

Description

Title of Invention:

THERAPEUTIC AGENT FOR CANCER, TESTING ASSISTANCE METHOD,
AND SCREENING METHOD FOR THERAPEUTIC AGENT

Technical Field

[0001]

The present invention relates to an agent for use in treatment of cancer via suppression of expression of an inflammation-associated gene targeting non-coding RNA expressed in senescent cells, and control of cell viability, a method for testing cancer, and a method for screening a therapeutic agent.

Background Art

[0002]

It is known that cancer is caused by various factors. One of them is "cellular senescence". The cellular senescence is a state in which various stresses such as oxidative stress from active oxygen species and the like, damage to DNA by radiation, anticancer agents and the like, and activation of oncogenes are placed on cells, so that cell cycling irreversibly stops. Like the DNA repair mechanism and apoptosis, the cellular senescence functions as a suppression mechanism against a

cancer that is caused by accumulation of genetic mutations caused by damage to DNA and mitochondrial insults. However, senescent cells accumulated in the body are known to highly express various inflammatory proteins, induce chronical inflammation with a senescence-associated secretory phenotype (SASP) secreted into peripheral tissues, and cause many diseases and pathological conditions associated with aging, such as cancer. Therefore, the control of senescent cells in the body or SASP may lead to suppression of cancer development or treatment of cancer.

[0003]

While the induction and control mechanisms for cellular senescence have not fully clarified yet, senolytic drugs selectively inducing cell death in senescent cells and senomorphic drugs suppressing SASP secreted by senescent cells are expected to have a prophylactic effect or a therapeutic effect for cancer developing due to aging.

[0004]

For this reason, senolytic drugs and senomorphic drugs are being studied as new prophylactic or therapeutic drugs for cancer. Patent Literature 1 discloses a complex for suppressing cellular senescence, by which a mitochondrial membrane potential reducing agent for removing dysfunctional mitochondria to suppress SASP and a p53 inhibitor are delivered together to target

cells. Patent Literature 2 discloses a cellular senescence-suppressing agent capable of suppressing the senescence of stem cells.

[0005]

However, the complex disclosed in Patent Literature 1 is intended to remove dysfunctional mitochondria associated with cellular senescence, and thus does not contribute to complete removal of senescent cells. The cellular senescence-suppressing agent disclosed in Patent Literature 2 is recognized to have an antioxidant action, but is not considered to be capable of eliminating cellular senescence.

Citation List

Patent Literature

[0006]

Patent Literature 1: Japanese Patent Laid-Open No. 2021-24852

Patent Literature 2: Japanese Patent Laid-Open No. 2018-52879

Non Patent Literature

[0007]

Non Patent Literature 1: Casella G et al. Nucleic Acids Res 2019, Vol 47(14), pp. 7294-7305

Non Patent Literature 2: Ting T et al, Science, 2011, Vol.6017, pp.593-596. doi: 10.1126/science.1200801. Epub 2011 Jan 13.

Non Patent Literature 3: Kishikawa T et al, JCI Insight. 2016, Jun 2;1(8):e86646. doi: 10.1172/jci.insight.86646.

Non Patent Literature 4: Solovyov A Cell Rep, 2018, Vol.23(2), pp.512-521. doi: 10.1016/j.celrep.2018.03.042.

Non Patent Literature 5: Franses J W et al, Oncologist. 2018, Vol. 23(1): pp. 121-127, Published online 2017 Aug 31, doi:10.1634/theoncologist.2017-0234.

Non Patent Literature 6: Billingsley K J, Scientific Reports 2019, volume 9, Article number: 4369.

Non Patent Literature 7: Oezguer E et al. Clin Chim Acta. 2021, Vol.514, pp.74-79. doi: 10.1016/j.cca.2020.12.008. Epub 2020 Dec 31.

Non Patent Literature 8: Cambier L et al, Scientific Reports, 2021, vol. 11, Article number: 94.

Non Patent Literature 9: Nogalski, M T et al, Nature Communications. 2019, vol. 10, Article number: 90.

Non Patent Literature 10: Bersani F et al, PNAS, 2015, 112(49), pp.11548-15153. <https://doi.org/10.1073/pnas.1518008112>.

Non Patent Literature 11: Nogalski, M T et al, PNAS, 2020, Vol. 117 (50), pp. 31891-31901, <https://doi.org/10.1073/pnas.2017734117>.

Non Patent Literature 12: Kojima R et al., Nature Communications, 2018, vol.9, Article number: 1305(2018), DOI:10.1038/s41467-018-03733-8.

Summary of Invention

Technical Problem

[0008]

As will be described in detail below, the present inventors have found that non-coding RNA which is not expressed in normal cells is highly expressed in senescent cells and cancer cells, and controls the expression of inflammatory genes. Further, it has been found that in senescent cells and cancer cells which highly express non-coding RNA, suppression of expression of the non-coding RNA induces cell death. Accordingly, an object is to provide a therapeutic drug for cancer, which targets the non-coding RNA, suppresses harmful SASP, and eliminates senescent cells in tumor microenvironment. Another object is to provide a test method and a test agent for use in diagnosis of a cancer by examining target non-coding RNA, and a molecule which the expression changes with the target, and epigenomes in the relevant gene region. A new target molecule for cancer treatment has been found. Accordingly, another object is to provide a method for screening a therapeutic drug using the target molecule.

Solution to Problem

[0009]

The present invention relates to the following therapeutic agents, testing methods, and screening method for therapeutic agent.

(1) A pharmaceutical composition which is a therapeutic agent and/or a prophylactic agent for a cancer caused by senescent cells, the pharmaceutical composition suppressing expression or inhibiting activity of hSATII RNA, and/or increasing expression of CTCF.

It has been found that increased expression or inhibited activity of hSATII RNA and decreased expression of CTCF are closely related to malignant transformation of cancer. Therefore, a substance capable of regulating these enables treatment of a cancer associated with senescent cells.

[0010]

(2) The pharmaceutical composition according to (1), wherein the pharmaceutical composition is a nucleic acid medicine which suppresses the expression or inhibits the activity of hSATII RNA and/or increases the expression of CTCF.

In particular, as illustrated in the present specification, a nucleic acid or a compound that regulates the expression and the activity of hSATII RNA and CTCF is effective as a medicine.

[0011]

(3) The pharmaceutical composition according to (1) or (2), which targets cancer cells and stromal cells.

The pharmaceutical composition targeting hSATII has an effect on both cancer cells and stromal cells that

form tumor microenvironment. Therefore, effective treatment can be performed.

[0012]

(4) The pharmaceutical composition according to (3), which is used in combination with a chemotherapeutic agent or radiotherapy.

When cellular senescence is induced by a chemotherapeutic agent or radiotherapy, cell death is induced in not only cancer cells but also stromal cells by the present pharmaceutical composition. Therefore, cell death is induced even in cancer cells, and stromal cells forming tumor microenvironment, which remain without cell death induced by existing chemotherapy, radiotherapy or the like. Therefore, the present pharmaceutical composition contributes to not only effective treatment of cancer, but also prevention of recurrence.

