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(54) **DELETION BEARING BARD1 ISOFORMS AND USE THEREOF**

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C12N 15/63	(2006.01)

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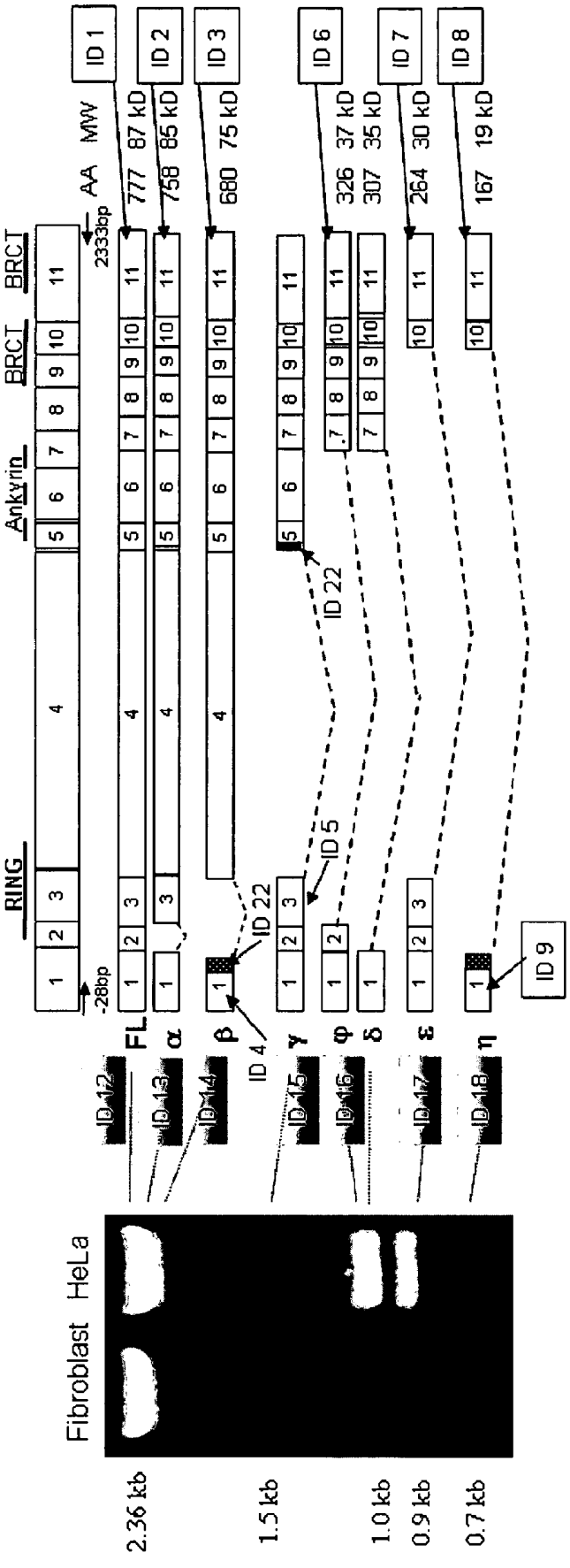
(57) **ABSTRACT**

The present invention relates to new protein isoforms, use thereof, methods of preparation thereof, methods of detection thereof, antibodies thereof, combination of antibodies thereof, use of these antibodies and combinations thereof and use of antagonists of those isoforms for the treatment of gynaecological cancers.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Ankyrin
BRCT
BRCT
-28bp
2333bp
AA
MW
ID 1
ID 2
ID 3
ID 6
ID 7
ID 8
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758
85 kD
680
75 kD
326
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307
35 kD
264
30 kD
167
19 kD
FL
α
β
γ
φ
δ
ε
η
ID 4
ID 5
ID 22
ID 22
ID 9
ID 12
ID 13
ID 14
ID 15
ID 16
ID 17
ID 18
Fibroblast
HeLa
2.36 kb
1.5 kb
1.0 kb
0.9 kb
0.7 kb

Fig.1B

Fig. 1A

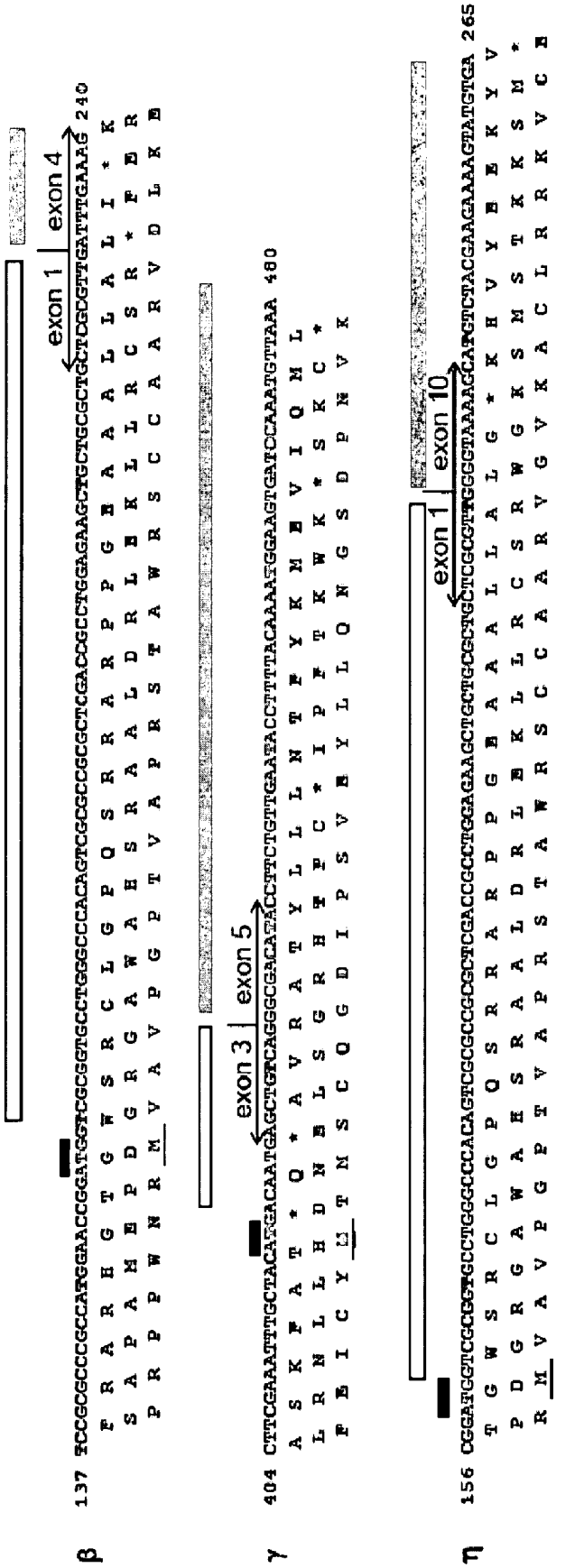
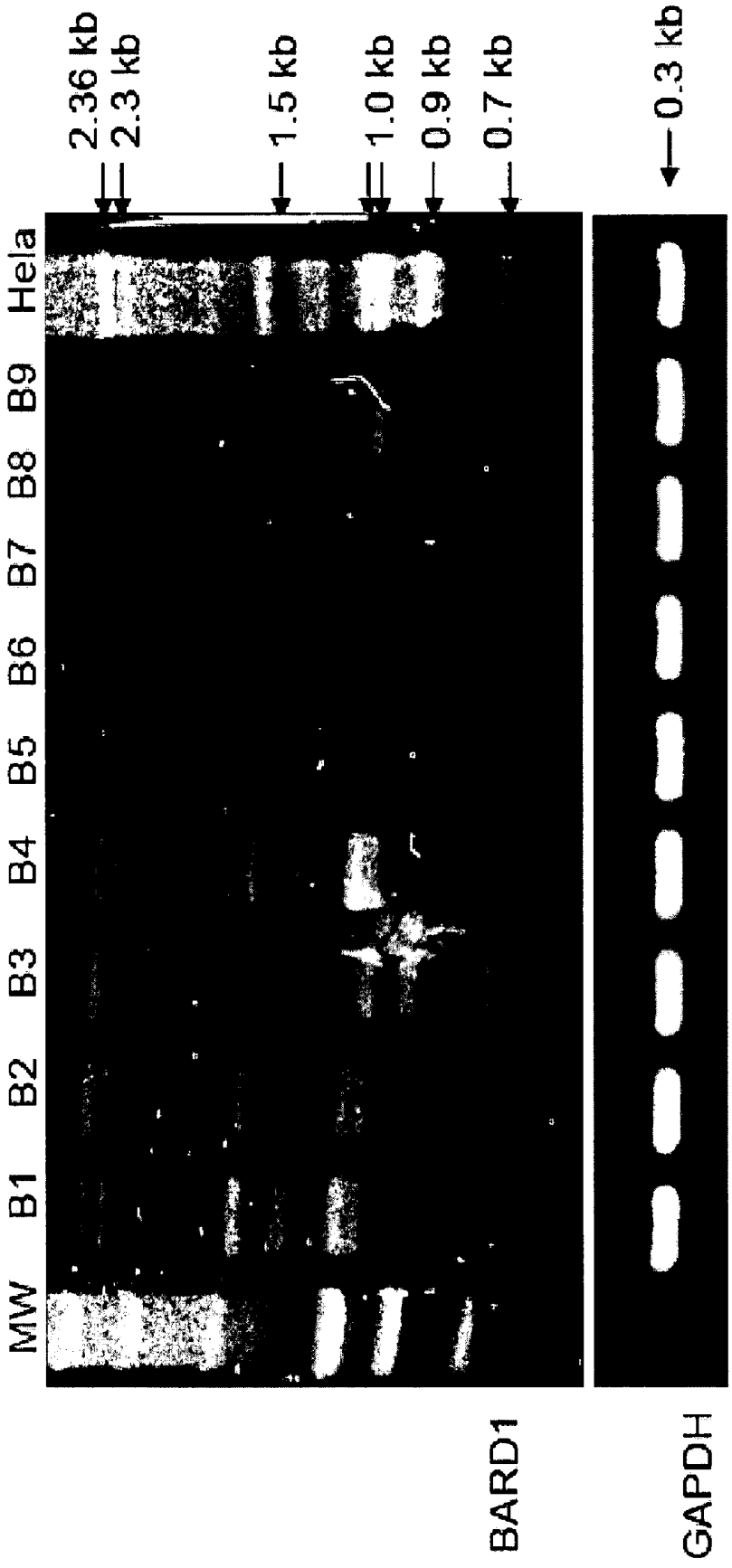


Fig. 1C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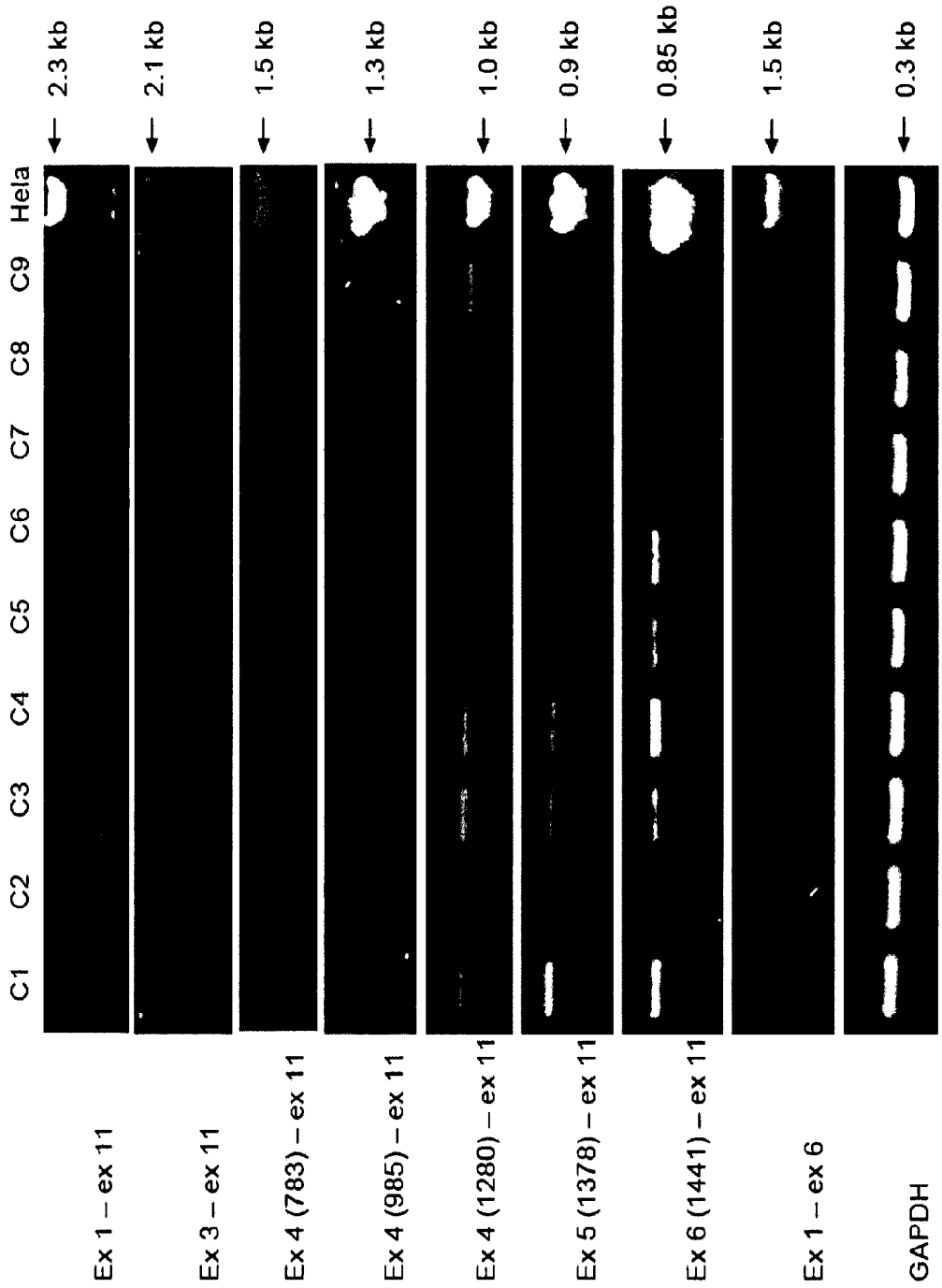
0.3 kb
MW
B1
B2
B3
B4
B5
B6
B7
B8
B9
Hela
BARD1
GAPDH

