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Published:
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(88) Date of publication of the international search report:
27 December 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/38532 A3

(54) Title: REAGENTS AND METHODS FOR IDENTIFYING AND MODULATING EXPRESSION OF GENES REGULATED BY CDK INHIBITORS

(57) Abstract: This invention provides methods and reagents for identifying genes involved in cell cycle progression, growth promotion, modulation of apoptosis, cellular senescence and aging, and methods for identifying compounds that inhibit or potentiate cellular senescence.

INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/US 00/28082

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/47 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data, EMBASE, MEDLINE, CAB Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	B.-D. CHANG ET AL.: "Transient overexpression of p21 WAF1/CIP1 induces cell death and features of senescence in a human fibrosarcoma line." PROCEEDING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING, vol. 40, March 1999 (1999-03), pages 94-95, XP000914903 the whole document --- -/--	1-14,16, 18-20, 22, 24-39, 41,42, 44-94, 96-109, 111-155, 170,171, 200-224

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 May 2001	08. 06. 2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hix, R

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	H. XIAO ET AL.: "Sodium butyrate induces NIH3T3 cells to senescence-like state and enhances promoter activity of p21 waf/CIP1 in p53-independent manner." BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 237, 1997, pages 457-460, XP000914900	175,211, 214
Y	the whole document	1-14,16, 18-20, 22, 24-39, 41,42, 44-94, 96-105

X	M. HSIAO ET AL.: "Functional expression of human p21 WAF1/CIP1 gene in rat glioma cells suppresses tumor growth in vivo and induces radiosensitivity." BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 233, 1997, pages 329-335, XP000914899	175
Y	the whole document	1-14,16, 18-20, 22, 24-39, 41,42, 44-94, 96-109, 111-155, 183-198, 200-210, 214-224

Y	M. VOGT ET AL.: "Independent induction of senescence by p16INK4a and p21CIP1 in spontaneously immortalized human fibroblasts." CELL GROWTH AND DIFFERENTIATION, vol. 9, no. 2, February 1998 (1998-02), pages 139-146, XP000923096	1-14,16, 18-20, 22, 24-39, 41,42, 44-94, 96-109, 111-155, 183-198, 200-210, 214-224
	the whole document	

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	ZENG Y.-X. ET AL: "Regulation of p21(WAF1/CIP1) expression by p53-independent pathways." ONCOGENE, (1996) 12/7 (1557-1564)., XP000922595 the whole document	12-14, 16, 18-20, 22, 24-26, 38,39, 41,42, 44-51, 92-94, 96-105, 179-181
Y	AFSHARI C.A. ET AL: "A role for a p21-E2F interaction during senescence arrest of normal human fibroblasts." CELL GROWTH AND DIFFERENTIATION, (1996) 7/8 (979-988)., XP000922599 the whole document	52-91, 107-109, 111-123, 186-198, 200-210
Y	M. JOHNSON ET AL.: "Evidence for a p53-independent pathway for upregulation of SDI1/CIP1/WAF1/p21 RNA in human cells." MOLECULAR CARCINOGENESIS, vol. 11, no. 2, October 1994 (1994-10), pages 59-64, XP000922631 the whole document	179-181
P,X	CHANG B.-D. ET AL: "Role of p53 and p21(waf1/cip1) in senescence -like terminal proliferation arrest induced in human tumor cells by chemotherapeutic drugs." ONCOGENE, (26 AUG 1999) 18/34, page 4808-4818 XP000922555 the whole document	1-14,16, 18-20, 22, 24-39, 41,42, 44-94, 96-109, 111-155, 170,171, 200-224
A	M. NAKANISHI ET AL.: "The C-terminal region of p21 SDI1/WAF1/CIP1 is involved in proliferating cell nuclear antigen binding but does not appear to be required for growth inhibition." THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 29, 21 July 1995 (1995-07-21), pages 17060-17063, XP000914898 the whole document	

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	M. NAKANISHI ET AL.: "Identification of the active region of the DNA synthesis inhibitory gene p21 Sdi1/CIP1/WAF1" THE EMBO JOURNAL, vol. 14, no. 3, 1995, pages 555-563, XP002039812 the whole document	
P,X	B.-D. CHANG ET AL.: "Effects of p21 Waf1/Cip1/Sdi1 on cellular gene expression: Implications for carcinogenesis, senescence, and age-related diseases." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 97, no. 8, 11 April 2000 (2000-04-11), pages 4291-4296, XP000921392 the whole document	1-14,16, 18-20, 22, 24-39, 41,42, 44-94, 96-109, 111-155, 170,171, 173-177, 179-198, 200-224
P,X	B.-D. CHANG ET AL.: "p21 Waf1/Cip1/Sdi1 - induced growth arrest is associated with depletion of mitosis-control proteins and leads to abnormal mitosis and endoreduplication in recovering cells." ONCOGENE, vol. 19, no. 17, 20 April 2000 (2000-04-20), pages 2165-2170, XP000922527 the whole document	1-14,16, 18-20, 22, 24-39, 41,42, 44-94, 96-109, 111-155, 170,171, 173-177, 179-198, 200-224
E	WO 00 61751 A (CHANG BEY DIH ;UNIV ILLINOIS (US); RONINSON IGOR B (US)) 19 October 2000 (2000-10-19) the whole document	1-14,16, 18-20, 22, 24-39, 41,42, 44-94, 96-109, 111-155, 170,171, 173-177, 179-198, 200-224