[0013]

(5) A testing assistance method of cancer, comprising detecting expression of hSATII RNA and/or CTCF in a sample.

Inflammatory genes in which expression is induced due to cellular senescence, such as a SASP factor, and the like can be expressed even by inflammation that is not associated with cellular senescence. However, it has been revealed that a change in expression of hSATII RNA or CTCF is a phenomenon specific to cellular senescence,

and is also related to cancer development. Therefore, by identifying such an expression change, a cancer caused by cellular senescence can be detected.

[0014]

(6) The testing assistance method according to (5), comprising determining that a cancer develops when the expression of hSATII RNA increases and/or the expression of CTCF decreases.

The pharmaceutical composition according to (1) to (4) can be expected to exhibit a high effect on a cancer to which cellular senescence is closely related. An optimal therapeutic agent can be provided by examining whether cancer is caused by cellular senescence.

[0015]

(7) A testing assistance method of cancer, comprising detecting an epigenomic change of a hSATII DNA region in a sample.

Before the expression of hSATII RNA increases and/or the expression of CTCF decreases in the process of inducing a cellular senescence-like phenotype in cancer cells, epigenomic changes such as swelling and increased accessibility of a hSATII region of genomic DNA occur. Therefore, the method for detecting the epigenomic change can be an effective test method for detecting a cancer.

[0016]

(8) The testing assistance method according to (7), the cancer is caused by cellular senescence.

The epigenomic changes such as swelling and increased accessibility of the hSATII region of genomic DNA may be changes occurring in a very early stage of cancer development, and therefore it may be possible to detect early stage of cancer by detecting the epigenomic change.

[0017]

(9) A method for screening a therapeutic agent and/or a prophylactic agent for a cancer caused by cellular senescence, comprising adding a candidate compound to a medium for senescent cells, the candidate compound being selected at least one selected from the group of decreased expression of hSATII RNA, suppressed activity of hSATII RNA, increased expression of CTCF, decreased expression of a SASP factor, shrinkage of the hSATII DNA region and decreased chromatin accessibility as an index.

By using a change specific to cellular senescence as an index, a novel therapeutic agent for cancer can be screened. In particular, epigenomic changes such as increased expression and activation of hSATII RNA, decreased expression of CTCF, and swelling and increased accessibility of the hSATII region of genomic DNA are phenomena specific to senescent cells. Therefore, detection of the changes enables screening of senolytic drugs and senomorphic drugs that potentially become therapeutic drugs for cancer.

[0018]

(10) A method for treating a cancer associated with cellular senescence, comprising detecting cancer or stromal cells by the testing assistance method according to any one of (5) to (8) for whether senescent cells are involved in development or progression of a cancer, and administering the therapeutic drug according to (1) to (4) to treat a disease.

(11) A method for treating a cancer associated with cellular senescence, comprising administering a compound that suppresses expression or inhibits activity of hSATII RNA, and/or increases expression of CTCF.

Brief Description of Drawings

[0019]

[Figure 1A] (a) shows a scheme of screening of transcripts related to increased accessibility of chromatin, (b) shows a Volcano plot showing a change in ATAC-seq peak for IMR-90 cells in the proliferation phase and cells induced cellular senescence by an X-ray, and (C) shows 652 transcripts for which the accessibility of chromatin shown in (b) changes in the Volcano plot of RNA-seq data (GSE130727). In (b) and (c), the horizontal axis FC represents a fold change, and the vertical axis represents FDR.

[Figure 1B] Figure 1B shows the peaks in ATAC-seq and RNA-seq (GSE130727) data of the hSATII gene loci of IMR-

90 cells in the proliferation phase and cells induced cellular senescence by an X-ray. The results of two biological replicates are shown. In each of ATAC-Seq and RNA-Seq, upper two rows and lower two rows represent the results for IMR-90 cells in the proliferation phase and after cellular senescence induction, respectively.

[Figure 2] Figure 2 is a diagram showing the results of analyzing the expression of hSATII RNA in various cancer cells.

[Figure 3A] Figure 3A is a heat map of SASP-associated gene expression in SVts8 cells and X-ray-induced senescent SVts8 cells in which centromere-derived non-coding RNA (hSAT α) or pericentromere-derived non-coding RNA (hSATII) is overexpressed.

[Figure 3B] Figure 3B is a scattering diagram showing a change in accessibility of chromatin in SVts8 cells in which hSAT α (X axis) or hSATII (y axis) RNA is overexpressed, where vector-expressing cells are used for comparison.

[Figure 3C] Figure 3C shows enrichment analyses of gene sets related to cellular senescence (upper) and inflammatory reaction (lower) in SVts8 cells in which hSAT α or hSATII RNA is overexpressed.

[Figure 3D] Figure 3D is a diagram showing the results of RT-qPCR analysis of an effect of hSATII RNA knockdown on SASP gene expression in SVts8 cells of proliferation

phase or SVts8 cells induced cellular senescence by an X-ray. *** $P < 0.001$.

[Figure 4A] Figure 4A shows the results of analysis of gene ontology of 280 hSATII RNA-binding proteins identified by mass analysis (left). Among them, 26 proteins are classified as chromatin-binding proteins (GO: 0003682). The top ten genes and the numbers of unique peptides are arranged on the right side.

[Figure 4B] Figure 4B is a diagram showing the results of performing a hSAT α or hSATII RNA pull-down assay with a SVts8 cell lysate, and analyzing the binding with CTCF by Western blotting.

[Figure 4C] Figure 4C is a diagram showing the results of expressing CTCF with a FLAG tag (wild type), CTCF Δ ZF1-11 (mutant type) or CTCF Δ ZF3-6 (mutant type) in HEK-293T cells, and analyzing the expression of each molecule by Western blotting.

[Figure 4D] Figure 4D is a diagram showing the results of RIP-qPCR analysis of binding of CTCF WT with a FLAG tag, CTCF Δ ZF1-11 or Δ ZF3-6, which is expressed in HEK-293T cells, to hSATII RNA. NS: Not significant, *** $P < 0.001$.

[Figure 5A] Figure 5A is a diagram showing the results of RT-qPCR analysis of an effect of hSATII RNA expression on the expression of a SASP-like inflammation-associated gene in SVts8 cells in which CTCF is forcibly expressed. The relative expression level is shown as a value

obtained by normalization with respect to a value for vector-expressing cells. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

[Figure 5B] Figure 5B is a diagram showing the results of RT-qPCR analysis of the expression of a SASP-like inflammation-associated gene in SVts8 cells in which expression of CTCF is suppressed by siRNA. The relative expression level is normalized with respect to a numerical value for cells treated with control siRNA. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

[Figure 5C] Figure 5C is a diagram showing the results of RT-qPCR analysis of the expression of hSATII RNA, CTCF, cellular senescence and inflammation-associated genes of TIG-3 cells in which cellular senescence is induced by passage culture. The expression level of each gene is shown as a value obtained by normalization with respect to the expression level in the early stage of passage. *** $P < 0.001$.