Fig. 2

Fig. 3


C1
C2
C3
C4
C5
C6
C7
C8
C9
Hela
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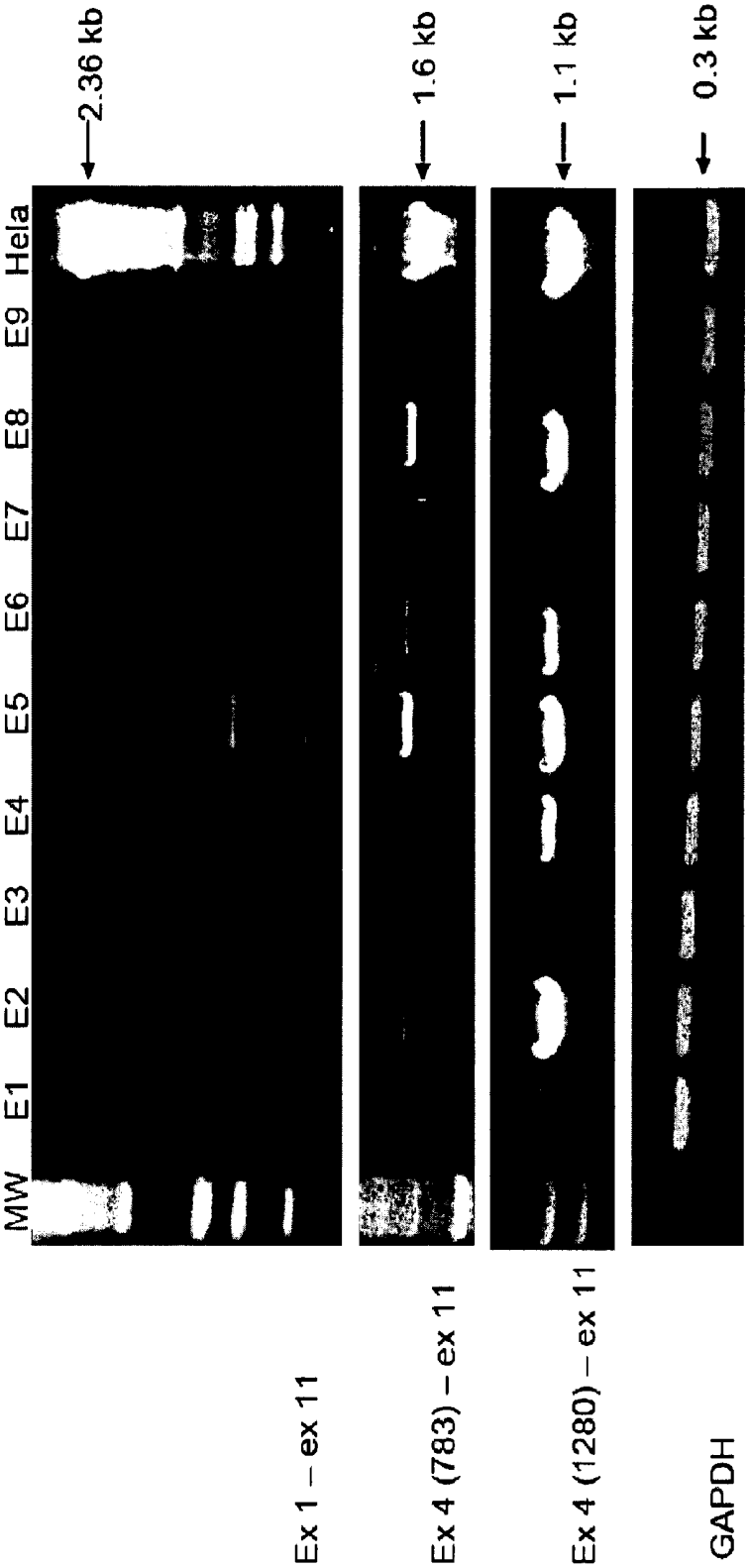
1.5 kb
1.3 kb
1.0 kb
0.9 kb
0.85 kb
1.5 kb
0.3 kb
Ex 1 – ex 11
Ex 3 – ex 11
Ex 4 (783) – ex 11
Ex 4 (985) – ex 11
Ex 4 (1280) – ex 11
Ex 5 (1378) – ex 11
Ex 6 (1441) – ex 11
Ex 1 – ex 6
GAPDH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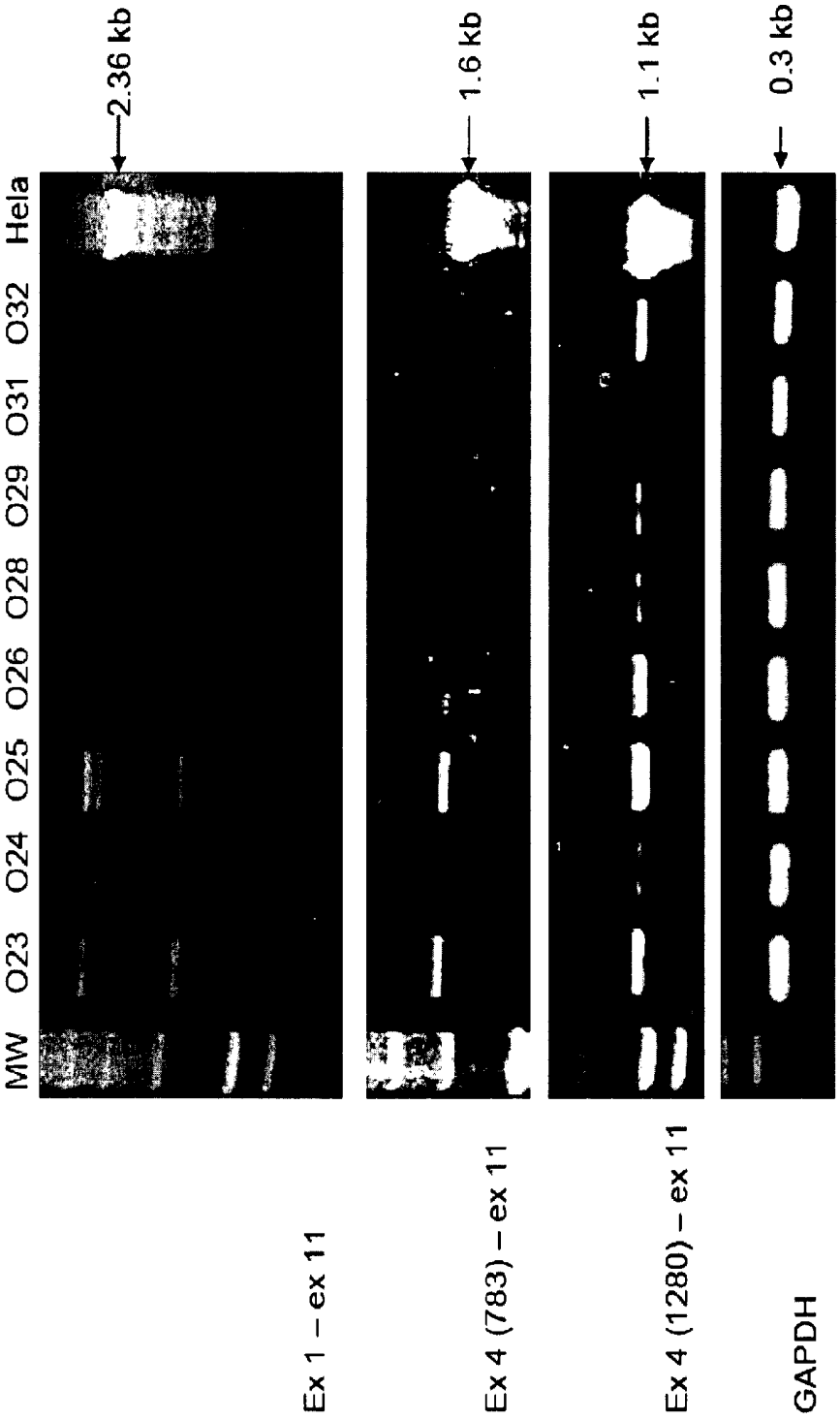
MW
E1
E2
E3
E4
E5
E6
E7
E8
E9
Hela
Ex 1 – ex 11
Ex 4 (783) – ex 11
Ex 4 (1280) – ex 11
GAPDH

Fig. 4A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1.6 kb
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MW
O23
O24
O25
O26
O28
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O31
O32
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Ex 1 – ex 11
Ex 4 (783) – ex 11
Ex 4 (1280) – ex 11
GAPDH

Fig. 4B

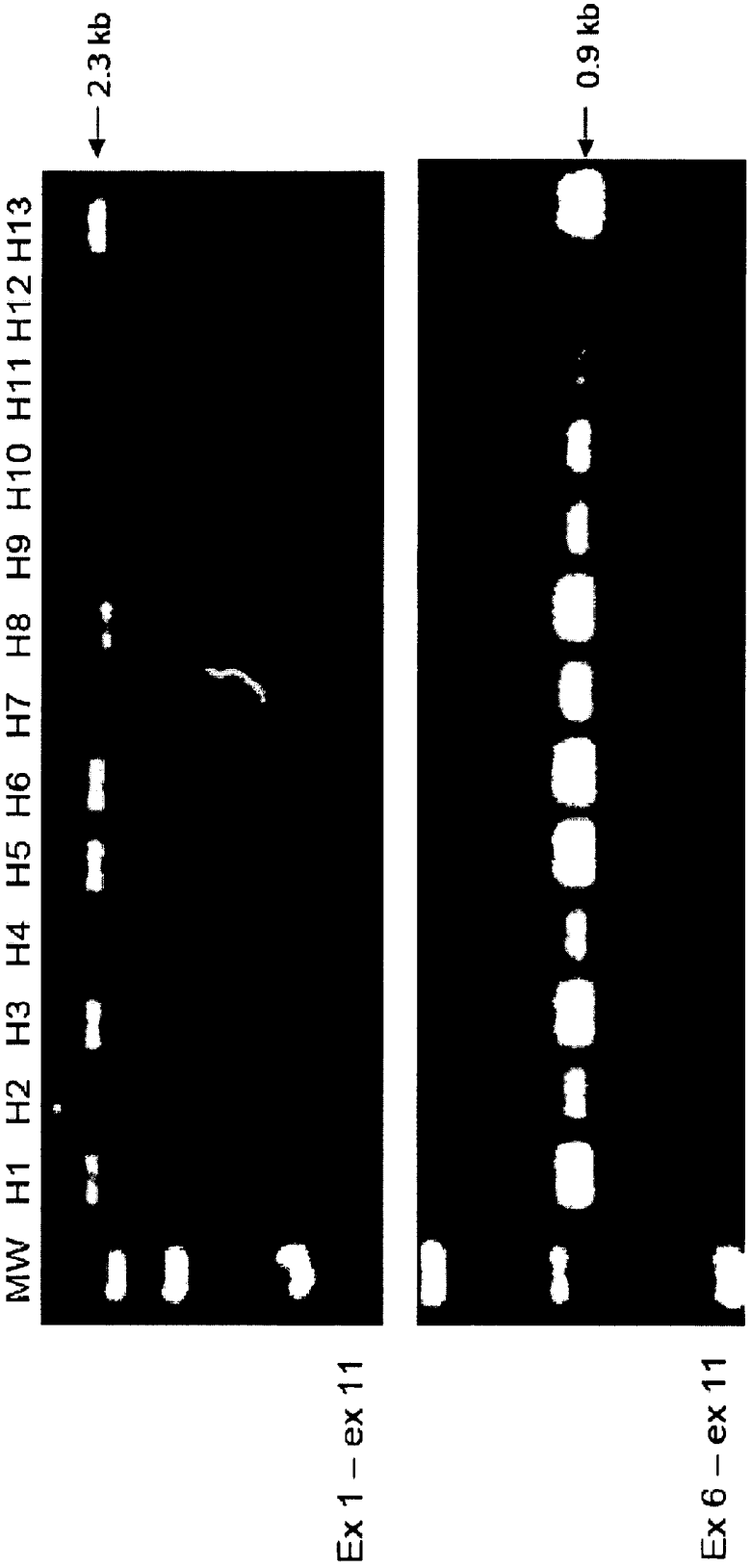
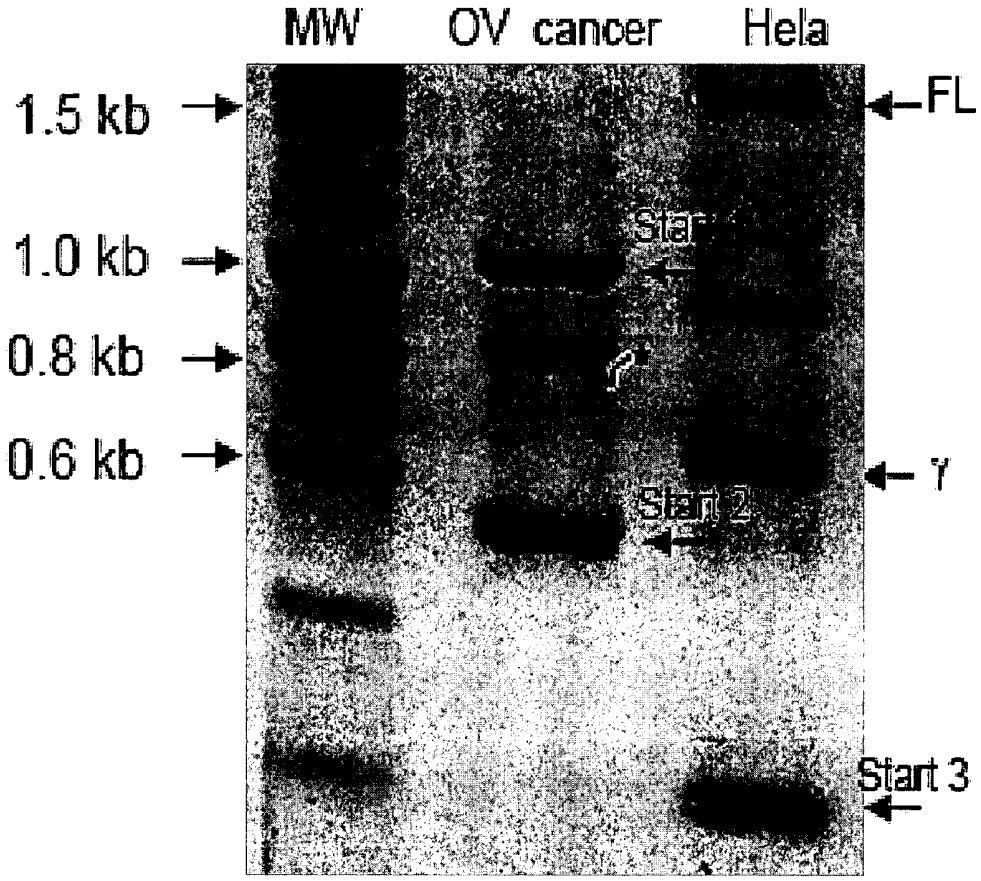

2.3 kb
0.9 kb
MW
H1
H2
H3
H4
H5
H6
H7
H8
H9
H10
H11
H12
H13
Ex 1 – ex 11
Ex 6 – ex 11

Fig. 5

Fig. 6A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Hela
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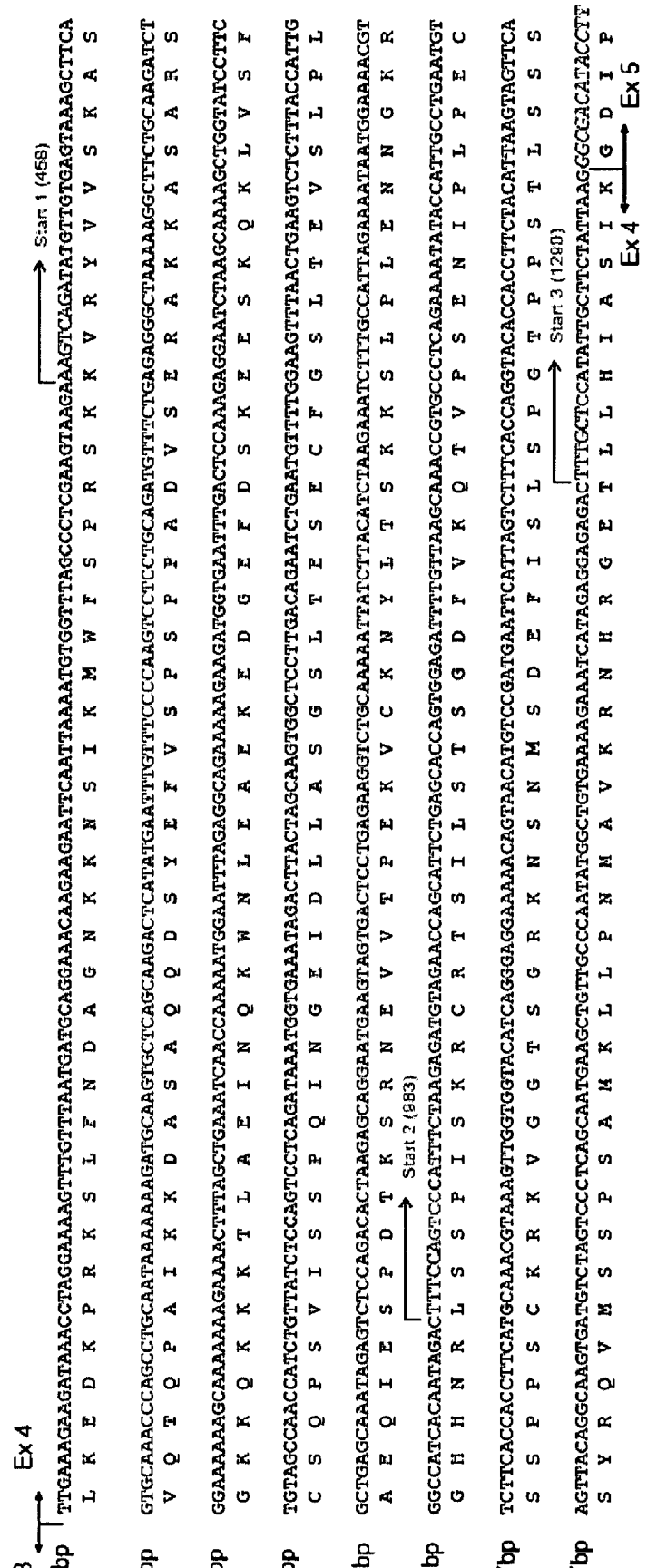
1.0 kb
0.8 kb
0.6 kb
FL
Start
?
Y
Start 2
Start 3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Ex 4
Start 1 (458)
Start 2 (883)
Start 3 (1260)
Ex 4
Ex 5
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604 bp
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844 bp
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1157bp
1277bp

