finger nuclease-coding sequences and a fusion gene comprising a cell reprogramming factor or transcription factor capable of reverting or reprogramming the differentiated colorectal tumour cell into the undifferentiated colorectal cancer stem-cell state.

In another aspect, there is provided a vaccine comprising the composition as defined herein or at least one dendritic cell stimulated with the method as defined herein.

In another aspect, there is provided a stimulated dendritic cell obtained from the method as defined herein.

In another aspect, there is provided use of a composition as defined herein or a vaccine as defined herein or an dendritic cell stimulated by the methods as defined herein, in the manufacture of a medicament for treating a colorectal cancer in a subject.

In another aspect, there is provided a method of producing an anti-colorectal cancer vaccine for a subject comprising autologous dendritic cells, comprising the steps of: (a) extracting and purifying peripheral blood mononuclear cells (monocytes) obtained from a sample from the subject; (b) cultivating the monocytes under conditions effective to induce monocytes to dendritic cell differentiation; (c) contacting the cultivated immature dendritic cells of (b) with a colorectal cancer stem cell, or fragment thereof, wherein the colorectal cancer stem cell comprises a differentiated colorectal tumour cell that has been reprogrammed with a baculoviral vector into an undifferentiated colorectal stem-cell state, wherein the baculoviral vector comprises zinc-finger nuclease-coding sequences and a fusion gene comprising a cell reprogramming factor or transcription factor capable of reverting or reprogramming the differentiated colorectal tumour cell into the undifferentiated colorectal cancer stem-cell state; (d) cultivating the colorectal cancer stem-cell loaded dendritic cells of (c) with a dendritic cell maturation-inducing agent; and (e) harvesting the colorectal cancer stem-cell loaded mature dendritic cells as the anti-cancer vaccine.

Brief Description of Drawings

The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

Fig.1

[Fig. 1] shows reprogramming of colorectal cancer (CRC) cells to generate induced pluripotent cancer (iPC) cells. (A) is a schematic diagram showing AAVS1-locus integration of the OSKM expression cassette provided by the baculovirus (BV) DNA donor BV-OSKM following BV-ZFN-induced DNA DBS. HR(L) & HR(R): Left and right arms for HR. FP & RP: Binding sites for PCR forward and reverse primers used for PCR genotyping. (B) shows iPC cell generation after BV transduction. Formation of iPS cell-like colony on mitomycin C-inactivated mouse embryonic fibroblasts (MEF) was observed. The phase contrast (left) and eGFP fluorescent (right) images of a colony derived from HCT8 cells are shown. The cells were cultured in a human iPS cell medium. Results from human CRC HCT8 subclones are shown. (C) shows the results of PCR genotyping to confirm the targeted integration of the OSKM cassette into the AAVS1 locus. The amplification of a 3- kb DNA fragment as shown in (A) is used to identify the targeted insertion. (D) shows expression of pluripotency markers in iPC cells derived from HCT-8 cells. Immunostaining was performed to detect the expression of Nanog, SSEA-4 and TRA-1-60 in an iPC cell colony. (E) shows hematoxylin and eosin-staining of tissue sections of a teratoma formed by the derived iPC cells. The histology of

differentiated tissues found in the teratoma demonstrates immature neuroglial tissue and neuroepithelial rosettes (ectoderm), cartilage (mesoderm), and gland (endoderm).

Fig.2

[Fig. 2] shows ectopic expression of OSKM leads to increased CSC-like properties in human CRC cells. (A) are graphs showing OSKM gene expression quantified by RT-qPCR. Primers were designed to amplify both endogenous and exogenous OSKM genes. The GAPDH gene expression was used to normalize and calculate the fold change. (B) is a graph that illustrates tumorsphere formation efficiency. The efficiencies of two OSKM-expressing clones, HCT3.11 and SW1.9, were tested. Results were normalized to their parent cells. (C) is the result of flow