[Figure 5D] Figure 5D is a diagram showing the results of Western blotting analysis of a decrease in amount of CTCF protein and expression of cellular senescence marker protein of TIG-3 cells in which cellular senescence is induced by passage culture.

[Figure 6A] Figure 6A is a Venn diagram showing a change in CTCF binding peaks which are based on ChIP-seq analysis.

[Figure 6B] Figure 6B shows a profile plot of signals in the region of center $\pm$ 2kb with regard to peaks obtained in ChIP-seq analysis (left), and a heat map divided into two clusters using the k-means algorithm (right). The P value of the Wilcoxon rank-sum test is shown.

[Figure 6C] Figure 6C is a diagram showing the results of ChIP-qPCR analysis of an effect of hSATII RNA on CTCF binding to ICR located between IGF2 and H19.

[Figure 6D] Figure 6D is a diagram showing the results of EMSA analysis of an effect of hSAT α or hSATII RNA on binding of CTCF to genomic DNA.

[Figure 6E] Figure 6E is a diagram showing RNA-seq, CTCF ChIP-seq and ATAC-seq profiles for gene loci of CXCL 10 and CXCL 11 that are typical SASP factors.

[Figure 6F] Figure 6F is a diagram showing the chromatin interaction reduced by hSATII RNA in the 3C-qPCR assay. The interaction between the constant region (C) and each target region (T) is shown. NS: Not significant, * $P < 0.05$.

[Figure 7A] Figure 7A is a diagram showing the results of RT-PCR experiment of the expression of hSATII RNA introduced into SVts8 cells by a retrovirus.

[Figure 7B] The left part of Figure 7B is a diagram showing the hSATII RNA expression increasing the number of multipolar cells. Microscopic images and the proportion of multipolar cells are shown. ** $P < 0.01$. The right part of Figure 7B is a diagram showing the

hSATII RNA expression increasing the number of cells in which chromosome bridges are formed. A microscopic image and the proportion of multipolar cells are shown. *** $P < 0.001$.

[Figure 7C] Figure 7C is a diagram showing the results of karyotype analysis of SVts8 cells in which hSATII RNA is overexpressed ($n = 20$). *** $P < 0.001$.

[Figure 7D] Figure 7D is a diagram showing the scaffold-independent proliferation ability of SVts8 cells in which hSATII RNA is overexpressed. * $P < 0.05$.

[Figure 7E] The left part of Figure 7E shows immunostaining images showing an effect of hSATII RNA expression on multi-polarization in SVts8 cells in which CTCF is overexpressed. The right part of Figure 7E is a diagram showing proportions of multipolar cells arose by hSATII RNA expression in SVts8 cells in which CTCF is overexpressed. * $P < 0.05$, ** $P < 0.01$.

[Figure 8A] Figure 8A is a diagram showing the results of RT-PCR experiment of the expression of MajSAT RNA introduced into mouse embryonic fibroblasts MEFs by a retrovirus.

[Figure 8B] The left part of Figure 8B is a diagram showing the MajSAT RNA expression increasing the number of multipolar cells. Microscopic images and the proportion of multipolar cells are shown. * $P < 0.05$.

The right part of Figure 8B is a diagram showing the MajSAT RNA expression increasing the number of cells in

which chromosome bridges are formed. Microscopic images and the proportion of cells in which chromosome bridges are formed are shown. * $P < 0.05$.

[Figure 8C] Figure 8C is a diagram showing the scaffold-independent proliferation ability of MEFs in which MajSAT RNA is overexpressed. *** $P < 0.001$.

[Figure 8D] Figure 8D is a diagram showing the results of karyotype analysis of MEFs in which MajSAT RNA is overexpressed ($n = 20$). *** $P < 0.001$.

[Figure 8E] MEFs in which MajSAT RNA was overexpressed were implanted under the skin of nude mice. Figure 8E shows the weights (left) and pictures (right) of tumors on Day 20 (MEF/MajSAT RNA #2) or Day 30 (MEF/Vector, MEF/MajSAT RNA #1, MEF/MajSAT RNA #3). ** $P < 0.01$.

[Figure 9A] Figure 9A shows the results of RT-qPCR analysis of hSATII and hSAT α RNA in extracellular vesicles derived from RPE-1/hTERT cells. * $P < 0.05$, *** $P < 0.001$.

[Figure 9B] The expression of a SASP-like inflammation-associated gene by designer exosomes formed by an EXOtic device was evaluated by RT-qPCR. Each value was normalized with EXOtic-Nluc-treated cells.

[Figure 9C] Figure 9C shows box plots of the quantified results of RNA-ISH using a hSATII RNA probe in colorectal cancer specimens. The hSATII RNA positive cell ratios in normal epithelial cells and tumor cells (left), and normal fibroblasts and cancer-associated

fibroblasts (right) are shown. The upper and lower hinges denote third and first quartiles, respectively, the upper and lower whiskers denote the maximum value and the minimum value, respectively, within a range of 1.5 times of the quartile, and the horizontal line in the box denotes a median value. ** $P < 0.01$, *** $P < 0.001$.

[Figure 9D] Figure 9D is a diagram schematically showing a mechanism in which the increased expression of pericentromere-derived hSATII RNA due to cellular senescence induces the expression of a SASP-associated inflammation gene.

[Figure 10A] Figure 10A is a diagram showing the results of analysis of the effect of suppressing hSATII expression by siRNA in pancreatic cancer cells and pancreatic stromal cells in which cellular senescence is induced.

[Figure 10B] Figure 10B is a diagram showing the results of RNA-ISH analysis of hSATII expression in specimen from a breast cancer patient. hSATII RNA expression is recognized in not only cancer cells but also stromal cells.

[Figure 11A] Pancreatic cancer cells were implanted into immunodeficient mice, the expression of hSATII RNA was then suppressed by siRNA, and the antitumor effect was analyzed. Figure 11A is a diagram showing the results.

[Figure 11B] In an experimental system in which pancreatic cancer cells and stromal cells in which

cellular senescence is induced are co-implanted into immunodeficient mice, the expression of hSATII RNA was suppressed by siRNA in the stromal cells, and the antitumor effect was analyzed. Figure 11B is a diagram showing the results.

[Figure 12A] Figure 12A shows the results of DNA-FISH analysis of the opened state of the hSATII DNA region and gene expression analysis between genes related to cellular senescence by RT-qPCR in induction of cellular senescence into TIG-3 cells by passage culture. NS: Not significant, *** $P < 0.001$.

[Figure 12B] Figure 12B is a scattering diagram obtained by plotting the chromatin accessibility of the hSATII DNA region from DNaseI sequence data of various kinds of cancer cell lines.

[Figure 12C] Figure 12C shows the results of scATAC-seq analysis of the opened state of the hSATII DNA region in specimens from breast cancer patients.

[Figure 12D] Figure 12D shows the results of DNA-FISH analysis of the opened state of the hSATII DNA region in specimens from breast cancer patients.

[Figure 12E] Figure 12E shows the results of analysis of the opened state of the hSAT DNA region in specimens from breast cancer patients.

[Figure 13] Figure 13 is a diagram schematically showing an aspect of screening.