Fig. 6B

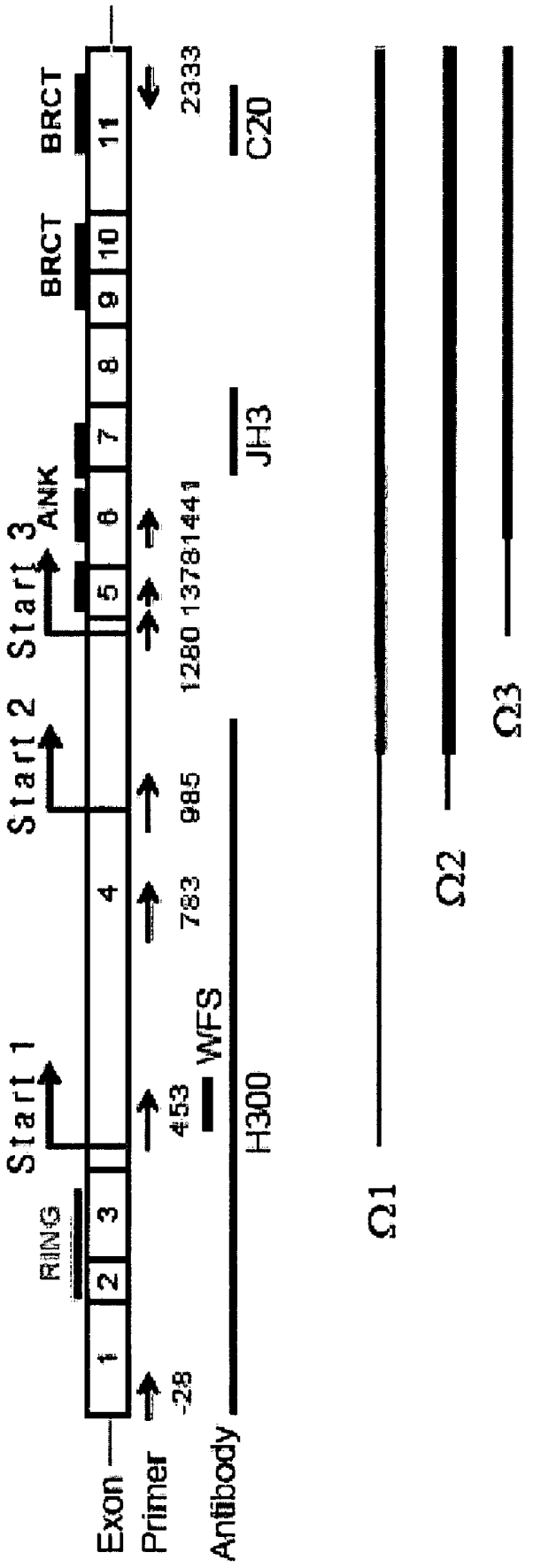
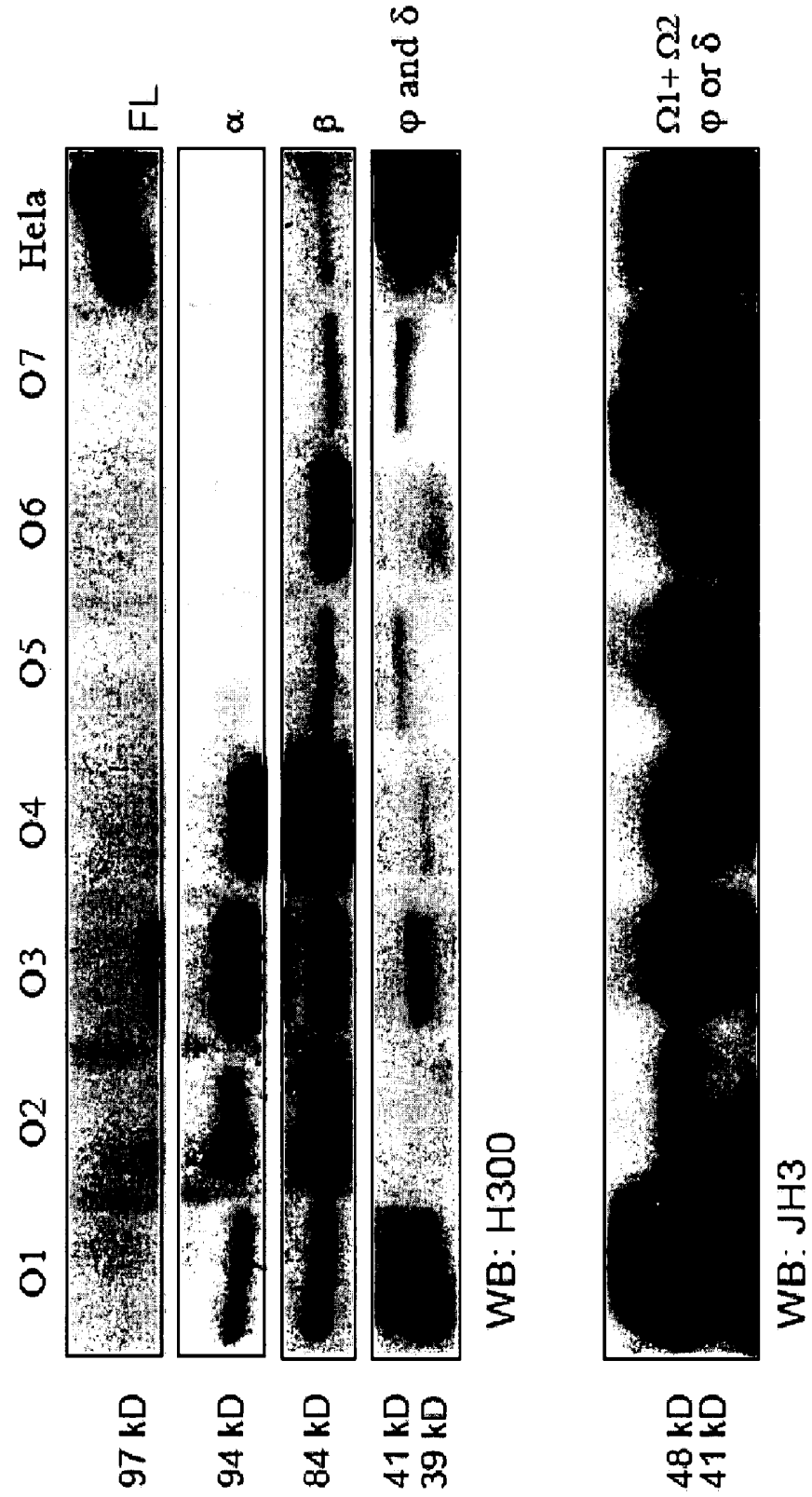

Exon
Primer
Antibody
-28
453
783
985
1280
13781441
2333
RING
WFS
H300
Start 1
Start 2
Start 3
ANK
BRCT
BRCT
C20
JH3
Ω1
Ω2
Ω3
1
2
3
4
5
6
7
8
9
10
11

Fig. 6C

Fig. 7


O1
O2
O3
O4
O5
O6
O7
Hela
FL
α
β
φ and δ
Ω1+ Ω2
φ or δ
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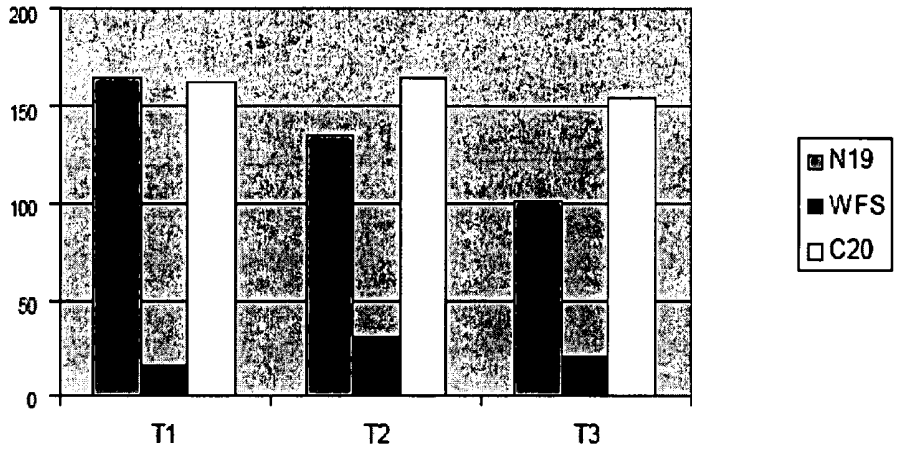
94 kD
84 kD
41 kD
39 kD
48 kD
41 kD
WB: H300
WB: JH3

Fig. 8A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150
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50
0
T1
T2
T3
N19
WFS
C20