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	REZNIKOFF C.A. ET AL: "Elevated p16 at senescence and loss of p16 at immortalization i human papillomavirus 16 E6, but not E7, transformed human uroepithelial cells." CANCER RESEARCH (1996) 56/13 (2886-2890). , XP000993535	175,176, 178
Y	the whole document	12,13, 15,17, 21, 23-38, 40,41, 43-93, 95-108, 110-155, 170, 172-174, 179-197, 199-236

X	TANIGUCHI K. ET AL: "Induction of the p16(INK4a) senescence gene as a new therapeuti strategy for the treatment of rheumatoid arthritis." NATURE MEDICINE, (1999) 5/7 (760-767). , XP000993540	175,176, 178
Y	the whole document	12,13, 15,17, 21, 23-38, 40,41, 43-93, 95-108, 110-155, 170, 172-174, 179-197, 199-236

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	D.A. ALCORTA ET AL.: "Involvement of the cyclin-dependent kinase inhibitor p16 (INK4a) in replicative senescence of normal human fibroblasts" PROC. NATL. ACAD. SCI. USA, vol. 93, November 1996 (1996-11), pages 13742-13747, XP001003075	175,176, 178
Y	the whole document	12,13, 15,17, 21, 23-38, 40,41, 43-93, 95-108, 110-155, 170, 172-174, 179-197, 199-236
X	L. UHRBOM ET AL.: "Induction of senescence in human malignant glioma cells by p16INK4a" ONCOGENE, vol. 15, no. 5, 31 July 1997 (1997-07-31), pages 505-514, XP000994730	175,176, 178
Y	the whole document	12,13, 15,17, 21, 23-38, 40,41, 43-93, 95-108, 110-155, 170, 172-174, 179-197, 199-236

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	A.J. BRENNER ET AL.: "Increased p16 expression with first senescence arrest in human mammary epithelial cells and extended growth capacity with p16 inactivation." ONCOGENE, vol. 17, no. 2, 16 July 1998 (1998-07-16), pages 199-205, XP000994736	175,176, 178
Y	the whole document	12,13, 15,17, 21, 23-38, 40,41, 43-93, 95-108, 110-155, 170, 172-174, 179-197, 199-236

X	D.F. JARRARD ET AL.: "p16/pRb pathway alterations are required for bypassing senescence in human prostate epithelial cells." CANCER RESEARCH, vol. 59, 15 June 1999 (1999-06-15), pages 2957-2964, XP000993538	175,176, 178
Y	the whole document	12,13, 15,17, 21, 23-38, 40,41, 43-93, 95-108, 110-155, 170, 172-174, 179-197, 199-236

Y	S.J. KUERBITZ ET AL.: "Deletion of p16INK4A/CDKN2 and p15INK4B in human somatic cell hybrids and hybrid-derived tumors." CELL GROWTH & DIFFERENTIATION, vol. 10, January 1999 (1999-01), pages 27-33, XP001004759 the whole document	225-236

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	M.S. STEINER ET AL.: "Adenoviral vector containing wild-type p16 suppresses prostate cancer growth and prolongs survival by inducing cell senescence." CANCER GENE THERAPY, vol. 7, no. 3, March 2000 (2000-03), pages 360-372, XP000994781	175,176, 178
P,Y	the whole document	12,13, 15,17, 21, 23-38, 40,41, 43-93, 95-108, 110-155, 170, 172-174, 179-197, 199-236

P,X	ALLAY J A ET AL: "Adenovirus p16 gene therapy for prostate cancer." WORLD JOURNAL OF UROLOGY, (2000 APR) 18 (2) 111-20. , XP000994791	175,176, 178
P,Y	the whole document	12,13, 15,17, 21, 23-38, 40,41, 43-93, 95-108, 110-155, 170, 172-174, 179-197, 199-236

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	STEINER M.S. ET AL: "p16/MTS1/INK4A suppresses prostate cancer by both pRb dependent and independent pathways." ONCOGENE, (2 MAR 2000) 19/10 (1297-1306). , XP001001577	175,176, 178
P,Y	the whole document	12,13, 15,17, 21, 23-38, 40,41, 43-93, 95-108, 110-155, 170, 172-174, 179-197, 199-236
A	G.P. NIELSON ET AL.: "Immunohistochemical survey of p16INK4A expression in normal human adult and infant tissues." LABORATORY INVESTIGATION, vol. 79, no. 9, September 1999 (1999-09), pages 1137-1143, XP000994720 the whole document	
A	R.S. ROBETORYE ET AL.: "Regulation of p21 Sdi1/Cip1/Waf1/mda-6 and expression of other cyclin-dependent kinase inhibitors in senescent human cells." MOLECULAR AND CELLULAR DIFFERENTIATION., vol. 4, no. 1, 1996, pages 113-126, XP000922642 the whole document	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/28082

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 76 to 91 and 144 to 155 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 156-169
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [X] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[X] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 12, 13, 24-38, 32, 44-93, 96-108, 111-155, 170, 173-176, 179-197, 200-224, partially and claims 1-11, 14, 16, 18-20, 22, 39, 41, 42, 94, 109, 171, 177, 198 completely.