[Figure 14] Figure 14 is a diagram schematically showing the function of hSATII RNA, and the concept of Seno-cancer therapy which targets hSATII RNA.

Description of Embodiments

[0020]

The present invention will be described in detail below showing data, but the present invention is not limited thereto.

[0021]

The therapeutic drugs shown below are effective as therapeutic drugs for cancers that develop due to aging. Particularly relevant are cancers in which inflammation is highly involved in cancer development, such as breast cancer, colorectal cancer and pancreatic cancer in which the number of patients increases with aging.

[0022]

The therapeutic drugs shown below target non-coding RNA expressed in senescent cells, and thus can suppress not only an already developed disease, but also development of cancer due to cellular senescence. Therefore, they are also effective in prevention of cancer development and prevention of recurrence. With regard to an administration method, administration can be performed by a commonly used administration method.

[0023]

In the present specification, the term "cellular senescence" (sometimes referred to simply as "senescence") refers to a state in which a continuous DNA damage response irreversibly stops the proliferation of normal cells. A cell undergoing cellular senescence is referred to as a senescent cell, and secretes SASP factors such as inflammatory protein and extracellular vesicles. The term "cellular senescence-like" refers to a state in which in normal cells or cancer cells, the expression of inflammatory genes increases, and inflammatory proteins, extracellular vesicles and the like are secreted. The concepts of the cellular senescence and the aging of human or animal individuals are different. The aging of individuals corresponds to physical age, whereas the cellular senescence corresponds to biological aging, where the number of senescent cells increases depending on how much stress has been placed on the cells. The definition of the "cancer" is broad, and includes the states of all neoplasms, regardless of whether they are malignant or benign. The cancer includes both solid cancer and non-solid cancer (hematologic cancer). The terms "cancer cells" and "tumor cells" have the same meaning. The term "stromal cells" collectively refers to cells forming supporting tissues for epithelial cells, and includes fibroblasts, immune cells such as lymphocytes and macrophages, vascular endothelial cells, and smooth muscle cells. The

term "cancer-associated fibroblasts" (CAFs) refers to stromal cells forming the cancer stroma, which are known to produce various growth factors having the action of promoting the proliferation of cancer cells.

[0024]

Substances secreted as SASP factors include inflammatory proteins such as inflammatory cytokines, chemokines, matrix metalloproteinases and growth factors, and extracellular vesicles such as exosomes. The inflammatory proteins specifically include inflammatory cytokines such as TNF- α , IL-1 and IL-6, CC chemokines, among which CCL-1 to CCL28 have been identified, CXC chemokines, among which CXCL1 to CXCL17 have been identified, C chemokines including XCL1 and XCL2, CX3C chemokines including CX3CL1, matrix metalloproteinases, among which MMP1 to MMP28 have been identified, and growth factors such as TGF- β , BMP, FGF, PDGF and HGF. In addition, there can be various substances that are secreted in association with inflammation.

[0025]

Whether the disease or pathological condition is one on which the therapeutic drug of the present invention exhibits an effect can be determined by tests that will be described in detail below. Conducting a test beforehand to determine whether the present therapeutic drug exhibits an effect is important not only in

selection of an appropriate therapeutic method for a patient but also in health economics.

[0026]

[Analysis of transcript in which expression changes in senescent cells]

Therapeutic targets for cancers caused by cellular senescence will be described below showing data. The experiment techniques used hereinafter, such as assay methods, follow methods commonly carried out in the art unless otherwise specified.

[0027]

It has been reported that cells which have undergone cellular senescence show abnormal karyomorphisms, where expression of an inflammatory gene is induced via activation of cytosolic DNA sensing pathways. Further, it is known that a dramatical change is induced in a chromatin structure by cellular senescence, so that gene expression changes. Thus, for the purpose of clarifying the mechanisms of changes in chromatin structure and gene expression in the process of cellular senescence, changes in chromatin accessibility and RNA expression in senescent cells and normal cells were compared.

[0028]

Using cells in proliferation phase and cells induced cellular senescence by an X-ray of human normal diploid fibroblasts IMR-90 (obtained from ATCC), ATAC-seq (assay for transposase-accessible chromatin sequencing) was

performed. It was revealed that 16325 regions (false discovery rate < 0.05) changed in the process of cellular senescence (Figure 1A, (a)).

[0029]

The results of ATAC-seq showed that for IMR-90 in which cellular senescence had been induced by an X-ray, there were 14356 peaks corresponding to an increase in chromatin accessibility and 1969 peaks corresponding to a decrease in chromatin accessibility (Figure 1A, (b)). For 16325 regions where chromatin accessibility changed, 652 transcripts were annotated using databases including GRCh37/hg19 and RepeatMasker.

[0030]

Reanalysis was performed on the 652 transcripts using data in which the expression levels of the transcripts are published (GSE130727, Non Patent Literature 1) (Figure 1A(c)). The expression levels were analyzed using RNA-seq data (GSE130727) of the IMR-90 cells in the proliferation phase and in senescent phase induced by an X-ray. The results revealed that for the IMR-90 cells in which cellular senescence had been induced by an X-ray, the accessibility of hSATII (human satellite II) gene loci which are repeated sequences present in pericentromeric regions was high (Figure 1A, (b)), and the expression thereof markedly increased as compared to that in the cells in the proliferation phase ($\log_{10}$ fold change = 2.8, Figure 1A, (c)).

[0031]

Combined ATAC-seq data and RNA-seq data (GSE130727) of IMR-90 cells in the proliferation phase and IMR-90 cells in which cellular senescence had been induced by an X-ray show that senescent cells had higher chromatin accessibility of hSATII gene loci, and an increased number of transcripts as compared to proliferative cells (Figure 1B). Although data are not shown, hSATII RNA expression associated with cellular senescence was detected even in IMR-90 cells in which cellular senescence had been induced by passage culture or the oncogene H-Ras^{V12} had been expressed, and normal diploid fibroblasts TIG-3 which had continuously undergone passage culture for a long period.

[0032]

For hSATII RNA, it is reported that enhancement of RNA transcription is recognized in pancreatic cancer cells (Non Patent Literature 2), and hSATII RNA is highly expressed in colorectal cancer, Parkinson disease and viral infectious diseases, and it is disclosed that hSATII RNA can be a candidate for a cancer diagnosis marker (Non Patent Literature 3 to 9). Further, it is indicated that proliferation of cancer cells and tumors is suppressed using LNA targeting hSATII RNA (Non Patent Literature 10 and 11).

[0033]

Thus, the expression levels of hSATII RNA in cell lines derived from lung cancer, colorectal cancer, stomach cancer, breast cancer and pancreatic cancer, and two types of normal cells (RPE-1, TIG-3) were analyzed by RT-qPCR (Figure 2). The cells were obtained from the following. A549, BSY1, HBC4, HCC2998, HCT15, HCT116, MKN1, MKN74, NCI-H460, ST4, DMS273, HT29, NCI-H226, NCI-H23 (human cancer cell line panel JFCR39), MDA-MB-468, SW620, MDA-MB-231 (ECACC), RPE-1, MCF7 (JCRB Cell Bank), RPE-1, T47D (ATCC), MiaPaCa (Riken Cell Bank). For the normal cells, cells in which cellular senescence had been induced with an anticancer agent that is doxorubicin, and cells which had undergone ordinary culture, and the expression level of hSATII RNA was analyzed. The expression level differed in cancer cells, but it was recognized that in all the cells, hSATII RNA was more highly expressed than in normal cells from ordinary culture. It was also revealed that even in normal cells, the expression markedly increased when cellular senescence was induced.