Fig. 8B

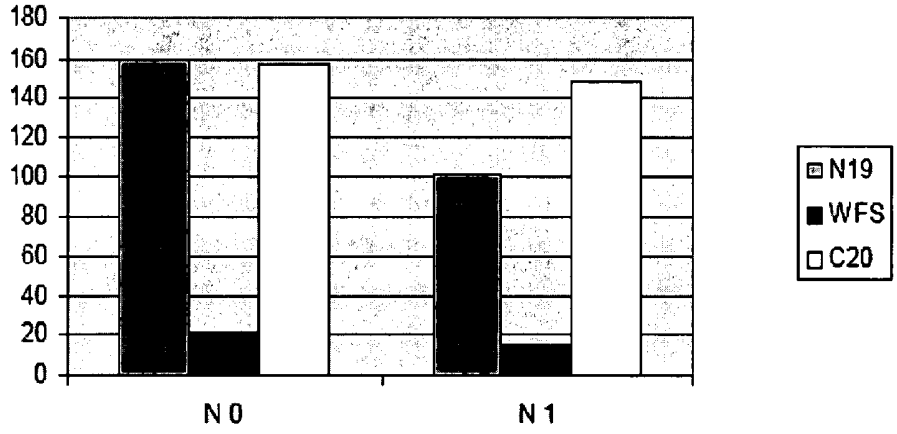

180
160
140
120
100
80
60
40
20
0
N 0
N 1
N19
WFS
C20

Fig. 8C

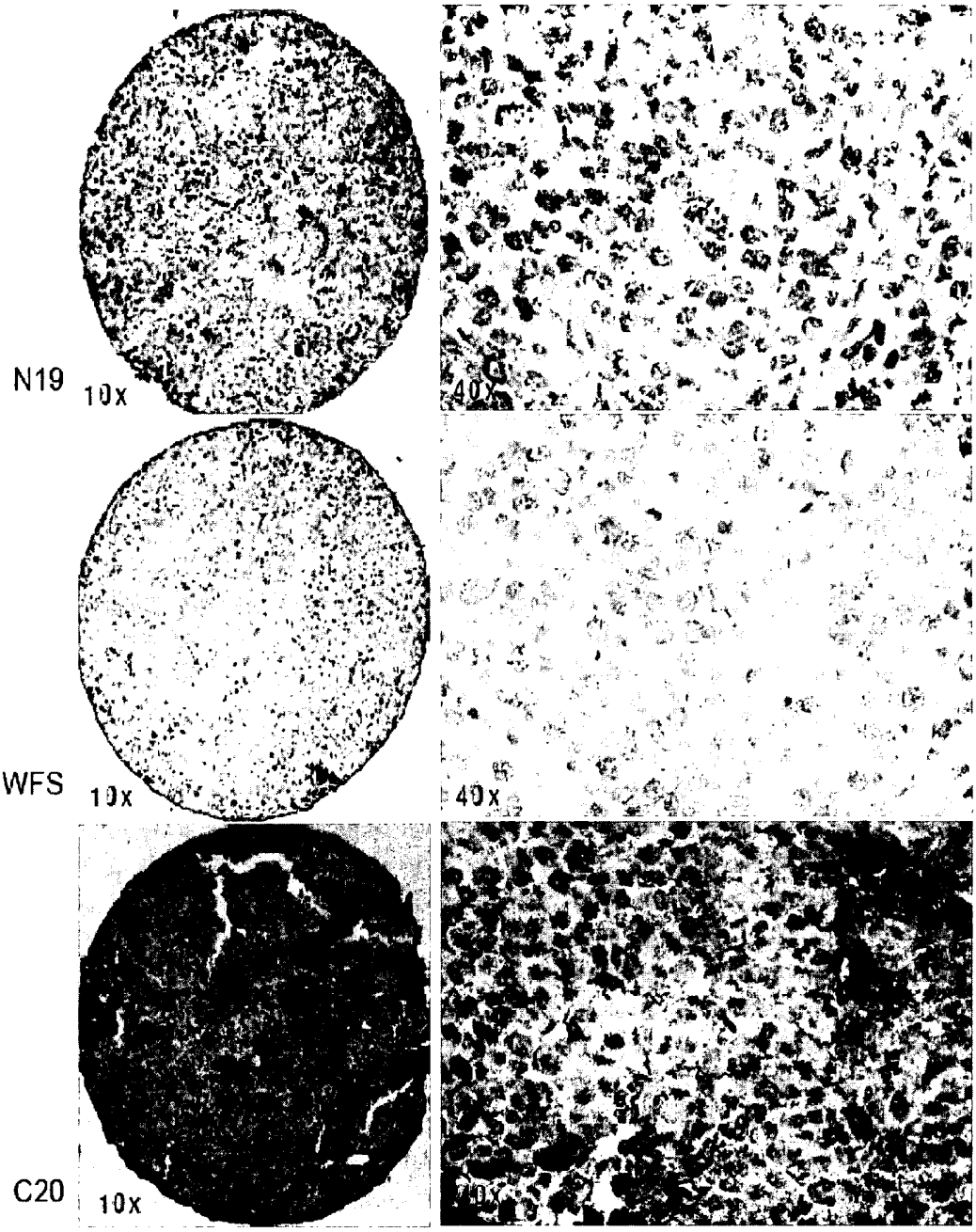


Fig. 8D

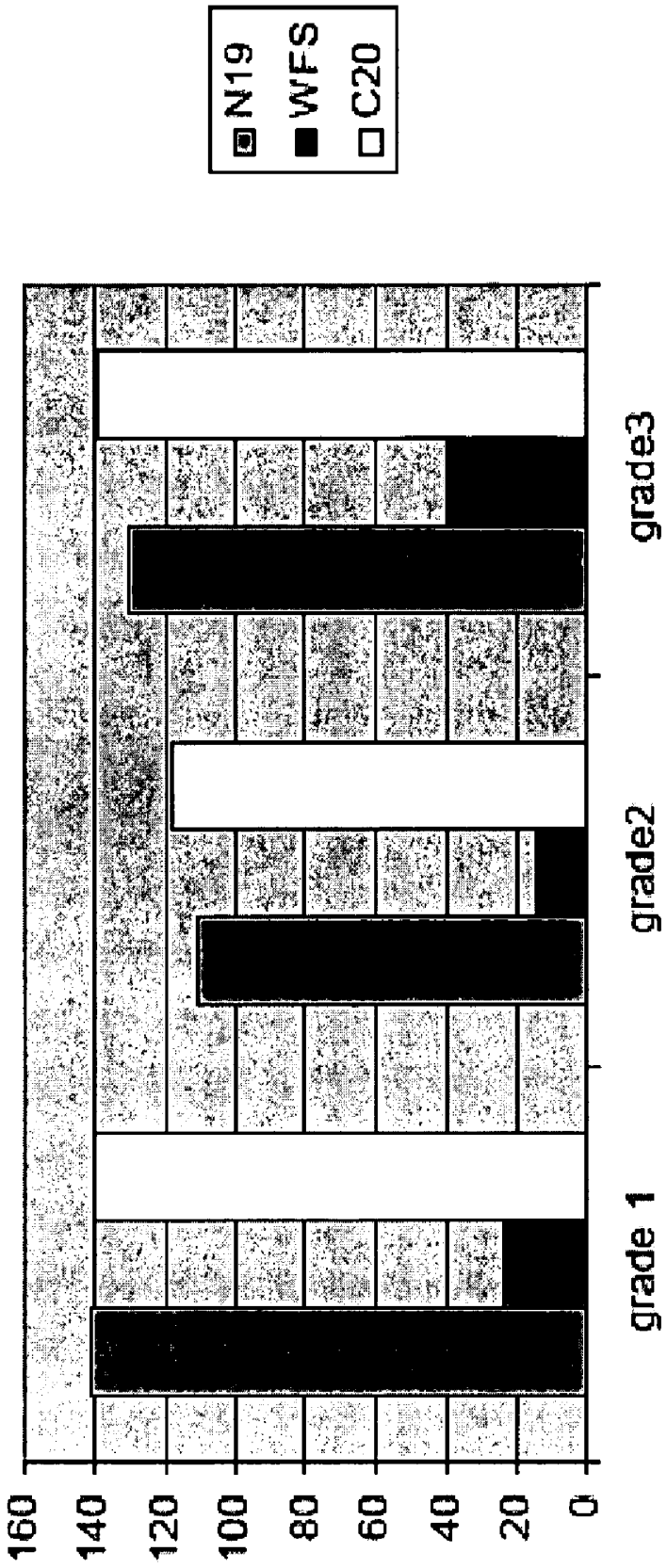


Fig. 9A

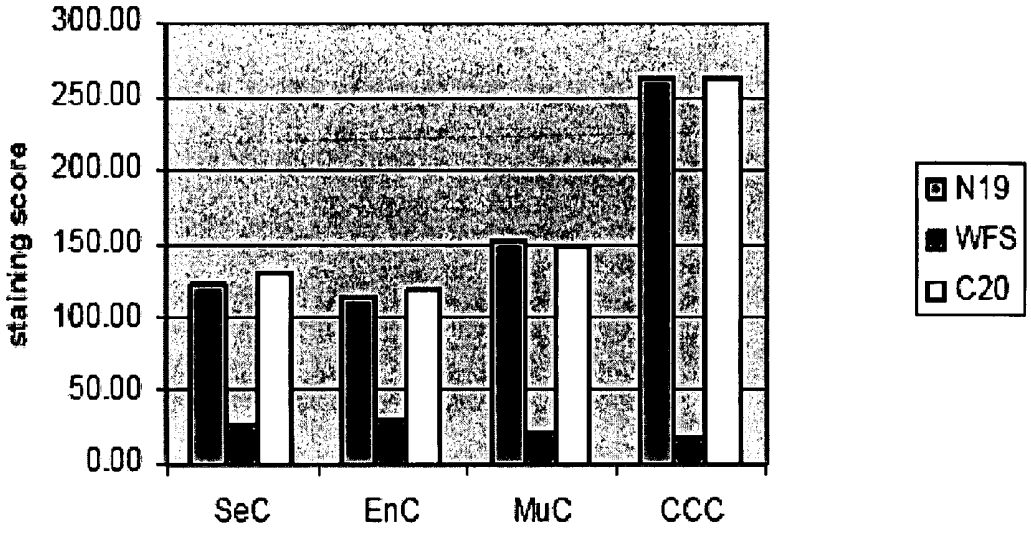
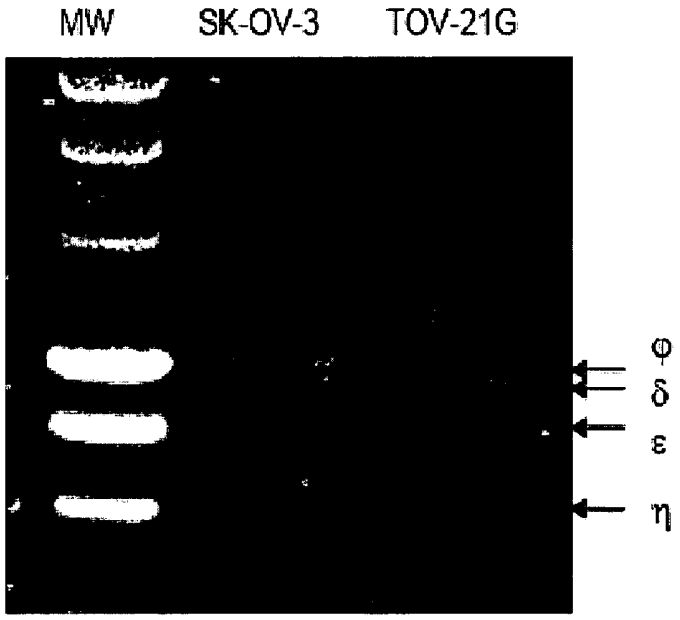


Fig. 9B



RT-PCR: BARD1

Fig. 9C

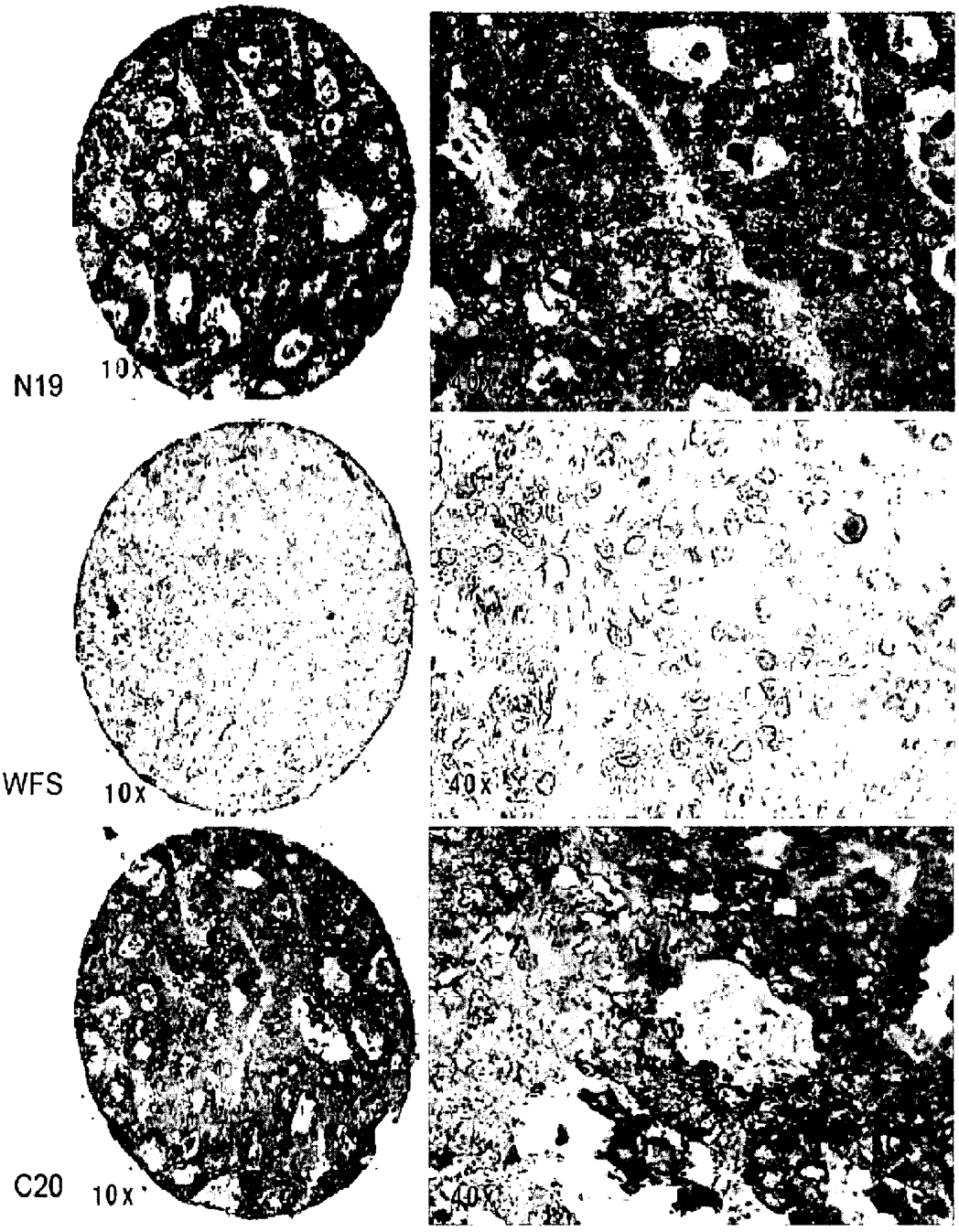
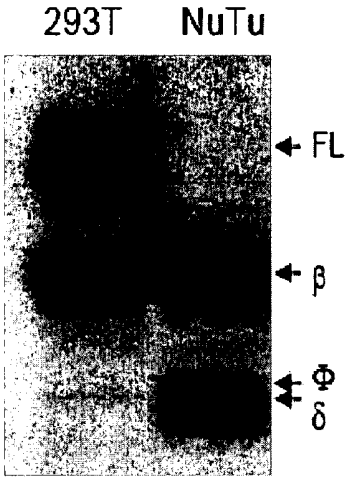
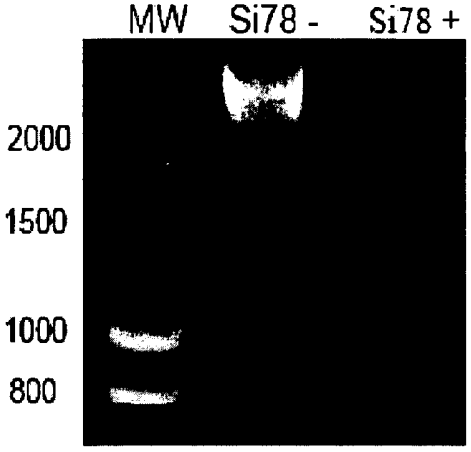


Fig. 10A



WB: H300

Fig. 10B



RT-PCR

g. 10C

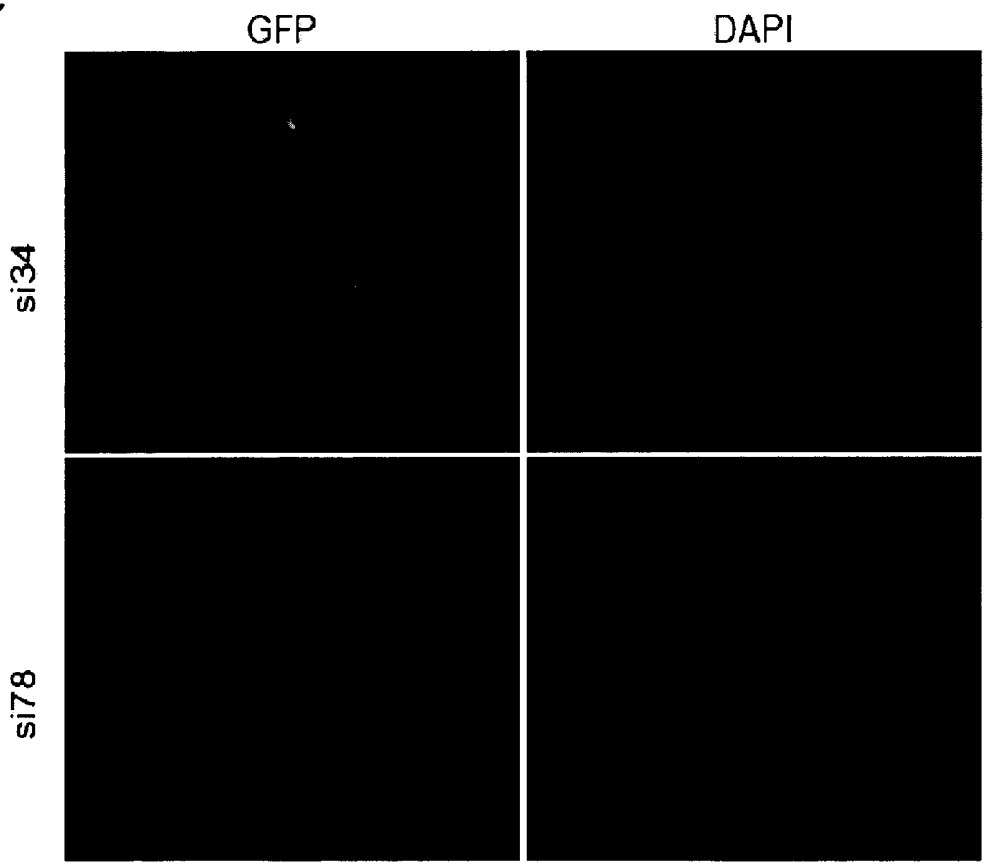


Fig. 10D

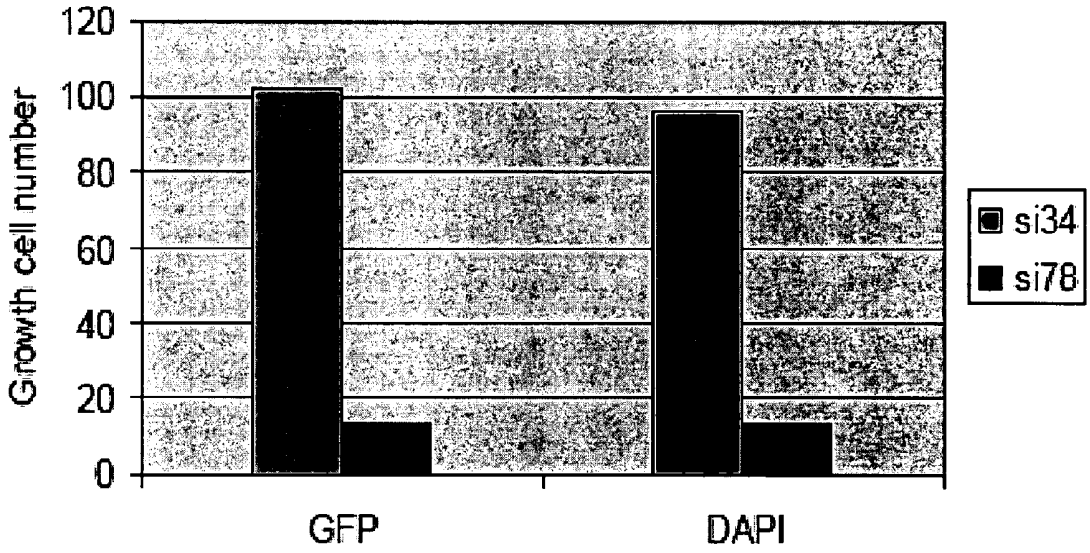
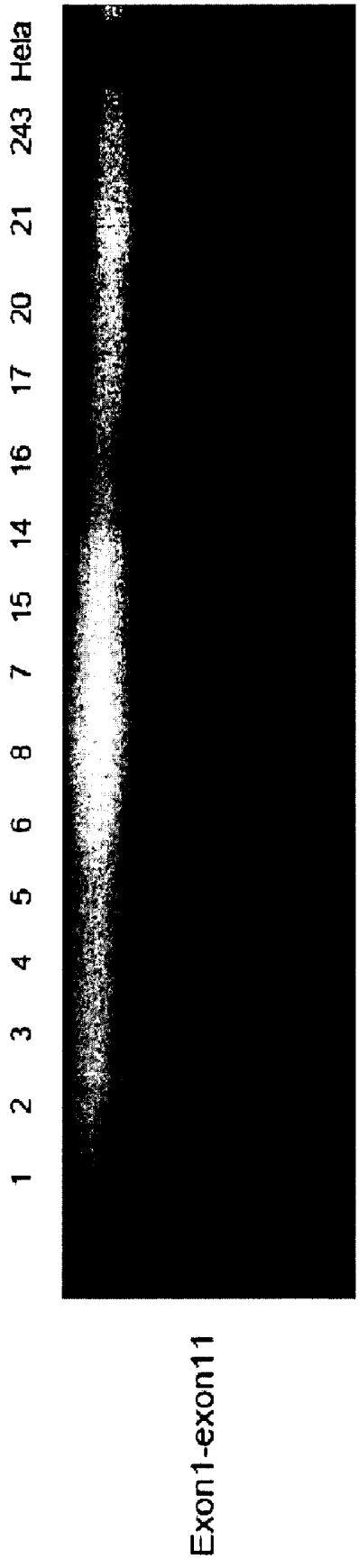


Fig. 11



DELETION BEARING BARD1 ISOFORMS AND USE THEREOF

FIELD OF THE INVENTION

[0001] The present invention relates to new protein isoforms, use thereof, methods of preparation thereof, methods of detection thereof, antibodies thereof, combination of antibodies thereof, use of these antibodies and combination thereof and use of antagonists of those isoforms for the treatment of gynaecological cancers.

