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(54) Title: PROMOTER INDUCIBLE BY REACTIVE OXYGEN SPECIES AND VECTOR COMPRISING THE SAME

(57) Abstract: The invention provides promoters inducible by reactive oxygen species (ROS), capable of driving the expression of a gene of interest, particularly in a tumor cell. More particularly, it refers to promoters inducible by reactive oxygen species, that may be used to drive the expression of a gene of interest, such as a therapeutic gene, or a reporter gene for use in image diagnosis. The promoters comprise at least a fragment of a promoter sequence responsive to said reactive oxygen species, and corresponding to a gene highly expressed in cancer cells, wherein the fragment of the promoter sequence responsive to reactive oxygen species (ROS) is selected from the group of: the VE element of the VEGF promoter, the E6 element of the promoter of the EGR-1 gene, the MMP-1 element and a chimeric promoter containing an E6 element and a VE element. It also provides vectors carrying a human therapeutic or non therapeutic gene of interest, operably linked to said promoter sequence and compositions comprising the same.



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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 08/05327

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07H 21/04; C12N 15/00 (2008.04)

USPC - 435/320.1; 536/24.1

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)
USPC - 435/320.1; 536/24.1;Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
PubWEST(USPT,PGPB,EPAB,JPAB) (see search terms below)Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(USPT,PGPB,EPAB,JPAB); Medline, Google: promoter, reactive oxygen species, ROS, VEGF, chimeric, E6 element, VE element, spacing, vector, suicidal gene, tissue image, EGR-1, CArG, Early Growth Response-1 gene, vascular endothelial growth factor, gene therapy, inducible. GenCore: SEQ ID NO: 1 and SEQ ID NO: 12

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	Greco et al. Novel chimeric gene promoters responsive to hypoxia and ionizing radiation. Gene Ther., 2002, Vol 9(20):1403-11; Abstract; pg 1403, col 1 and col 2; pg 1404, col 1 and col 2; pg 1406, col 2; pg 1407, col 1 and col 2; pg 1408, col 2; and Fig 1 and Fig 5	1, 6, and 18 ----- 2-4, 7-9, 11-13, 15-17 and 30-32
Y	WO 2006/078317 A1 (WHITTEN et al.) 27 Jul 2006 (27.07.2006) para [0113]-[0114]; SEQ ID NO: 15	2-4, 7-9, 11-13, 15-17 and 30-32
Y	US 2006/0024780 A1 (ALDAZ et al.) 02 Feb 2006 (02.02.2006) para [0024], [0037], and [0132]	3-4, 8-9, 11-13, 15-17, 31 -32
Y	US 2006/0199778 A1 (ELLIS-BEHNKE et al.) 07 Sep 2006 (07.09.2006) para [0118] and [0149]	16
Y	US 2006/0127880 A1 (HARRIS et al.) 15 Jun 2006 (15.06.2006) para [0035]	17

☐ Further documents are listed in the continuation of Box C.

* 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 application or patent 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

10 October 2008 (10.10.2008)

Date of mailing of the international search report

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Name and mailing address of the ISA/US

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US 08/05327

***** SUPPLEMENTALPAGE *****

Continuation of: Box III (unity of invention is lacking)

The inventions listed as Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Groups I-III do not include the inventive concept of a promoter inducible by reactive oxygen species operatively linked to a gene expressing proteins detectable through optical systems, as required by Group IV.

Although Groups I-IV do share the technical feature of a promoter inducible by reactive oxygen species, this shared technical feature does not represent a contribution over the prior art. Specifically, the article entitled "Oxidant-induced Vascular Endothelial Growth Factor Expression in Human Keratinocytes and Cutaneous Wound Healing" by Sen et al. (THE JOURNAL OF BIOLOGICAL CHEMISTRY 2002, 277(36):33284-33290) teaches said promoter: "H₂O₂ potently induces vascular endothelial growth factor (VEGF) expression in human keratinocytes. Deletion mutant studies with a VEGF promoter construct revealed that a GC-rich sequence from bp -194 to -50 of the VEGF promoter is responsible for the H₂O₂ response" (Abstract). As the above promoter was known at the time, as evidenced by the teaching of Sen et al, this cannot be considered a special technical feature that would otherwise unify the groups.

Groups I-III lack unity because SEQ ID NO: 1-3 do not share a significant structural element that is essential to the common property or activity. In addition, US 2005/027027205 A1 to Gargill et al teaches SEQ ID NO: 1 (bp 131-166 of SEQ ID NO: 27688). Therefore, unity of invention is lacking.

Groups I-IV therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of:

a. type of material



a sequence listing



table(s) related to the sequence listing

b. format of material



on paper



in electronic form

c. time of filing/furnishing



contained in the international application as filed



filed together with the international application in electronic form



furnished subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 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. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
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:

3. ☒ Claims Nos.: 19-27
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-18, 30-33, drawn to a promoter inducible by reactive oxygen species and its use, wherein claims 2, 7, 30 are limited to SEQ ID NO: 1.

Groups II and III, claims 1-12, 14-18, 30-33, drawn to a promoter inducible by reactive oxygen species and its use, wherein claims 2, 7, 30 are limited to SEQ ID NO: 2 and 3, respectively.

Groups IV, claims 28 and 29, drawn to a diagnosis through the use of a promoter inducible by reactive oxygen species operatively linked to a gene expressing proteins detectable through optical systems.

*****Continued in extra Sheet*****

1. ☐ 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 additional fees, this Authority did not invite payment of additional fees.
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.:
Claims 1-18 and 30-33, wherein claims 2, 7, 30 are limited to SEQ ID NO: 1.

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.