A recombinant mammalian fibrosarcoma cell comprising a recombinant expression construct encoding a mammalian p21 gene, method for identiying a compound that inhibits p21-mediated modulation of cellular gene expression, mammalian cell comprising a recombinant expression construct encoding a mammalian p21 inhibitor gene,or a construct encoding a reporter gene under transcriptional control of a promoter for a mammlian gene whose expression is induced or repressed by p21, method for identifying a compound that inhbits or potentiates p21 mediated modulation of cellular gene expression, or inhibits senescence in a mammalian cell by assaying for repression or induction of genes repressed or induced by p21, method for identifying a compound that potentiates senescence using a cell comprising a promoter for a mammalian gene whose expression is modulated by p21, method for identifying a compound that promotes induction of senescence in a mammalian cell by assaying the mammalian cell for repression or induction of genes that are repressed or induced by p21 gene expression, compound that inhibits or potentiates p21 modulation of cellular gene expression, compound that inhibits senescence in a mammalian cell where the mammalian cell is assayed for repression or induction of genes that are repressed or induced by p21 gene expression, method for producing an anti-apopototic or mitogenic factor from a mammalian cell by producing p21 gene expression in a mammalian cell and culturing the cell to produce an anti-apototic or mitogenic factor, mammalian cell culture medium conditioned by growth of a mammalian cell that expresses p21, method for obtaining a plurality of nucleic acid species enriched for a gene involved in cell cycle progression or genes that encode secreted proteins with parcrine function involving inducing expression of p21 in a mammalian cell, method for identifying a plurality of cellular genes that are markers of cellular senescence involving inducing expression of p21, recombinant expression construct encoding a reporter gene under the transcriptional control of a promoter for a mammalian gene whose expression is inhbited or induced by p21.

2. Claims: 12, 13, 24-38, 32, 41, 44-93, 96-108, 111-155, 170, 173-176, 179-197, 200-224, partially and claims 15, 17, 21, 23, 40, 43, 95, 110, 172, 178, 199, 225-236 completely

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A recombinant mammalian fibrosarcoma cell comprising a recombinant expression construct encoding a mammalian p16 gene, method for identiying a compound that inhibits p16-mediated modulation of cellular gene expression, mammalian cell comprising a recombinant expression construct encoding a mammalian p16 inhibitor gene, or a construct encoding a reporter gene under transcriptional control of a promoter for a mammlian gene whose expression is induced or repressed by p16, method for identifying a compound that inhbits or potentiates p16 mediated modulation of cellular gene expression, or inhibits senescence in a mammalian cell by assaying for repression or induction of genes repressed or induced by p16, method for identifying a compound that potentiates senescence using a cell comprising a promoter for a mammalian gene whose expression is modulated by p16, method for identifying a compound that promotes induction of senescence in a mammalian cell by assaying the mammalian cell for repression or induction of genes that are repressed or induced by p16 gene expression, compound that inhibits or potentiates p16 modulation of cellular gene expression, compound that inhibits senescence in a mammalian cell where the mammalian cell is assayed for repression or induction of genes that are repressed or induced by p16 gene expression, method for producing an anti-apopototic or mitogenic factor from a mammalian cell by producing p16 gene expression in a mammalian cell and culturing the cell to produce an anti-apototic or mitogenic factor, mammalian cell culture medium conditioned by growth of a mammalian cell that expresses p16, method for obtaining a plurality of nucleic acid species enriched for a gene involved in cell cycle progression or genes that encode secreted proteins with parcrine function involving inducing expression of p16 in a mammalian cell, method for identifying a plurality of cellular genes that are markers of cellular senescence involving inducing expression of p16, recombinant expression construct encoding a reporter gene under the transcriptional control of a promoter for a mammalian gene whose expression is inhbited or induced by p16.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 156-169

Present claims 156 to 169 relate to products defined by reference to a desirable characteristic or property, namely that the product acts as an inhibitor of p21-mediated modulation of cellular gene expression or as an inhibitor or potentiator of senescence and as products of processes whereby the products are identified only by their desirable characteristics.

The claims cover all products having this characteristic or property. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int...ional Application No
PCT/US 00/28082

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0061751 A	19-10-2000	AU 4079000 A	14-11-2000