BACKGROUND OF THE INVENTION

[0002] The tumor suppressor BARD1 (BRCA1 Associated Ring Domain) has multiple functions with and without BRCA1. N-terminal RING finger domains of BARD1 and BRCA1 confer an interaction module, and are essential for heterodimer formation. Mutations disrupting this interaction are found to be associated with cancer, indicating that the heterodimer has essential tumor suppressor functions, presumably attributable to its ubiquitin ligase activity. By itself, BARD1 has a function in apoptosis by stabilizing p53 and facilitating its phosphorylation, another important tumor suppressor function. BARD1 and BRCA1 were also reported to be involved in mitosis and specifically spindle formation.

[0003] Mutations and upregulated expression of BARD1 were found in breast and ovarian cancer. They were associated with poor prognosis, suggesting that cancer-associated BARD1 might be deficient in tumor suppressor functions. RT-PCR was performed to characterize the cDNA structure of cancer-associated BARD1 iso forms in breast, ovarian, cervical, and uterine cancers and cancer cell lines. Interestingly, all gynaecological cancers expressed a number of BARD1 isoforms derived from differential splicing, which was not the case for non-gynaecological cancers such as lymphomas. In cervical cancers, however, differentially spliced isoforms were not found but a truncated transcript, derived from alternative transcription initiation. Ovarian cancer and uterine cancer cells expressed a mixture of iso forms generated by both mechanisms. Specific repression of isoforms in a cancer cell line deficient of full length BARD1 leads to a complete growth arrest. This suggests that isoforms, which are expressed in gynaecological cancers and lack the central part and exons encoding the BRCA1-interacting RING finger domain, are essential for tumor cell growth.

[0004] BARD1 was found to be an interacting protein with BRCA1 (Wu et al., 1996, *Nat. Genet.* 14, 430-440). BARD1 and BRCA1 form a stable heterodimer with function in DNA repair, transcription regulation, RNA processing, ubiquitination and cell cycle regulation (Irminger-Finger et al., 2006, *Nature Reviews* 6, 382-391). Depletion of BARD1 leads to genomic instability, loss of polarity, premalignant phenotype, and embryonic lethality in knock out mice. As a tumour suppressor, BARD1 (SEQ ID NO: 1) also has a BRCA1 independent function in mediating p53-dependent apoptosis (Irminger-Finger et al., 2001, *Molecular cell* 8, 1255-1266). It binds to p53, facilitating its phosphorylation and stabilisation (Feki et al., 2005, *Oncogene* 24, 3726-3736). Recently a novel function of BARD1 in mitosis was found. The role of BRCA1/BARD1 in mitotic spindle assembly may contribute to its function in maintaining chromosome stability and tumour suppression. Furthermore, BARD1, by interacting with acidic coiled-coil protein TACC1, BRCA1, BRCA2 and Aurora B, plays a role in controlling mitosis completion and genetic stability.

[0005] BARD1 is expressed in most proliferative tissues, with maximum expression in spleen and testis (Ayi et al., 1998, *Oncogene* 17, 2143-2148). Furthermore, it is upregulated in response to hypoxia, and genotoxic stress (Irminger-Finger et al., 2001, *Molecular cell* 8, 1255-1266; Jefford et al., 2004, *Oncogene* 23, 3509-3520), and hormone signalling (Feki et al., 2005, above). This upregulation of BARD1 thus induces apoptosis pathways and tumour suppression (Irminger-Finger et al., 2001, above).

[0006] More than 600 mutations, comprising deletions, insertions miss-sense, and nonsense mutation have been identified in BRCA1. Since BARD1 is a tumour suppressor both as a heterodimer with BRCA1 and on its own, BARD1 mutations should also predispose to cancer. However, BARD1 mutations are less frequent. After screening a panel of sporadic breast, ovarian and endometrial cancers, three missense alterations were identified in the BARD1 gene at the amino acid positions Q564H, V695L, and S761N (That et al., 1998, *Human Molecular Genetics* 7, 195-202). Five alterations were discovered in an Italian cohort with familial breast and ovarian cancers that was chosen for its absence of BRCA1 and BRCA2 gene alterations in its proband (Ghimenti et al., 2002, *Genes, chromosomes & cancer* 33, 235-242). Apart from mutation, BARD1 shows aberrantly elevated expression and localization to the cytoplasm in cancer cells, as compared to the normal tissue where it is localized to the nucleus. Elevated BARD1 staining in the cytoplasm was correlated with poor prognostic factor for breast and ovarian cancer (Wu et al., 2006, *Int. J. Cancer* 118, 1215-1226).

[0007] Consistent with BARD1 isoform lacking exon 2 through 6 as well as no full length (FL), BARD1 was found in a rat ovarian cancer cell line that is resistant to apoptosis (Feki et al., 2005, above). This isoform lacks most of the RING domain and the entire ankyrin repeats, a region required for the apoptosis and p53 binding (Feki et al., 2005, above). The same iso form was later reported in Hela cells. Deletion of N-terminal epitopes of BARD1 was also found in majority of ovarian cancer (Wu et al., 2006, above). It was therefore hypothesized that specific isoforms of BARD1 might have lost its tumour suppressor functions and acquired tumourigenic properties. To elucidate BARD1 function in cancer, experiments were performed to characterize BARD1 expression pattern in various types of cancer and determine their structure and potential function in cancer cell growth (Li et al., 2007, *Int. J. Biochem. Cell. Biol.* 39(9):1659-1672).

[0008] Diagnostics and therapies of gynaecological diseases comprise some of the most severe unmet clinical needs, including breast, ovarian, cervical and uterine cancers. Therefore, there is a need for developing new substances and related methods for better diagnosing and treating such diseases.

SUMMARY OF THE INVENTION

[0009] The present invention is directed towards to new protein isoforms, antibodies thereof, and related methods useful for the treatment of gynaecological cancers.

[0010] It is an object of the invention to provide new protein isoforms, antibodies thereof and related methods which are suitable for or the treatment of and/or prevention of and/or delaying the progression of gynaecological cancers, notably breast, ovarian, cervical and uterine cancers.

[0011] A first aspect of the invention provides a method for detecting the presence of gynaecological cancer related proteins (including breast cancer, ovarian cancer, endometrial and cervical cancer) according to any one of claims **1** to **27**.

[0012] A second aspect of the invention provides an isolated polypeptide according to any one of claims **28** to **32**.

[0013] A third aspect of the invention provides an isolated nucleic acid consisting of a nucleotide sequence according to any one for claims **33** to **34**, recombinant expression vectors thereof, host cells transfected or transformed with a recombinant expression vector according to the invention and a process for producing cells capable of expressing a polypeptide according to the invention.

[0014] A fourth aspect of the invention provides the use of a nucleic acid according to the invention.

[0015] A fifth aspect of the invention provides an isolated antibody according to any one of claims **37** to **38**.

[0016] A sixth aspect of the invention resides in a combination of antibodies according to any one of claims **39** to **46** and use thereof.

[0017] A seventh aspect of the invention provides a method for detecting the level of cellular expression of proteins according to claim **47**.

[0018] An eighth aspect of the invention resides in the use of an antibody or a combination of antibodies according to the invention in an assay.

[0019] A ninth aspect of the invention provides a recombinant vector comprising a nucleic acid according to the invention.

[0020] A tenth aspect of the invention resides in a host cell transfected with the recombinant vector according to the invention.

[0021] An eleventh aspect of the invention provides a process for producing cells capable of expressing a polypeptide according to the invention.

[0022] A twelfth aspect of the invention resides in a kit comprising at least one polypeptide according to the invention. In a preferred embodiment, the kit according to the invention is useful for the detection of at least one gynaecological cancer related protein in a biological sample of a subject suspected of or suffering from a gynaecological cancer or at high risk of developing a gynaecological cancer.

[0023] A thirteenth aspect of the invention provides an immunoassay kit for detecting gynaecological cancer in a biological sample, the kit comprising at least one antibody according to the invention or a fragment thereof or a combination of antibodies according to the invention. In a preferred embodiment, the immunoassay kit according to the invention is useful for detection of at least one gynaecological cancer related protein in a biological sample of a subject suspected of or suffering from a gynaecological cancer.

[0024] A fourteenth aspect of the invention resides in the use of an antagonist of a polypeptide according to the invention for the manufacture of a medication for the treatment of a gynaecological cancer, including breast, ovarian, cervical and uterine cancers. In a preferred embodiment, the antagonist is an antibody according to the invention.

[0025] A fifteenth aspect according to the invention provides a method of treating a disease comprising the administration of a therapeutically effective amount of an antagonist of a polypeptide according to the invention in a mammal in need thereof; wherein the disease is a gynaecological cancer, including breast, ovarian, cervical and uterine cancers.

[0026] Other features and advantages of the invention will be apparent from the detailed description, figures and sequence listings.

DESCRIPTION OF THE FIGURES

[0027] FIG. **1**. Structure of BARD1 isoforms. (A) RTPCR amplification of FL BARD1 coding region in normal skin fibroblast and Hela cells. (B) Diagram of BARD1 exons and structural domains compared to exon structure of FL BARD1 and isoforms α, β, γ, ϕ, δ, ϵ, and η. Approximate locations of structural domains are indicated as RING, Ankyring, and BRCT above BARD1 molecule structure. Small arrows mark positions of forward and reverse primers used for RT-PCR. Open reading frame corresponding to known BARD1 sequence is presented by empty boxes, alternative reading frame is indicated as spotted boxes. Amino acids and calculated molecular weight are indicated. The respective sequence IDs are listed on the left side for DNA sequences and on the right side for protein sequences (C) Sequences of splice junctions of isoforms β, γ, and η are presented. Known BARD1 ORF is marked with a grey bar, alternative ORF with an empty bar. Possible translation initiation methionines are labelled black bar (underlined) within alternative ORF of isoforms β, γ, and η. The sequence IDs are indicated.

[0028] FIG. **2**. RT-PCR of breast cancer cell lines (B1-B9) for amplification of FL BARD1. Hela cells were used as a control.

[0029] FIG. **3**. RT-PCR of cervical cancer cell lines (C1-C9) for amplification of regions as indicated. Nucleotide position of the forward primers are indicated. Hela cells were used as a control.

[0030] FIG. **4**. Amplification of FL BARD1 and truncated isoform from exon 4 through exon 11 in endometrial and ovarian cancer cell lines. (A) RT-PCR in endometrial cancer cell lines. (B) RT-PCR in ovarian cancer cell lines. Hela cells were used as a control.

[0031] FIG. **5**. RT-PCR of BARD1 expression in haematology tumour cell lines (H1-H13). No splice isoforms are visible.

[0032] FIG. **6**. Alternative initiation of transcription in exon 4. (A) Nested PCR with 5' GeneRacer of ovarian cancer sample and Hela cells. Forward primer was 5' nested primer and reverse primer located in exon 6. The bands sequenced were indicated by arrows. (B) mRNA and protein sequence of BARD1 exon 4. Positions of new initiations of transcription found by 5' GeneRacer are indicated (Start 1, 2 and 3). (C) Diagram of BARD1 structure and three new transcripts initiation isoforms (Ω1, Ω2, Ω3). Primers and antibodies used in the experiment were shown. The translated regions were shown in thick lines, non-translated in thin lines.

[0033] FIG. **7**. Western blot of ovarian cancer cell lines probed with BARD1 antibodies H300 and JH3 in ovarian cancer cell lines. MW of different BARD1 isoforms was indicated. Hela cells were used as a control.

[0034] FIG. **8**. Immunohistochemical staining of ovarian cancer tissue arrays. (A) Correlation of BARD1 expression and tumour size in ovarian cancer. (B) Correlation of BARD1 expression and lymph node metastasis in ovarian cancer. (C) Immunohistochemistry of a patient in stage T3 showed both N19 and WFS were negative while C20 was strongly positive, which indicates that only omega iso forms are expressed. (D) Correlation of BARD1 expression with different pathology grades in ovarian cancer.

[0035] FIG. **9**. BARD1 expression in different pathologic types of ovarian cancer. (A) Immunohistochemical staining in different pathologic types. Clear cell carcinoma has the highest score. SeC, serous carcinoma; EnC, endometriod carcinoma; CCC, clear cell carcinoma; MuC, mucinous carcinoma. (B) RT-PCR for amplification of FL BARD1 in clear cell carcinoma cell line. (C) Immunohistochemistry of clear cell carcinoma showed strong staining by both N19 and C20, but was negative for WFS.

[0036] FIG. **10**. Function of isoforms in cell viability. (A) Western Blot probed with BARD1 antibody H300 showed only iso forms in NuTu cells (rat ovarian cancer). (B) RT-PCR showed that BARD1 expression was repressed by siRNA78. (C) Fluorescence microscopy of GFP and DAPI in NuTu cells transduced with siRNAs-GFP constructs. (D) Histogram of survival cells in si78 (targeting exon 9, repressing isoform) and si34 (targeting exon 2) transduced NuTu cells.

[0037] FIG. **11**. RT-PCR of BARD1 expression in lung cancer cell lines. Hela cells were used as a control. No splice isoforms are visible.

DETAILED DESCRIPTION OF THE INVENTION

[0038] As used herein, "treatment" and "treating" and the like generally mean obtaining a desired pharmacological and physiological effect. The effect may be prophylactic in terms of preventing or partially preventing a disease, symptom or condition thereof and/or may be therapeutic in terms of a partial or complete cure of a disease, condition, symptom or adverse effect attributed to the disease. The term "treatment" as used herein covers any treatment of a disease in a mammal, particularly a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; or relieving the disease, i.e., causing regression of the disease and/or its symptoms or conditions.

[0039] The term "subject" as used herein refers to mammals. For examples, mammals contemplated by the present invention include human, primates, domesticated animals such as cattle, sheep, pigs, horses and the like.

[0040] The term "isolated" is used to indicate that the molecule is free of association with other proteins or polypeptides, for example as a purification product of recombinant host cell culture or as a purified extract.

[0041] The term "antibody" comprises antibodies binding to at least one isoform according to the invention or fragment thereof, chimeric antibodies recognizing and/or binding selectively to at least one iso form according to the invention or fragment thereof, fully human, humanized, genetically engineered or bispecific or multispecific antibodies as well as fragments thereof such as single chain antibodies (scFv) or domain antibodies against at least one isoform according to the invention or fragment thereof and the like. Antibodies of this invention may be monoclonal or polyclonal antibodies, or fragments or derivative thereof having substantially the same antigen specificity. The term "selectively" indicates that the antibodies preferentially recognize and/or bind to at least one target polypeptide or epitope of an isoform according to the invention, i.e., with a higher affinity than any binding to any other antigen or epitope, i.e. the binding to the target polypeptide can be discriminated from non-specific binding to other antigens such as other proteins not belonging to the group of the iso forms according to the invention. Examples of antibodies or combinations thereof according to the invention are presented herein. The binding affinity of an antibody can be readily determined by one of ordinary skill in the art, for example, by Scatchard analysis (Scatchard et al., 1949, *Ann NY Acad. Sci.*, 51, 660-672).

[0042] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method.