[0034]

[Change given by hSATII RNA expression]

Analysis was performed on what kind of effect hSATII RNA expression had in a biological sense. With SVts8 cells which are an immortalized human fibroblast line (a cell line obtained by transfecting TIG-3 cells with a temperature-sensitive SV40-Large T antigen gene), hSATII

RNA was overexpressed. The results of RNA-Seq showed that the overexpression of hSATII RNA induced the expression of SASP-like inflammatory genes. On the other hand, the overexpression of human satellite α RNA (hSAT α) located in the centromeric region did not lead to the expression of inflammatory genes (Figure 3A). Also, the regions of inflammatory genes were shown to be opened by the overexpression of hSATII RNA, but were not opened in the case of hSAT α RNA (Figure 3B). The analysis of the expression of genes related to inflammatory reaction and SASP suggested that the overexpression of hSATII RNA increased the expression of SASP-like inflammatory genes (Figure 3C). The present inventors found, for the first time, that hSATII RNA was expressed in senescent cells, and controlled the expression of inflammatory genes.

[0035]

Further, in SVts8 cells of the proliferation phase, and in SVts8 cells of cellular senescence had been induced by an X-ray, hSATII RNA was knocked down with siRNA. The expression of CXCL10, IL1A and IFNB1 which are SASP-associated genes was analyzed. The hSATII RNA was knocked down by introducing siRNA into SVts8 cells with Lipofectamine RNAiMAX Transfection Reagent (Thermo Fisher Scientific). The following siRNAs were used to perform transfection at a concentration of 20 nM. After 2 days, the expression of each gene was analyzed by RT-qPCR.

[0036]

hSATII siRNA

#1: 5'-UUUCCAUUC AUUCCAUUC-3' (SEQ ID NO: 1)

#2: 5'-AAUCAUCGAA UGGUCUCGA-3' (SEQ ID NO: 2)

Control

5'-AUGAACGUGA AUUGCUCAA-3' (SEQ ID NO: 3)

[0037]

The expression levels of hSATII, CXCL10, IL1A and IFNB1 were analyzed by RT-qPCR. The total RNA was extracted with TRIzol Reagent (Thermo Fisher Scientific), and contaminating DNA was removed by a TURBO DNA-free kit (Applied Biosystems), followed by reverse transcription with PrimeScript RT Master Mix (TaKaRa). The RT-qPCR was performed by a StepOne Plus PCR system (Thermo Fisher Scientific) using SYBR Premix Ex TaqII (TaKaRa). The following sequences were used as primers.

[0038]

hSATII

Forward: 5'-AATCATCGAA TGGTCTCGAT-3' (SEQ ID NO: 4)

Reverse: 5'-ATAATTCCAT TCGATTCCAC-3' (SEQ ID NO: 5)

CXCL10

Forward: 5'-CCAGAATCGAAGGCCATCAA-3' (SEQ ID NO: 6)

Reverse: 5'-CATTTCTTGCTAACTGCTTTCAG-3' (SEQ ID NO: 7)

IL1A

Forward: 5'-AACCAGTGCTGCTGAAGGA-3' (SEQ ID NO: 8)

Reverse: 5'-TTCTTAGTGCCGTGAGTTTCC-3' (SEQ ID NO: 9)

IFNB1

Forward: 5'-AAACTCATGAGCAGTCTGCA-3' (SEQ ID NO: 10)

Reverse: 5'-AGGAGATCTTCAGTTTCGGAGG-3' (SEQ ID NO: 11)

[0039]

The knockdown of hSATII RNA decreased the expression of CXCL10, IL1A and INFB1 in senescent cells. Figure 3D shows a fold change (FC) as a relative expression level when the expression in proliferative cells treated with control siRNA is 1. Similar results were obtained for senescent cells from passage culture of IMR-90 cells (data not shown). These data suggest that hSATII RNA changes its chromatin accessibility with cellular senescence, whereby the expression of a SASP-like inflammation-associated gene is controlled.

[0040]

[Mechanism of controlling inflammation-associated gene by hSATII RNA]

An attempt was made to identify hSATII RNA-binding proteins for analyzing the mechanism of promoting the expression of a SASP-like inflammation-associated gene by hSATII RNA. An association between hSAT α RNA located in the centromeric region and a specific protein has been reported, but there has not been a report of an association of a specific protein for hSATII RNA located in the pericentromeric region. Thus, RNA pull-down and mass analysis were performed and identified 280 hSATII RNA-binding proteins.

[0041]

Among these proteins, 26 chromatin-binding proteins (GO: 0003682) were identified by gene ontology analysis, but analysis was performed with a focus on CTCF because it exhibited a high strength score in mass analysis, and was reported to contribute to maintenance of a chromatin steric structure in many cases (Figure 4A). Whether hSATII RNA and CTCF were bound was confirmed by an RNA pull-down assay. The SVts8 cell lysate was analyzed by Western blotting after the RNA pull-down assay. It was confirmed that hSATII RNA was bound to CTCF, but hSAT α RNA was not (Figure 4B).

[0042]

It is known that binding of CTCF to genomic DNA is important for maintaining an appropriate steric structure of a genome. Since CTCF is an RNA-binding protein, RNA immunoprecipitation (RIP) was performed. Wild-type CTCF (WT), and ZF (Zinc Finger)-deleted mutants of CTCF Δ ZF1-11 and Δ ZF3-6 were expressed in HEK293T cells (Figure 4C). The hSATII RNA was found to bind to wild-type CTCF, but was not found to bind to the CTCF mutants Δ ZF1-11 and Δ ZF3-6 (Figure 4D). CTCF has 11 ZF domains, and it is said that binding between ZF1 or ZF10 and RNA is important for forming a chromatin loop to control gene expression. However, although data are not shown, it was found that hSATII RNA did not bind either to ZF1 or to ZF10. It has been revealed that ZF3-ZF6-deleted mutants of CTCF (CTCF Δ ZF3-6) known as a DNA-binding domain

cannot bind to hSATII RNA, therefore ZF3-ZF6 is important for binding to hSATII RNA.