[0043] The term "antagonists" is defined as a molecule that antagonizes completely or partially one or more activity of biological molecule. Preferred antagonists according to the invention antagonize the biological function of at least of the iso forms according to the invention and does not antagonize FL BARD1 biological activity. The term "antagonist" includes but is not limited to: BARD 1 iso forms specific antibodies of any sort (polyclonal, monoclonal, antibody fragments, antibody variants), chimaeric proteins, natural or unnatural proteins with BARD 1 iso form antagonizing activities, small molecules, nucleic acid derived polymers (such as DNA and RNA aptamers, siRNAs, PNAs, or LNAs), peptidomimetics, fusion proteins, or gene therapy vectors driving the expression of such antagonists. An antagonist, as an isolated, purified or homogeneous protein according to the invention, may be produced by recombinant expression systems as described herein or purified from naturally occurring cells.

[0044] Suitable expression of polypeptides according to the invention, variants or fragments, antagonists, thereof include prokaryotes, yeast or higher eukaryotic cells. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast and mammalian cellular hosts are described for example in Pouwels et al., 1985, *Cloning Vectors: A laboratory manual*, Elsevier, N.Y.

[0045] The expression "gynaecological cancer" includes breast cancer, ovarian cancer, endometrial cancer and cervical cancer.

[0046] The expression "risk of developing a future gynecological cancer" refers to a higher risk of developing a gynaecological cancer than an individual (such as a mammal), who does not present the iso form.

[0047] The expression "biological sample" refers to cells, tissue samples or cell components (such as cellular membranes or cellular components) obtained from a subject suspected of or suffering from gynaecological cancer or at high risk of developing a gynaecological cancer. Examples include blood, serum, plasma and tissue samples.

[0048] The expression "kit" comprises at least one polypeptide according to the invention or at least one antibody according to the invention or a fragment thereof or a combination thereof as described herein coupled to a solid matrix and instructional material. The solid matrix as referred herein may include nitrocellulose paper, glass slide, microtitre plates and wells.

[0049] Table 1 below presents the Sequence identity numbers and associated molecules:

TABLE 1

SEQ ID NO.	Molecule
1	Amino acid sequence for human BARD1 full length
2	Amino acid sequence for human BARD1- alpha
3	Amino acid sequence for human BARD1- beta (Reading frame 1 in exon 1)
4	Amino acid sequence for human BARD1- beta (Reading frame 2 in exon 1)
5	Amino acid sequence for human BARD1- gamma
6	Amino acid sequence for human BARD1- phi
7	Amino acid sequence for human BARD1- epsilon
8	Amino acid sequence for human BARD1- eta (Reading frame 1 in exon 1)
9	Amino acid sequence for human BARD1- eta (Reading frame 2 in exon 1)
10	Amino acid sequence for human BARD1- omega 1
11	Amino acid sequence for human BARD1- omega 2
12	Nucleotide sequence for human BARD1 full length
13	Nucleotide sequence for human BARD1 alpha (Exon 2 deleted), Exon 1 linked to exon 3; Exons (1-3-4-5-6-7-8-9-10-11); Exon 3 starts at 232 (TAATTGTGT . . .), 2473 nucleotides, ATG at position 74, Translates into 758 amino acids starting "MPDNRQPRNR". Calculated molecular weight 84.56 kDa
14	Nucleotide sequence for human BARD1 beta (Exons 2 and 3 deleted), Exon 1 linked to exon 4; Exons (1-4-5-6-7-8-9-10-11); Exon 4 starts at 232 (ATTTGAAAG . . .), 2324 nucleotides, translates into 680 amino acids starting with "MVAVPGPTVA". Calculated molecular weight: 75.46 kDa
15	Nucleotide sequence for human BARD1 gamma (Exon 4 deleted), Exon 3 linked to exon 5; Exons (1-2-3-5-6-7-8-9-10-11); Exon 5 starts at 438 (GGCGACATACC . . .), 1456 nucleotides, translates into 126 amino acids. Calculated molecular weight: 14.34 kDa
16	Nucleotide sequence for human BARD1 phi (Exon 3-6 deleted), Exon 2 linked to exon 7; Exons (1-2-7-8-9-10-11); Exon 7 starts at 244 (TAATATATTTGG . . .), 1008 nucleotides, translates into 327 amino acids starting with "MPDNRQPRNR", Calculated molecular weight 37.13 kDa
17	Nucleotide sequence for human BARD1 epsilon (Exons 4-9 deleted), Exon 3 linked to exon 10; Exons (1-2-3-10-11); Exon 10 starts at 393 (GGGTAAAAGC . . .), 825 nucleotides, translates into 263 amino acids. Calculated molecular weight: 30.36 kDa, starting with "MPDNRQPRNR"
18	Nucleotide sequence for human BARD1 eta (Exons 2-9 deleted), Exon 1 linked to Exon 10 (Exons1-10-11); Exon 10 starts at 232 (GGGTAAAA . . .), 702 nucleotides, translates into 219 amino acids
19	Nucleotide sequence for human BARD1 omega 1, translates into 264 amino acids
20	Nucleotide sequence for human BARD1 omega 2, translates into 449 amino acids
21	Amino acid sequence for human BARD1 beta fragment
22	Amino acid sequence for human BARD1 gamma fragment
23	Nucleotide sequence for human BARD1 omega 3, translates into 347 amino acids
24	Amino acid sequence for human BARD1 omega 3
25	Amino acid sequence for synthetic peptide 1
26	Nucleotide sequence for 5' primer from exon 11
27	Nucleotide sequence for reverse primer from exon 11
28	Nucleotide sequence for 5' primer 1 from exon 6
29	Nucleotide sequence for reverse primer from exon 6
30	Amino acid sequence for synthetic peptide 2
31	Nucleotide sequence for 5' primer from exon 1
32	Nucleotide sequence for reverse primer from exon 11
33	Nucleotide sequence for 5' primer from exon 3
34	Nucleotide sequence for 5' primer 1 from exon 4
35	Nucleotide sequence for 5' primer 2 from exon 4
36	Nucleotide sequence for 5' primer 3 from exon 4
37	Nucleotide sequence for 5' primer 4 from exon 4
38	Nucleotide sequence for 5' primer 2 from exon 6

[0050] According to one aspect of the invention, is provided a method for detecting the presence of gynaecological cancer related proteins (including breast cancer, ovarian cancer, endometrial and cervical cancer) in a biological sample, comprising the steps of:

(a) Determining one or more of the following in a sample from a female mammal (including tissue biopsies or blood samples):

[0051] i. The expression level of a protein of SEQ ID NO: 1 through a detectable signal proportional to the said level of expression; and

[0052] iia. The expression level of at least one protein of an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7; SEQ ID NO: 8 and SEQ ID NO: 9 through a detectable signal proportional to the said level of expression; and/or

[0053] iib. The expression and/or expression level of at least one protein of an amino acid sequence selected from the group consisting of SEQ ID NO: 10; SEQ ID NO: 11 and SEQ ID NO: 24 through a detectable signal proportional to the said level of expression;

(b) Optionally comparing the expression levels obtained under step (i) with the expression level obtained under steps (iia) and/or (iib);

(c) Detecting a signal indicative of a ratio lower than a 1:1 ratio between the expression level obtained under step (i) and the expression level obtained under steps (iia) and/or (iib); or detecting a signal indicative of the expression/expression level determined under step (iib).

[0054] According to a further aspect of the invention, is provided a method according to the invention comprising the steps of:

(a) Determining one or more of the following in a sample from a female mammal (including tissue biopsies or blood samples):

[0055] i. The expression level of a protein of SEQ ID NO: 1 through a detectable signal proportional to the said level of expression; and

[0056] iia. The expression level of at least one protein of an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7; SEQ ID NO: 8 and SEQ ID NO: 9 through a detectable signal proportional to the said level of expression; and/or

(b) Optionally comparing the expression levels obtained under step (i) with the expression level obtained under steps (iia);

(c) Detecting a signal indicative of a ratio lower than a 1:1 ratio between the expression level obtained under step (i) and the expression level obtained under step (iia).

[0057] According to another further aspect of the invention, is provided a method according to the invention, wherein the signal obtained under detection step (c) is indicative of a ratio lower than a 1:1 ratio between the expression level obtained under step (i) and the expression level obtained under step (iia).

[0058] According to another further aspect of the invention, is provided a method according to the invention, wherein the signal indicative of a ratio lower than a 1:1 ratio between the expression level obtained under step (i) and the expression level obtained under step (iia), obtained under step (c), is of or lower than a ratio about 1:2.

[0059] According to another further aspect of the invention, is provided a method according to the invention, wherein the signal obtained under the detection step (c) is indicative of a gynaecological cancer.

[0060] According to another further aspect of the invention, is provided a method according to the invention comprising the steps of:

(a) Determining one or more of the following in a sample from a female mammal (including tissue biopsies or blood samples):

[0061] i. The expression level of a protein of SEQ ID NO: 1 through a detectable signal proportional to the said level of expression; and

[0062] iib. The expression and/or expression level of at least one protein of an amino acid sequence selected from the group consisting of SEQ ID NO: 10; SEQ ID NO: 11 and SEQ ID NO: 24 through a detectable signal proportional to the said level of expression;

(b) Optionally comparing the expression levels obtained under step (i) with the expression level obtained under step (iib);

(c) Detecting a signal indicative of the expression/expression level obtained under step (iib).

[0063] According to another further aspect of the invention, is provided a method according to the invention, wherein the signal obtained under detection step (c) is indicative of a ratio lower than a 1:1 ratio between the expression level obtained under step (i) and the signal obtained under step (iib).

[0064] According to another further aspect of the invention, is provided a method according to the invention, wherein the signal indicative of a ratio lower than a 1:1 ratio between the expression level obtained under step (i) and the expression level obtained under steps (iib) is of or lower than a ratio about 1:5.

[0065] According to another further aspect of the invention, is provided a method according to the invention, wherein the signal obtained under the detection step (c) is indicative of a gynaecological cancer or a risk of developing a future gynaecological cancer in the subject.

[0066] According to another further aspect of the invention, is provided a method according to the invention, wherein the method further comprises a comparison step (d) of the expression levels obtained under steps (i), (iia) and/or (iib), respectively, with expression levels in a normal control, wherein the normal control includes expression levels measured in a biological sample from an individual not suspected to suffer from a gynaecological cancer.

[0067] According to another aspect of the invention, is provided a method for detecting the presence of gynaecological cancer related proteins (including breast cancer, ovarian cancer, endometrial and cervical cancer) in a biological sample, comprising the steps of:

(i) Reacting a sample from a female mammal (including tissue biopsy, blood sample) with at least one antibody, a fragment thereof or a combination thereof, which is specific to a protein of SEQ ID NO: 1; and

(ii) Reacting the said sample with at least one antibody, fragment thereof, or a combination thereof, which is specific to at least one protein comprising an amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7; SEQ ID NO: 8 and SEQ ID NO: 9; and/or

(iii) Reacting the said sample with at least one antibody, a fragment thereof or a combination thereof, which is specific

to at least one protein comprising an amino acid sequence selected from SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 24;

(iv) Detecting (a) a protein of SEQ ID: 1; and (b) a protein comprising an amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7; SEQ ID NO: 8 and SEQ ID NO: 9; and/or (c) a protein comprising an amino acid sequence selected from SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 24; wherein the detection is achieved through the detection of the interaction of each said antibody, fragment thereof or combination thereof, used under steps (i) and (ii) and/or (iii) with the corresponding said at least one protein, wherein the presence of the interaction correlates with the concentration of the protein in the biological sample;

(v) Detecting a signal indicative of a ratio lower than a 1:1 ratio between the said interaction detection signal obtained under step (iv) for a protein of SEQ ID NO: 1 and the said interaction detection signal obtained under step (iv) for either a protein comprising an amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7; SEQ ID NO: 8 and SEQ ID NO: 9; or for a protein comprising an amino acid sequence selected from SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 24; or detecting a signal indicative of an interaction signal detected under step (iv) for a protein comprising an amino acid sequence selected from SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 24.

[0068] According to a further aspect of the invention, is provided a method for detecting the presence of gynaecological cancer according to the invention, wherein the signal detected under step (v) indicative of a ratio lower than a 1:1 ratio between the said interaction detection signal obtained under step (iv) for a protein of SEQ ID NO: 1 and the said interaction detection signal obtained under step (iv) for a protein comprising an amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7; SEQ ID NO: 8 and SEQ ID NO: 9, is indicative of a gynaecological cancer.

[0069] According to another further aspect of the invention, is provided a method for detecting the presence of gynaecological cancer according to the invention, wherein the signal detected under step (v) indicative for a protein comprising an amino acid sequence selected from SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 24, is indicative of a gynaecological cancer or a risk of developing a future gynaecological cancer in the subject.

[0070] According to another further aspect of the invention, is provided a method for detecting the presence of gynaecological cancer according to the invention, wherein steps (ii) and/or (iii) further comprise a washing step (iiia) wherein the unbound antibodies are washed off from the sample.

[0071] According to another further aspect of the invention, is provided a method for detecting the presence of gynaecological cancer according to the invention, wherein the antibodies are conjugated to a detectable moiety.

[0072] According to another further aspect of the invention, is provided a method for detecting the presence of gynaecological cancer according to the invention, wherein the antibodies used under step (ii) is a combination of antibodies wherein the combination comprises (a) at least one antibody against exon 1 from full length BARD 1 (SEQ ID NO: 12); (b) at least one antibody against one exon selected from exon 4, exon 5, exon 6, exon 7, exon 8 and exon 9 from full length BARD 1 (SEQ ID NO: 12); and (c) at least one antibody against exon 10 and/or exon 11 from full length BARD 1 (SEQ ID NO: 12).

[0073] According to another further aspect of the invention, is provided a method for detecting the presence of gynaecological cancer according to the invention, wherein the antibodies used under step (ii) is a combination of antibodies wherein the combination comprises (a) at least one antibody against exon 1 from full length BARD 1 (SEQ ID NO: 12); (b) at least one antibody against one exon selected from exon 4, exon 5 and exon 6 from full length BARD 1 (SEQ ID NO: 12); and (c) at least one antibody against exon 10 and/or exon 11 from full length BARD 1 (SEQ ID NO: 12).

[0074] According to another further aspect of the invention, is provided a method for detecting the presence of gynaecological cancer according to the invention, wherein the antibodies used under step (ii) is a combination of antibodies wherein the combination comprises (a) at least one antibody against exon 1 from full length BARD 1 (SEQ ID NO: 12); (b) at least one antibody against exon 4 from full length BARD 1 (SEQ ID NO: 12); and (c) at least one antibody against exon 10 and/or exon 11 from full length BARD 1 (SEQ ID NO: 1).

[0075] According to another further aspect of the invention, is provided a method for detecting the presence of gynaecological cancer according to the invention, wherein the antibodies used under step (ii) is a combination of antibodies wherein the combination comprises (a) an antibody against exon 1 from full length BARD 1 (SEQ ID NO: 12); (b) an antibody against exon 4 from full length BARD 1 (SEQ ID NO: 12); and (c) an antibody exon 11 from full length BARD 1 (SEQ ID NO: 12).

[0076] According to another further aspect of the invention, is provided a method for detecting the presence of gynaecological cancer according to the invention, wherein an antibody against exon 4 from full length BARD 1 (SEQ ID NO: 12) is an antibody against a polypeptide of SEQ ID NO: 25.

[0077] According to another further aspect of the invention, is provided a method for detecting the presence of gynaecological cancer according to the invention, wherein the antibodies used under step (iii) is a combination of antibodies wherein the combination comprises (a) at least one antibody against exon 1 from full length BARD 1 (SEQ ID NO: 12); (b) at least one antibody against exon 7 from full length BARD 1 (SEQ ID NO: 12); and (c) at least one antibody against exon 10 and/or exon 11 from full length BARD 1 (SEQ ID NO: 12).

[0078] A method according to any one of claims **11** to **15** and **21**, wherein the antibodies used under step (iii) is a combination of antibodies wherein the combination comprises (a) at least one antibody against exon 1; (b) at least one antibody against exon 7; and (c) at least one antibody against exon 11 from full length BARD 1 (SEQ ID NO: 12).

[0079] According to another further aspect of the invention, is provided a method for detecting the presence of gynaecological cancer according to the invention, wherein the specific antibodies used under step (ii) is a combination of antibodies wherein the combination comprises (a) at least one antibody against exon 1 from full length BARD 1 (SEQ ID NO: 12; and at least one antibody against a sequence selected from the following group: SEQ ID NO: 21 and SEQ ID NO: 22.