[0043]

For SVts8 cells in which CTCF was forcibly expressed, and hSATII RNA was overexpressed, the expression of inflammation-associated genes was analyzed by RT-qPCR (Figure 5A). In situations where CTCF was overexpressed, even hSATII RNA was expressed, its effect was eliminated, and the expression of the inflammation-associated gene was not enhanced. Further, in proliferative cells, CTCF was knocked down with CTCF siRNA (Thermo Fisher Scientific, #HSS116455, #HSS116456, Negative Control (Duplex High GC Duplex) siRNA, #46-2000), and the expression of the inflammation-associated genes was analyzed (Figure 5B). The knockdown of CTCF enhanced the expression of the inflammation-associated gene. Unexpectedly, it was revealed that the knockdown of CTCF also increased the expression of hSATII RNA. These results suggested that the expression of inflammation-associated genes which is induced by hSATII RNA was likely to depend on the dysfunction of CTCF, and the CTCF controlled the expression of hSATII RNA in the process of cellular senescence. Then, the expression level of CTCF in TIG-3 cells in which senescence had been induced by passage culture was analyzed by RT-qPCR and Western blotting (Figures 5C and 5D). As the passage culture progressed, the expression level of CTCF

decreased (Figures 5C and 5D), and the expression levels of hSATII RNA and the inflammation-associated genes increased (Figure 5C). This suggested that CTCF controlled the expression of hSATII RNA in the process of cellular senescence.

[0044]

Further, although data are not shown, the expression of major satellite (MajSAT) RNA located in the pericentromeric region of mice was analyzed in a manner similar to that for human hSATII RNA. In mouse embryonic fibroblasts (MEF), DNA damage was generated with doxorubicin to induce cellular senescence. As a result, there was an increase in expression of MajSAT RNA as well as SASP-associated inflammation genes. That is, this indicated that the induction of MajSAT RNA was negatively correlated with the expression of CTCF even in the case of mice. Further, CTCF bound not to minor satellite (MinSAT) RNA present in the centromere, but to MajSAT RNA present in the pericentromere of the mice, and increased the expression of SASP-associated inflammation genes. These findings indicated that CTCF was important for controlling the expression of satellite RNA in the pericentromere and SASP-associated inflammation genes in cellular senescence.

[0045]

[Functional mechanism of hSATII RNA]

Since the ZF3-ZF6 DNA-binding domain of CTCF was related to binding to hSATII RNA, it was assumed that hSATII RNA directly bound to the ZF domain of CTCF to change the DNA binding ability, and the following studies were conducted. The hSATII RNA was expressed, and a ChIP-Seq assay was performed (Figures 6A and 6B). As was expected, the overexpression of hSATII RNA led to a change in binding peak of CTCF. The ability of CTCF to bind to ICR (imprinting control region) which is a typical binding site of CTCF located between IGF2 and H19 was analyzed by ChIP-qPCR and EMSA (Electrophoretic Mobility Shift Assay) (Figures 6C and 6D). The expression of hSATII RNA reduced the DNA binding ability of CTCF, and it was revealed that the binding was inhibited in a manner dependent on the concentration of RNA of hSATII.

[0046]

The hSATII RNA changes the interaction with chromatin via binding between CTCF and DNA. This suggests that hSATII RNA may be important for maintaining an appropriate steric structure of a genome. Thus, hSATII RNA was expressed, and a chromosome conformation capture (3C) assay was performed in the vicinity of the gene loci of CXCL10/CXCL11 which are SASP genes. The results revealed that the overexpression of hSATII RNA markedly depressed the interaction in T2 and T22 regions. Further, ATAC-seq analysis showed that chromatin

accessibility in the gene loci increased, and there was an increase in expression of SASP-associated inflammation genes. A similar phenomenon was also found in senescent cells induced by an X-ray (Figures 6E and 6F). These results suggested that an increase in expression of hSATII RNA in senescent cells caused a change in conformation of the chromatin structure in SASP gene loci. A molecular mechanism behind the association between control of CTCF and SASP gene expression in cellular senescence had not been clarified, but it became evident that satellite RNA in pericentromere inhibits the function of CTCF to influence the chromatin interaction, which leads to a change in expression of the SASP-associated inflammation-associated gene.

[0047]

[Effect of hSATII RNA on tumor]

It has been reported that the satellite RNA of humans and mice may induce chromosomal instability, leading to tumor formation. SATII RNA was forcibly expressed in SVts8 cells (Figure 7A), multi-polarization and formation of chromosome bridges, which are typical characteristics of chromosomal instability, were analyzed by immunostaining (Figure 7B). Microtubules were stained with an anti- α -tubulin antibody (Sigma-Aldrich), centrosomes were stained with pericentrin (Abcam), and DNA was stained with DAPI (Thermo Fisher Scientific). The proportion of multipolar cells significantly

increased in the cells in which hSATII RNA had been forcibly expressed (Figure 7B, left). The proportion of cells in which chromosome bridges were formed also significantly increased in the cells in which hSATII RNA had been forcibly expressed (Figure 7B, right).

[0048]

Further, the cells in which hSATII RNA had been forcibly expressed showed phenotypes that are found in tumor cells, such as an abnormal number of chromosomes and scaffold-independent proliferation. Karyotype analysis was performed, and the results showed that the number of cells having an abnormal number of chromosomes significantly increased in the cells in which hSATII RNA had been forcibly expressed (Figure 7C). Whether hSATII RNA expression caused scaffold-independent proliferation was analyzed by a soft agar colony formation assay. The hSATII RNA expression led to a significant increase in the number of colonies that proliferate in a scaffold-independent manner (Figure 7D).

[0049]

Next, hSATII RNA and CTCF were overexpressed at the same time to examine whether chromosomal instability induced by hSATII RNA was influenced. The hSATII RNA and CTCF were forcibly expressed in SVts8 cells, and the proportion of multipolar cells was analyzed in a manner similar to that in Figure 7B. The results revealed that the expression of CTCF eliminated chromosomal instability

induced by hSATIII RNA (Figure 7E). This suggested that CTCF was involved in chromosomal instability induced by hSATIII RNA, thus being a risk factor for tumor formation. [0050]

The results of ectopically expressing MajSAT RNA using mouse embryonic fibroblasts (MEFs) are shown (Figure 8). In a manner similar to that in Figure 7, MajSAT RNA was forcibly expressed in MEFs (Figure 8A), and multi-polarization and formation of chromosome bridges were observed with a microscope (Figure 8B). They both significantly increased due to the forcible expression of MajSAT RNA. Analysis was also performed on scaffold-independent proliferation (Figure 8C) and the karyotype (Figure 8D), and the results showed that the expression of MajSAT RNA led to a significant increase in the number of transformed cells that proliferate in a scaffold-independent manner, and the abnormality of the number of chromosomes. Further, these cells, which were implanted under the skin of nude mice, exhibited a tumorigenic potential (Figure 8E). Therefore, it is concluded that satellite RNA in the pericentromere may promote cancer development.

[0051]

Next, the functions of hSATIII RNA in microenvironment of tumors were given attention, and analyzed. Extracellular vesicles secreted from cancer cells and senescent stromal cells are known to be

involved in the development and progress of tumors in tumor microenvironment. Doxorubicin (DXR) or X-ray radiation (XRA) at 40-gray was applied to RPE-1/hTERT cells (obtained from ATCC) to induce cellular senescence in the cells. Extracellular vesicles were collected from these cells, and subjected to RT-qPCR, and the results showed that the extracellular vesicles derived from senescent cells contained a significantly larger amount of hSATII RNA than extracellular vesicles derived from proliferative cells. However, there was no increase in the amount of hSAT α RNA (Figure 9A).

[0052]

It has been reported, based on analysis of RNA-seq data, that hSATII RNA is detected from extracellular vesicles (exosomes) secreted from a plurality of human colorectal cancer cell lines. These lead to an inference that hSATII RNA derived from senescent stromal cells moves to peripheral cells through extracellular vesicles, and functions as a SASP-like inflammation factor. Although data are not shown, the present inventors have confirmed that extracellular vesicles derived from senescent cells induce scaffold-independent proliferation and chromosomal instability in normal cells. For evaluating the involvement of hSATII RNA contained in extracellular vesicles, designer exosomes containing hSATII RNA were established by a synthetic biological method using an EXOtic device (EXOsomal transfer into

cells) (Non Patent Literature 12). The prepared designer exosomes were introduced into SVts8 cells, and expression of inflammation-associated genes was analyzed by RT-qPCR. The designer exosomes containing hSATII RNA were found to promote the expression of SASP-like inflammation-associated genes (Figure 9B). That is, the prepared designer exosomes were found to have oncogenic activity similar to that of extracellular vesicles derived from senescent cells. This indicates that hSATII RNA contained in exosomes secreted from senescent cells promotes SASP-like inflammation-associated gene expression and chromosomal instability in stromal cells in tumor microenvironment.

[0053]

The expression of hSATII RNA in tumor microenvironment was analyzed by an RNA in situ hybridization (RNA-ISH) method using surgical resection sample from primary colorectal cancer patient. The results showed that the number of colorectal cancer cells in which hSATII RNA was expressed was significantly larger than the number of normal epithelial cells (Figure 9C, left). For the cancer-associated fibroblasts, the proportion of hSATII RNA-positive cells was significantly higher than that of fibroblasts of normal stromal tissues (Figure 9C, right). This suggests that senescent stromal cells which express hSATII RNA promote tumor formation in tumor microenvironment via secretion of extracellular

vesicles containing SASP-like inflammation factors and hSATII RNA (Figure 9D).

[0054]

As described above, it is considered that senescent stromal cells which express hSATII RNA may form tumor microenvironment and promote tumor formation. Thus, analysis was performed on whether suppression of the expression of hSATII RNA had an effect on suppression of proliferation of cancer cells and stromal cells. The siRNA of hSATII was introduced into pancreatic cancer cells PANC-1 and normal pancreatic stromal cells hPSC (obtained from ScienCell), followed by analysis of expression by RT-qPCR (Figure 10A, upper side). Gemcitabine was added to a culture medium to induce cellular senescence, resulting in increased expression of hSATII RNA in both the types of cells. The cell viability assay, Cell Titer-Glo (Promega), performed at the same time showed that for the pancreatic cancer cells, suppression of the expression of hSATII RNA by siRNA led to a significant decrease in cell viability (Figure 10A, lower side). On the other hand, for the normal pancreatic stromal cells, suppression of the expression of hSATII RNA by siRNA does not induce cell death, but when cellular senescence is induced by gemcitabine, cell death is induced even in the pancreatic stromal cells (Figure 10A, lower side). These results indicate that when an anticancer agent is administered to

induce cellular senescence in stromal cells, and the expression of hSATII RNA is suppressed, cell death is induced not only in cancer cells, but also in stromal cells forming tumor microenvironment. Therefore, suppression of hSATII RNA enables more effective cancer treatment.

[0055]

Although gemcitabine that is typically used in treatment of pancreatic cancer was used here because pancreatic cancer cells were used, cellular senescence can be induced by any anticancer agent. Cellular senescence can be induced not only by an anticancer agent but also by radiotherapy. This suggests that when in cancer treatment with an anticancer agent or radiation, treatment causing a decrease in expression of hSATII RNA is performed in parallel, a higher effect is obtained. Further, cancer cells and stromal cells which have survived against treatment with an anticancer agent or the like are considered to be senescent due to the treatment, and thus, these cells can be killed by concurrently administering a pharmaceutical composition that causes a decrease in expression of hSATII RNA. Therefore, it is possible to suppress recurrence of cancer by hSATII RNA expression suppressor in combination with anticancer agents selected by the type of cancer or radiotherapy.

[0056]

With a specimen from a breast cancer patient, the expression of hSATII RNA was analyzed. The results of RNA-ISH showed that hSATII RNA was expressed not only in cancer cells but also in stromal CAFs (Figure 10B). Therefore, a pharmaceutical composition that suppresses hSATII RNA expression may provide a therapeutic drug which targets not only cancer cells but also stromal cells forming tumor microenvironment.

[0057]

Analysis was performed on whether suppression of hSATII RNA expression had a therapeutic effect on cancer. Pancreatic cells PANC-1 were implanted into immunodeficient mice, siRNA of hSATII was then introduced into the cells, and the tumor size was measured (Figure 11A). The results showed that in pancreatic cancer, suppression of hSATII expression by siRNA significantly suppressed the proliferation of cancer cells than administration of control siRNA. That is, a pharmaceutical composition that suppresses the expression of hSATII RNA was shown to inhibit the proliferation of cancer.

[0058]

Next, analysis was performed on suppressing effect of a hSATII RNA expression in a system of co-implantation of cancer cells and stromal cells. In normal pancreatic stromal cells hPSC, cellular senescence was induced by X-ray irradiation (Day 0). Thereafter, the cells were

transfected with siRNA targeting hSATII, or control siRNA (Day 10 and Day 12). Pancreatic cancer cells MIAPaCa2 and hPSC were mixed at a ratio of 1 : 2, and implanted into the mice, and the tumor size was measured once per week (see Figure 11B, upper side). Both the results of the tumor volume and the tumor weight on the 35th day after the implantation (Day 48) showed that the proliferation of the tumor in the group which had undergone inhibition of hSATII RNA expression was more significantly suppressed as compared to that in the control. Suppression of the expression of hSATII RNA in the stromal cells in which cellular senescence was induced was shown to lead to suppression of tumor proliferation.

[0059]

The present inventors found that the hSATII DNA region swelled before the expression of hSATII RNA by DNA-FISH analysis of the hSATII DNA region in the process of inducing cellular senescence into TIG-3 cells through passage culture (Figure 12A). Although DNA-FISH was performed using a probe of SEQ ID NO: 12, any sequence may be used as long as the probe is capable of detecting the hSATII DNA region. Further, the chromatin accessibility of the hSATII DNA region was analyzed from a DNaseI sequence data of a plurality of cancer cell lines, and the results showed that the chromatin

accessibility in cancer cells evidently increased as compared to that in normal fibroblasts (Figure 12B).