[0080] According to another further aspect of the invention, is provided a method according to the invention, wherein the biological sample is isolated from a human subject.

[0081] According to another further aspect of the invention, is provided a method according to the invention, wherein the biological sample is blood.

[0082] According to another further aspect of the invention, is provided a method according to the invention, wherein the steps (b) and/or (c) in any one of claims **1** to **10** or the detection steps (iv) and/or (v) in any one of claims **23** to **25**, are assayed for with an assay selected from an ELISA assay and a western blotting assay.

[0083] According to another further aspect of the invention, is provided a method according to the invention, wherein the comparison step (b) or the detection under step (iv) are assayed for with an assay selected from an ELISA assay wherein the biological sample is a blood sample.

[0084] According to another aspect of the invention, is provided an isolated polypeptide comprising at least one sequence of amino acids having at least 80% identity or homology (such as at least 85%, at least 90%, at least 95%, at least 98%) with a sequence of amino acids selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 24.

[0085] According to a further aspect of the invention, is provided an isolated polypeptide according to the invention, having a sequence of amino acids having at least 80% identity or homology (such as at least 85%, at least 90%, at least 95%, at least 98%) with a sequence of amino acids selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7; SEQ ID NO: 8 and SEQ ID NO: 9.

[0086] According to another further aspect of the invention, is provided an isolated polypeptide according to the invention, having a sequence of amino acids having at least 80% identity or homology (such as at least 85%, at least 90%, at least 95%, at least 98%) with a sequence of amino acids selected from SEQ ID NO: 10; SEQ ID NO: 11 and SEQ ID NO: 24.

[0087] According to another further aspect of the invention, is provided an isolated polypeptide according to the invention, having a sequence of amino acids selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7; SEQ ID NO: 8 and SEQ ID NO: 9.

[0088] According to another further aspect of the invention, is provided an isolated polypeptide according to the invention, having a sequence of amino acids selected from SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 24.

[0089] According to another aspect of the invention, is provided an isolated nucleic acid consisting of a nucleotide sequence encoding a polypeptide according to the invention.

[0090] According to a further aspect of the invention, is provided an isolated nucleic acid consisting of a nucleotide sequence according the invention selected from the group consisting of SEQ ID NO: 13; SEQ ID NO: 14; SEQ ID NO: 15; SEQ ID NO: 16; SEQ ID NO: 17; SEQ ID NO: 18, SEQ ID NO: 19; SEQ ID NO: 20 and SEQ ID NO: 23.

[0091] According to another aspect of the invention, is provided a use of a nucleic acid according to claim **33** or **34** for expressing recombinant polypeptides for analysis, characterization and therapeutic use.

[0092] According to a further aspect of the invention, is provided a use of a nucleic acid according to the invention as probes or primers.

[0093] According to another aspect of the invention, is provided an isolated antibody that selectively binds at least one polypeptide according to the invention.

[0094] According to a further aspect of the invention, is provided an isolated antibody according to the invention that selectively binds at least one polypeptide according to the invention.

[0095] According to another aspect of the invention, is provided a combination of antibodies comprising (a) at least one antibody against exon 1 from full length BARD 1 (SEQ ID NO: 12); (b) at least one antibody against one exon selected from exon 4, exon 5, exon 6, exon 7, exon 8 and exon 9 from full length BARD 1 (SEQ ID NO: 12); and (c) at least one antibody against exon 10 and/or exon 11 from full length BARD 1 (SEQ ID NO: 12).

[0096] According to a further aspect of the invention, is provided a combination of antibodies according to the invention comprising (a) at least one antibody against exon 1 from full length BARD 1 (SEQ ID NO: 12); (b) at least one antibody against one exon selected from exon 4, exon 5 and exon 6 from full length BARD 1 (SEQ ID NO: 12); and (c) at least one antibody against exon 10 and/or exon 11 from full length BARD 1 (SEQ ID NO: 12).

[0097] According to another further aspect of the invention, is provided a combination of antibodies according to the invention comprising (a) at least one antibody against exon 1 from full length BARD 1 (SEQ ID NO: 12); (b) at least one antibody against exon 4 from full length BARD 1 (SEQ ID NO: 12); and (c) at least one antibody against exon 10 and/or exon 11 from full length BARD 1 (SEQ ID NO: 1).

[0098] According to another further aspect of the invention, is provided a combination of antibodies according to the invention comprising (a) an antibody against exon 1 from full length BARD 1 (SEQ ID NO: 12); (b) an antibody against exon 4 from full length BARD 1 (SEQ ID NO: 12); and (c) an antibody exon 11 from full length BARD 1 (SEQ ID NO: 12).

[0099] According to another further aspect of the invention, is provided a combination of antibodies according to the invention, wherein the antibody against exon 4 from full length BARD 1 (SEQ ID NO: 12) is an antibody against a polypeptide of SEQ ID NO: 25.

[0100] According to another aspect of the invention, is provided a combination of antibodies comprising (a) at least one antibody against exon 1 from full length BARD 1 (SEQ ID NO: 12); (b) at least one antibody against exon 7 from full length BARD 1 (SEQ ID NO: 12); and (c) at least one antibody against exon 10 and/or exon 11 from full length BARD 1 (SEQ ID NO: 12).

[0101] According to another further aspect of the invention, is provided a combination of antibodies according to the invention comprising (a) at least one antibody against exon 1; (b) at least one antibody against exon 7; and (c) at least one antibody against exon 11 from full length BARD 1 (SEQ ID NO: 12).

[0102] According to another aspect of the invention, is provided a combination of antibodies comprising at least one antibody against exon 1; and at least one antibody against a sequence selected from the following group: SEQ ID NO: 21 and SEQ ID NO: 22.

[0103] According to another aspect of the invention, is provided a method for detecting the level of cellular expression of proteins of comprising the step of:

(i) Contacting at least one antibody according to the invention or a fragment thereof, or a combination of antibodies accord-

ing to the invention with cells to be tested under appropriate conditions for binding of the said antibodies, combination thereof or combination of antibodies to at least a protein having a sequence of amino acids selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 24 on the cells;

(ii) Determining the level of binding of said at least one antibody, combination thereof, or combination of antibodies to the cell as indicative level of expression of the said protein.

[0104] According to another aspect of the invention, is provided a use of an antibody according to the invention or a combination of antibodies according to the invention in an assay.

[0105] According to a further aspect of the invention, is provided a use according to the invention wherein the assay is western blots, immunohistochemistry, ELISA or FACS assays.

[0106] According to a further aspect of the invention, is provided a use of an antibody according to the invention or a combination of antibodies according to the invention in a method according to the invention.

[0107] According to another aspect of the invention, is provided a recombinant expression vector comprising a nucleic acid molecule according to the invention, wherein the vector optionally comprises an expression control sequence, allowing expression in prokaryotic or eukaryotic host cells of the encoded polypeptide, operably linked to the nucleic acid molecule.

[0108] According to another aspect of the invention, is provided a host cell transfected or transformed with a recombinant expression vector according to the invention or a nucleic acid according to the invention.

[0109] According to another aspect of the invention, is provided a process for producing cells capable of expressing a polypeptide according to the invention comprising genetically engineering cells with a vector according to the invention or a nucleic acid according to the invention.

[0110] According to another aspect of the invention, is provided a kit comprising at least one polypeptide according to the invention.

[0111] According to another aspect of the invention, is provided an immunoassay kit for detecting gynaecological cancer in a biological sample, the kit comprising at least one antibody according to the invention or a fragment thereof or a combination of antibodies according to the invention.

[0112] According to another aspect of the invention, is provided a use of an antagonist of a polypeptide according to the invention for the manufacture of a medicament for the treatment of a gynaecological cancer, including breast, ovarian, cervical and uterine cancers. In a particular embodiment, the antagonist is an antibody or a combination of antibodies according to the invention.

[0113] According to another aspect of the invention, is provided a method of treating a disease comprising the administration of a therapeutically effective amount of an antagonist of a polypeptide according to the invention in a mammal in need thereof; wherein the disease is a gynaecological cancer, including breast, ovarian, cervical and uterine cancers.

[0114] The BARD1 isoforms, polypeptides and antibodies of the invention may be useful in the Prognostic and diagnostic of gynaecological cancers

[0115] The N-terminus of BARD1 interacts with BRCA1, and is essential for BARD1's well established tumour suppressor function. Therefore loss of the N-terminus, such as observed in the omega isoforms, correlates with loss of tumour suppressor function. In the absence of further mutations, it is possible that the cell would not be a cancer cell.

[0116] However, absence of the BARD1 N-terminus, such as observed in the omega isoforms, is an indication of a predisposition to develop a cancer because of the absence of an important tumour suppressor function. Detection of BARD1 forms lacking the N-terminus such as omega iso forms or more generally iso forms with a start in exon 3 or downstream of exon 3 or forms of N-terminally proteolytically cleaved BARD1 can be used as a predictive tool to establish predisposition to a cancer. Specifically, detection of omega isoforms is predictive of a high risk of developing a gynaecological cancer. Moreover, in many cases, at the time of testing, a cancer will already have developed in the absence of this tumour suppressor function, and the detection of omega iso forms will correlate in these cases with the presence of a gynaecological cancer. Consequently, if omega isoforms are detected in a patient, further investigation will be appropriate to establish whether the patient already has a gynaecological cancer. If the patient is found not to have a cancer at the time of initial testing, then the patient will have to be closely monitored to detect the appearance of a gynaecological cancer rapidly after its event.

[0117] The exons in the middle part of BARD1, such as observed in the splice isoforms (alpha, beta and more importantly phi, delta, epsilon and eta), are important for the well established tumor suppressor function together with BRCA1 residing in exons 2 and the apoptotic function of BARD1 residing in exons 5 through 8. Therefore loss of exons in this region, such as observed in the splice iso forms gives BARD1 proliferation-inducing properties, making it oncogenic on its own. Therefore, absence of exons in the middle part of BARD1 is indicative of the presence of a gynaecological cancer. Absence of such splice isoforms, however, is not indicative of the absence of a cancer. Detection of splice iso forms can be used as a diagnostic tool to establish the presence of a gynaecological cancer.

[0118] BARD1-based diagnostic screening for gynaecological cancers or high risk of developing such cancers will in any case have to be undertaken in combination with other diagnostic methods as gynaecological cancers could, in some cases, also occur without expression of BARD1 splice or omega isoforms.

[0119] References cited herein are hereby incorporated by reference in their entirety. The present invention is not to be limited in scope by the specific embodiments described herein, which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of the invention. Indeed, various modifications of the invention, in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

[0120] The invention having been described, the following examples are presented by way of illustration, and not limitation.

EXAMPLES

[0121] The following abbreviations refer respectively to the definitions below:
kDa (Kilo Dalton), μg (microgram), μl (microliter), min (minute), mM (millimolar), sec (second), BRCA1 (Breast cancer 1), BSA (bovine serum albumin), CCC (clear cell carcinoma), CIP (calf intestinal phosphatase), DAB (diaminobenzidine), DAPI (4',6-diamidino-2-phenylindole), EDTA (Ethylenediaminetetraacetic acid), EnC (Endometriod carcinoma), FL (Full length), GFP (Green Fluorescent Protein), HRP (horse radish peroxidase), LNA (Nitro(imidazole/triazole)-linked acridine), MuC (mucinous carcinoma), MLV (Murine Leukemia virus), MW (molecular weight), ORF (Open reading frame), PNA (peptide nucleic acid), RT-PCR (reverse transcriptase polymerase chain reaction), SDS (Sodium Dodecyl Sulfate), SeC (serous carcinoma), siRNA (small interfering RNA), TAE (Tris-acetate-EDTA), TBS (Tris buffered saline), TNM (Tumor Node Metastatis), UV (ultraviolet), WFS (Anti-BARD1 antibody WFS).

Example 1

Structure of BARD1 Isoforms

[0122] To unravel the expression pattern of BARD1, the structure of BARD1 isoforms was determined in human normal fibroblasts and in Hela cells by RT-PCR. BARD1 was highly expressed in normal fibroblasts, and there was almost no expression of BARD1 iso forms when primers for amplifying the entire coding region were used. In Hela cells, spliced isoforms of BARD1 were highly expressed together with FL BARD1 (FIG. **1**A). These iso forms were cloned and sequenced and their structure, exon composition, and calculated molecular weight (MW) were determined (FIG. **1**B). FL BARD1 (SEQ ID NO: 12) translates into a protein of 777 amino acids or a calculated MW of 87 kDa (SEQ ID NO: 1).
[0123] Isoform α has a deletion of exon 2 (SEQ ID NO:13) and produces a 85 kDa protein of 758 amino acids (SEQ ID NO: 2). Isoform β, derived from deletion of exon 2 and 3 (SEQ ID NO:14), translates into a protein of 680 amino acid or 75 kDa, but would use a translation start in an alternative reading frame of exon 1 (SEQ ID NO: 3 and SEQ ID NO: 4). Deletion of exon 4 in isoform γ (SEQ ID NO:15) disrupts the open reading frame. However, isoform φ and δ, missing exons 3 to 6 (SEQ ID NO:16) or 2 to 6, could produce a 37 or 35 kDa protein of 326 amino acids (SEQ ID NO: 6) or 307 amino acids; only δ was reported previously in HeLa (Tsuzuki et al., 2005, above) and ovarian cancer cells (Feki et al., 2005, above); and isoform ϵ lacking exons 4 to 9 (SEQ ID NO:17) with a predicted MW of 30 kDa, composed of 264 amino acids (SEQ ID NO:7), and isoform η is composed of exons 1, 10, and 11 (SEQ ID NO:18), which is not in frame but initiation of translation could occur in an alternative reading frame and translate into a 19 kDa protein of 167 amino acids (SEQ ID NO: 8 and SEQ ID NO: 9). All these iso forms might loose either the RING domain or the ANK and BRCT domain, which are the important regions for BARD1 function as a tumour suppressor, and consequently lead to a tumourogenesis function.

Example 2

Expression of BARD1 in Different Cancer Cell Lines

[0124] To further investigate the structure of BARD1 isoforms, RT-PCR was performed on RNA from different gynaecological cancer cell lines to characterize BARD1 expression. Primers located in various exons of BARD1 were used to amplify different regions of BARD1 for breast, cervical, endometrial, and ovarian cancer cell lines. A specific BARD1 expression pattern in cell lines derived from different cancers was observed. Firstly, in breast cancer cell lines FL BARD1 was expressed together with smaller isoforms: β, φ, δ, and ϵ which were more abundant than FL BARD1. Another group showed no expression at all when primers were used for amplification of FL BARD1 (FIG. **2**).
[0125] In all cervical cancer lines, neither FL BARD1 nor splice isoforms were found, when RT-PCR was performed to amplify exon 1 to exon 11. Different forward primers more downstream were then used to amplify potentially 5' truncated forms of BARD1, and BARD1 expression was detected when using primers at different sites in exon 4. Finally, BARD1 expression was found in all samples when forward primer in exon 5 (FIG. **3**) was used. It seems that these BARD1 isoforms were initiated in exon 4 in cervical cancer cell lines. Two new transcription initiations were found within exon 4 for ovarian cancer. One was at the nucleotide position of 458 (start 1), which was at the beginning of exon 4 and the other was at the 983 nucleotide (start 2) in exon 4. In Hela cells, the new transcription initiation located at the end of exon 4, at nucleotide position 1290 (start 3). Start 1 and 2 transcript at the same ATG within exon 4 and produce a protein of approximately 44 kDa, and start 3 could produce a protein about 27 kDa. The mRNA and translated sequence structure was shown in FIG. **5**B. The new isoforms initiating in exon 4 were named Ω1, Ω2 and Ω3. In our RT-PCR experiments, the forward primer within exon 4 at nucleotide position 783 detected isoform Ω1 (SEQ ID NO: 10), and primes at nucleotide position 985 and 1280 detected isoform Ω2 (SEQ ID NO: 11. Isoform Ω3 (SEQ ID NO: 24) could be detected by forward primer within exon 5 at nucleotide position 1378.
[0126] RT-PCR was performed in endometrial cancer and ovarian cancer cell lines by using forward primers within exon 4 (FIGS. **4**A and B). FL BARD1 and isoforms were expressed in some of the samples. In other samples, which showed neither FL BARD1 nor isoforms, BARD1 was detected by forward primers within exon 4.
[0127] In summary (Table 2 below), using RT-PCR in breast cancer either little or no FL was observed, whereas splice isoforms or only omega iso forms were observed. In cervical cancer, only omega isoforms were observed, in endometrial and ovarian cancer, either little or no FL was observed but rather the presence of splice isoforms. Endometrial and ovarian cancer lines also expressed omega isoforms. By Western Blot, very little or no FL, expression of all splice iso forms and of omega iso forms were observed in ovarian cancer. Overall, in all gynaecological cancers there is little or no FL BARD1, but rather the expression of splice and/or omega isoforms was observed. In all cases, when FL and iso forms were expressed, FL was much less abundant than the iso forms.