[0060]

Next, for tumor tissues, analysis was performed on whether the hSAT DNA region was opened. With specimens from breast cancer patients, the hSAT DNA region was analyzed by scATAC-seq (Figure 12C) and DNA-FISH (Figure 12D). scATAC-seq analysis was performed for types of breast cancer (triple negative breast cancer; TNBC, luminal type breast cancer; Luminal), and the results suggested a possibility that the hSAT DNA region was opened, i.e., transcription actively occurred. Further, for the luminal type breast cancer, it was shown that there was a correlation between the swelling of the DNA region and the age, and an association with cellular senescence was suggested (Figure 12C). Further, the DNA-FISH analysis revealed that in tissues from breast cancer patients, the hSAT DNA region in breast cancer cells swelled as compared to that in the normal mammary gland epithelium, and the hSAT DNA region in CAFs swelled as compared to normal fibroblasts (Figure 12D). Further, the analysis was performed by cell type, and the results showed that even in patients in which the hSATII region was relatively closed in breast cancer cells, the hSATII DNA region was opened in stromal vascular endothelial cells and CAFs. These results suggest that treatment

which targets hSATII DNA may have an effect even in stromal cells (Figure 12E).

[0061]

As is apparent from the above results, decreased expression of hSATII RNA which is non-coding RNA in the pericentromere enables treatment of cellular senescence and associated cancer, and prevention of recurrence. As has been shown above, a substance which targets hSATII RNA expression and suppresses its expression can provide a new therapeutic drug for cancer. That is, by administering a drug which suppresses hSATII RNA expression, cancer cells and senescent stromal cells secreting SASP factors can be eliminated. Specifically, as shown above, nucleic acid medicines such as siRNA and LNA enables a decrease in hSATII RNA. For example, siRNAs represented by SEQ ID NOS: 1 and 2 are useful because they enable hSATII RNA to disappear with good efficiency. In addition, new LNA and siRNA may be designed, and used. Further, compounds that target hSATII RNA expression, stability and activity (specifically, inhibition of binding to CTCF, or the like) can be screened, and used.

[0062]

Whether the disease is caused by cellular senescence can be confirmed by examining hSATII RNA expression. For example, the use of a set of primers represented by SEQ ID NOS: 4 and 5 enables confirmation of hSATII RNA

expression by qPCR. The expression of hSATII RNA can also be evaluated by RNA-ISH. Since hSATII RNA is contained in extracellular vesicles such as exosomes, it is also possible to detect the amount of hSATII RNA in extracellular vesicles using body fluids such as blood, urine and ascitic fluid. SASP factors, the expression of which is promoted by hSATII RNA expression, may be measured. For SASP factors, RNA expression may be measured, or the amount of protein or the amount of secretion may be measured by ELISA. Samples used may be tissues of affected parts, for example, tissues with suspected cancer which have been taken through biopsy may be used, and extracellular vesicles derived from body fluid may be used. A decrease in CTCF expression may be examined. If expression of hSATII RNA, expression of a SASP factor, or decreased expression of CTCF is recognized, the pharmaceutical composition that suppresses hSATII RNA expression described above can be used for treatment. Since the hSATII DNA region swells and has increased chromatin accessibility before the expression of hSATII RNA, it may be possible to diagnose a cancer early by epigenomic analysis such as DNA-FISH analysis or ATAC sequence analysis, or analysis using genomic DNA fragments or extracellular vesicles in the body fluid.

[0063]

It is also possible to screen compounds using suppression of expression and activity of hSATII RNA, suppression of expression of SASP factors, increased expression of CTCF, or an epigenomic change such as shrinkage or decreased chromatin accessibility of the hSATII DNA region as an index. For example, by constructing a FRET-based system, low-molecular compounds can be screened with high sensitivity. Specifically, system can be constructed in which a bead bound to hSATII RNA labeled with fluorescent substance as a donor and a bead labeled with fluorescent substance which is bound to CTCF with a specific binding substance such as an antibody as an acceptor (Figure 13). By adding a library compound, followed by excitation at a specific fluorescence wavelength, whether hSATII RNA and CTCF are close to each other can be determined. Alternatively, when in culture of senescent cells with a candidate compound, the candidate compound causes suppression of hSATII RNA expression, increased expression of CTCF, or shrinkage or decreased chromatin accessibility of the hSATII DNA region, the compound can function as a therapeutic drug for a disease caused by cellular senescence. For suppression of expression or activity of hSATII RNA, increased expression of CTCF, and shrinkage and decreased accessibility of the hSATII region of genomic DNA, methods commonly used in the present field can be used. For the senescent cells, cellular

senescence induction methods commonly used in the field, such as passage culture, X-ray irradiation and doxorubicin exposure described above, can be used. The expression of hSATII RNA and CTCF can be analyzed by well-known methods such as RT-qPCR. The expression can be monitored by analysis of gene expression and secretion of SASP factors.

[0064]

As described above, the mechanism of inducing SASP by hSATII RNA, and its involvement in viability of senescent cells and cancer cells have been clarified. In senescent cells and cancer cells, the pericentromeric region of the chromosome is opened, and from the region, hSATII RNA which is non-coding RNA is actively transcribed. It has been discovered that by binding to CTCF having a function important for maintaining chromosomal structures, hSATII RNA inhibits the function, changes chromosome interaction, and induces transcription of inflammatory genes. By suppressing hSATII RNA expression in cancer cells and stromal senescent cells, cell death and arrest of proliferation were induced to suppress cancer proliferation. This indicates that hSATII RNA becomes a new target molecule in cancer treatment. Seno-cancer therapy having both the Senomorphic function of suppressing the expression of inflammatory SASP-associated genes by targeting hSATII RNA expressed in stromal cells in which cellular

senescence is induced and cancer cells, and the Senolytic function of selectively inducing cell death in senescent cells may create a novel therapeutic method (Figure 14).

Claims

[Claim 1]

A pharmaceutical composition which is a therapeutic agent and/or a prophylactic agent for a cancer caused by senescent cells, the pharmaceutical composition suppressing expression or inhibiting activity of hSATII RNA, and/or increasing expression of CTCF.

[Claim 2]

The pharmaceutical composition according to claim 1, wherein the pharmaceutical composition is a nucleic acid medicine which suppresses the expression or inhibits the activity of hSATII RNA and/or increases the expression of CTCF.

[Claim 3]

The pharmaceutical composition according to claim 1 or 2, which targets cancer cells and stromal cells.

[Claim 4]

The pharmaceutical composition according to claim 3, which is used in combination with a chemotherapeutic agent or radiotherapy.

[Claim 5]

A testing assistance method of cancer, comprising detecting expression of hSATII RNA and/or CTCF in a sample.

[Claim 6]

The testing assistance method according to claim 5, comprising determining that a cancer develops when the expression of hSATII RNA increases and/or the expression of CTCF decreases.

[Claim 7]

A testing assistance method of cancer, comprising detecting an epigenomic change of a hSATII DNA region in a sample.

[Claim 8]

The testing assistance method according to claim 7, the cancer is caused by cellular senescence.

[Claim 9]

A method for screening a therapeutic agent and/or a prophylactic agent for a cancer caused by cellular senescence,

comprising adding a candidate compound to a medium for senescent cells,

the candidate compound being selected at least one selected from the group of decreased expression of hSATII RNA, suppressed activity of hSATII RNA, increased expression of CTCF, decreased expression of a SASP factor, shrinkage of the hSATII DNA region and decreased chromatin accessibility as an index.