TABLE 2

CANCER TYPE	FL BARD 1	SPLICE ISOFORMS	OMEGA ISOFORMS
CERVICAL	0	0	+++
BREAST	+	++	0
	0	0	+++

TABLE 2-continued

CANCER TYPE	FL BARD 1	SPLICE ISOFORMS	OMEGA ISOFORMS
OVARIAN	+	++	0
(Based on WB data)	0	0	+++
	+	++	+++
ENDOMETRIAL	+	++	0
	0	0	+++
LYMPHOMA	++	(+)	0
LUNG CANCER	0	0	0
NORMAL CELLS (CONTROL)	+	0	0

[0128] By contrast, in lymphoma where FL and splice iso forms are expressed, FL is much more abundant than splice isoforms. In lung cancer, none of FL BARD1, omega isoforms or splice isoforms (as those seen in gynaecological cancers) was observed. Expression of splice isoforms is characteristic of gynaecological cancers, and non-gynaecological cancers either do not express any splice isoforms, or splice isoforms are expressed at very low levels, and always much less abundant than FL BARD1.

[0129] The relative prevalence of the different patterns observed in gynaecological cancers is indicated in Table 3 below. In none of the cervical cancer cell lines tested was the expression of FL BARD1 observed, and only BARD1 omega isoforms were present. In endometrial cancer, FL and isoforms were expressed in 55.6% of the cases, in 11.1% of the cases only spliced isoforms were present, and 33.3% of the cases showed omega isoforms. In breast cancer cells, 19.2% of the cases expressed FL BARD1 and isoforms, and most of the cell lines expressed omega isoforms, which accounted for about 80.8%. In ovarian cancer cell lines, 21.9% expressed FL and isoforms, 15.6% expressed spliced isoforms only, and 62.5% expressed transcripts comprising exon 4 through exon 11. All the tested cancer cell lines were derived from cancers which might be hormonally regulated. In all of the gynaecological cancer cell lines tested, FL BARD1 was either missing or it seemed less abundant than other smaller isoforms. All the cancer cell lines expressed one or the other form of BARD1.

[0130] As a comparison, RT-PCR was also performed in haematological tumour cell lines which are unlikely to be hormonally controlled. Thus it was concluded that FL BARD1 is often lost in gynaecological cancer cell lines, but instead either splice isoforms or omega iso forms are expressed.

TABLE 3

TYPE OF CANCER	FULL LENGTH ONLY	FULL LENGTH & SPLICE ISOFORMS	SPLICE ISOFORMS ONLY	OMEGA ISOFORMS
Cervical cancer	0	0	0	100% (9/9)
Breast cancer	0	19.2% (5/26)	0	80.8% (21/26)
Ovarian cancer	0	21.9% (7/32)	15.6% (5/32)	62.5% (20/32)
Endometrial cancer	0	55.6% (5/9)	11.1% (1/9)	33.3% (3/9)
Lymphoma	61.5% (8/13)	38.5% (5/13)	0	Not determined

[0131] From these results the following could be deduced:

[0132] In many cases, gynaecological cancers (breast, ovarian, endometrial or cervical) express splice isoforms, always in much higher abundance than FL BARD1. In some cases, gynaecological cancers (breast, ovarian, endometrial or cervical) express omega isoforms but no FL and no splice isoforms. In some cases, gynaecological cancers (breast, ovarian, endometrial or cervical) express both splice and omega isoforms. However, in lung cancer no FL BARD1 or the splice isoforms observed in gynaecological cancers or the omega iso form have been observed. Normal cells only express FL BARD1. Lymphoma cell lines express FL BARD1 and sometimes splice isoforms, but these are always in much lower abundance than FL BARD1.

Example 3

Protein Expression Patterns of BARD1 Isoforms (Detected by Western Blot or ELISA)

[0133] Splice iso forms could for example be detected with a combination of antibodies against exon 1 (such as for example antibody N19), exon 4 (e.g. antibody WSF), and exon 11 (e.g. antibody C20) (FIG. **6**).

[0134] The antibody against exon 1 recognizes FL BARD1 and all splice isoforms but not omega isoforms. The antibody against exon 11 recognizes FL BARD1, all splice isoforms and all omega isoforms. The antibody against exon 4 recognizes FL BARD1 and does not recognize splice isoforms.

[0135] In another embodiment, the above antibody against exon 4 would be directed against the sequence LKEDKPRKSLFNDAGNKKNSIKMWFSPRSK (SEQ ID NO: 25) located at the beginning of exon 4. Such an antibody would recognize only FL BARD1 but not splice isoforms or omega isoforms. It would recognize isoform beta.

[0136] Another possibility for detecting splice iso forms would be to use an antibody directed against the sequence MVAVPGPTVAPRSTAWRSCCAARV (SEQ ID NO: 21) which is characteristic of the beta and eta splice isoforms expressed from an alternative reading frame. This sequence is only present in beta and eta and allows their identification without cross-reaction with FL BARD1. Beta and eta are usually expressed together with other splice isoforms, so their presence would be indicative of expression of splice isoforms in general. Antibodies against the sequence GRHTFC (SEQ ID NO: 22) in the gamma splice isoform could achieve the same purpose. Alternatively, one could use an antibody directed against exon 7 (e.g. antibody JH3, see FIG. **6**), which would recognize all omega isoforms as well as the splice isoforms alpha, beta, phi and delta but not epsilon and eta. All the antibodies directed against exons 4, 5, 6 or 7 would also recognize FL BARD1. It would be a matter of calibrating the signal ratios to determine which pattern is being recognized.

1-4-11 Combination (N19-WSF-C20):

[0137] FL would give 1high-4-high-11high

[0138] Splice isoforms would give 1high-4very low-11high

[0139] Omega isoforms would give 1null-4very low/null-11high

[0140] Little FL and more splice would give 1high-4low-11high

[0141] Little splice and more FL would give 1high-4medium/high-11high

Read-Out of the Above 1-4-11 Test:

[0142] If 1 is lower than 11, then there are omega isoforms expressed, which is predictive of an increased risk of developing a gynaecological cancer.

[0143] If 4 is lower than 1, then there are splice iso forms expressed, which is indicative of the presence of a cancer.

[0144] If 4 is lower than 1 and 4 is low, then splice iso forms are present and more abundant than FL, which would be indicative of the presence of a gynaecological cancer.

1-7-11 Combination (N19-JH3-C20):

[0145] FL would give 1high-Thigh-11high

[0146] Splice isoforms would give 1high-7low-11high

[0147] Omega isoforms would give 1null-Thigh-11high

[0148] Little FL and more splice would give 1high-7low/medium-11high.

Example 4

Identification of BARD1 Protein Isoforms in Ovarian Cancer Cell Lines

[0149] As different BARD1 transcripts were observed in cancer cells, it has been investigated whether these isoforms were translated. Western Blot analysis was performed on protein extracts from ovarian cancer cell lines. Hela cells were used as control. BARD1 antibody H300 against epitopes expressed on exon 1 through 4, and antibody JH3 directed against a peptide antigen within exon 7 for C terminal, were used. FIG. **7** shows how it would be possible to detect FL, splice isoforms and omega isoforms in the same sample by Western blot. Individual iso forms could be identified through a combination of reactivity with a specific antibody and size on the gel.

[0150] When using H300, we found that FL BARD1, which migrates on the gel as a band of 97 kDa was detected in extracts from Hela cells, but none of the ovarian cancer samples showed the FL BARD1. We detected protein bands of 94 kDa, 84 kDa and 68 kDa in all these cases. Concluding from the structure for the mRNA expressed in ovarian cancer, the 94 kDa and 84 kDa bands corresponded to isoform α (deletion exon 2) and iso form β (deletion exon 2 and 3), respectively. The 68 kDa band remains unknown. In some of the samples, several smaller bands of 40 to 50 kDa were observed, which were weakly expressed. However, when probing with JH3, a very strong band of 48 kDa was detected, which was barely detected by H300 (FIG. **7**). This N-terminally truncated form was abundantly expressed in ovarian cancer samples. The observed MW of this protein corresponds to the calculated MW of isoforms Ω1 and Ω2, which was about 44 kDa when migration on gel slightly higher like FL BARD1 could account for 48 kDa. This 48 kDa protein could derive from isoforms Ω1 and Ω2 (SEQ ID NO: 10 and SEQ ID NO: 11), which is consistent with our RT-PCR result. It is also deduced that the other smaller band of about 41 kDa detected by JH3 could be isoform ϕ (deletion exon 3 to 6) or δ (deletion exon 2 to 6). The result of Western blots thus confirmed the results obtained by RT-PCR and provided evidence that there was little or no FL BARD1 expressed in ovarian cancer, but instead different splice and omega isoforms were expressed. Compared to the splice isoforms, isoforms Ω1 and 2 were most abundant. This figure shows that, at protein level, both splice and omega iso forms but no FL BARD1 are detectable in ovarian cancer cell lines.

Example 5

BARD1 Expression in Ovarian Cancer Patients

[0151] To investigate how BARD1 was expressed and correlated with carcinogenesis and cancer progression, immunohistochemical staining was performed on a tissue array of ovarian cancers. Different antibodies detecting epitopes at the N-terminus (N19) within exon 4 (WFS) and C-terminus (C-20) of BARD1 were used (FIG. **6**). For ovarian cancer, it was observed that WSF only weakly reacted with all samples, whereas C20 reacted more strongly with all samples. Overall, the N19 epitope seemed to be less abundant than the C20 epitope but more abundant than the WSF epitope. This indicates that there was little or no FL present but instead that there both splice and omega iso forms were present. Interestingly, the loss of N19 reactivity mostly happened in cancer of T3 stage or cancers with lymph node metastasis (FIG. **8**) indicating that omega isoforms correlate with T3 stage and metastatic stages of ovarian cancer. Loss of N-terminus (N-19) is correlated with advanced tumor stage and lymph node metastasis 8D. Furthermore, it was found that both N19 and C20 were highly expressed in clear cell carcinoma, which is the type of ovarian cancer with worst prognosis (FIG. **9**A), but not for WFS. This indicates that the over-expression of splice is forms is more prevalent in clear cell carcinoma and correlates with the worst prognosis. Expression of isoforms delta, phi, epsilon, but not FL correlated with clear cell carcinoma. This expression pattern was consistent with the expressed iso form ϕ and δ. The RT-PCR performed in ovarian cancer cell lines derived from clear cell carcinoma confirmed this hypothesis. Isoforms ϕ, δ, and ϵ were highly expressed in SK-OV-3 and TOV-21G cell lines, which are of clear cell type (FIG. **10**B).

Example 6

BARD1 Isoforms Role in Tumour Cell Growth

[0152] It has been previously shown that rat ovarian cancer cells NuTu-19 do not express FL BARD1 but abundantly express the alternatively spliced isoform BARD1 β and δ (Feki et al., 2005, above). NuTu/19 cells are resistant to apoptosis, but exogenous expression of wild-type BARD1 can induce apoptosis in these cells (Feki et al., 2005, above), consistent with the finding that regions of BARD1 that are required for apoptosis is missing in BARD1 isoform δ.

[0153] To elucidate the function of BARD1 iso forms, lentiviral vectors containing inducible BARD1 siRNAs, and co-expression of GFP were transduced to NuTu cells to repress BARD1 expression. Si78 which targets the sequence in exon 9 was used to repress BARD1 expression, and si34, which targeted human sequence but not the rat version in exon 2 was used as a control. As shown on Western Blot probed with BARD1 antibody H300 in NuTu cells, NuTu cells do not express FL BARD1, but it expressed isoforms 0 and the smaller bands which correspond to ϕ and δ (FIG. **10**A). After transduction and induction of siRNAs, RT-PCR was performed and si78 completely repressed BARD1 expression (FIG. **10**B). Then, fluorescence microscopy showing GFP expression and DAPI staining showed that NuTu cells transduced with si78 showed very few growing cells, and cells

became big and flat and stopped proliferating. More importantly, si78-expressing but not si3-expressing cells stopped growing and detached. Cells transduced with si34 looked normal and proliferated (FIGS. **8**C and D). SiRNA78 expression lead to growth arrest, siRNA34 had no effect. These experiments demonstrate that BARD1 β and δ are important for NuTu cell growth, and repressing these iso forms leads to a blockage of cell proliferation and subsequently cell death. BARD1 splice isoforms are thus causally involved in cancer-related cell proliferation. Therefore inhibiting these splice iso forms inhibits cell proliferation and leads to cell death. Molecules that reduce BARD1 splice isoform activity should act as cancer therapeutics by stopping cancer cell proliferation and killing these cells.

Material and Methods

Cancer Cell Lines

[0154] Breast cancer cell lines (B1-B26): MCF-7, MM231, T47D, Hs578T, SKBR3, MM435s, ZR-75-1, BT549, MM453, BT474, PA1, A2780ADR, BT20, HBL100, HMEC, MCF12A, MCF10A, MCF7/6, MCF12F, MM134VI, MM157, MM175VII, MM330, MM468, UCAA812, MM361.

[0155] Cervical cancer cell lines (C1-C9): HeLa, SW756, GH354, Ca Ski, C-4 I, C-33 A, HT-3, ME-180, SiHa.

[0156] Endometrial cancer cell line (E1-E9): KLE, RL95-2, AN3 CA, HEC-1-B, Ishikawa, Colo. 684, HEC-50, EN, EJ.

[0157] Ovarian cancer cell line (O1-O32): A2780, Caov-3, ES-2, NIH: OVCAR-3, SK-OV-3, TOV-21G, TOV-112D, OV-90, OV-MZ-1a, OV-MZ-1c, OV-MZ-2, OV-MZ-2a, OV-MZ-5, OV-MZ-6, OV-MZ-8, OV-MZ-9, OV-MZ-10, OV-MZ-12, OV-MZ-12b, OV-MZ-17b, OV-MZ-18, OV-MZ-20, OV-MZ-21, OV-MZ-22, OV-MZ-26, OV-MZ-27, OV-MZ-30, OV-MZ-32, OV-MZ-33, OV-MZ-35, OV-MZ-37, OV-MZ-38.

RNA Isolation and RT-PCR

[0158] Total RNA from cell lines and tissue specimens were extracted by isopycnic centrifugation as described previously (Kury et al., 1990, *Oncogene* 5, 1403-1408). For reverse transcription, 0.5 μg of RNA was used in 20 μl of reverse transcription buffer containing 1 μl of random primer, 1.25 μl of 10 mM dNTP's, 1 μl of M-MLV-Powerscript enzyme. The reaction took place at 65° C. 3 minutes followed by 55° C. 60 minutes and 94° C., 5 minutes. cDNA (2-4 μl) was used as a template for PCR with different primers (Table 4 below). It was performed with Taq polymerase in a final volume of 50 μl. Primary denaturation (94° C., 3 min) and final extension (72° C., 10 min) were the same for each PCR Annealing temperature and extension time were variable according to different primers. PCR product (15 μl) was used for analysis in 1% of agarose/TAE gel with EtBr and visualized under UV light.

TABLE 4

Forward primer		Reverse primer				
Sequence	Position (bp) (exon)	Sequence	Position (bp) (exon)	PCR product (bp)	Annealing Temp (° C.)	Extension (sec)
SEQ ID NO: 31	−28 (exon 1)	SEQ ID NO: 29	1481 (exon 6)	1508	56	100
		SEQ ID NO: 32	2333 (exon 11)	2361	56	140
SEQ ID NO: 33	228 (exon 3)	SEQ ID NO: 32	2333 (exon 11)	2105	56	130
SEQ ID NO: 34:	783 (exon 4)	SEQ ID NO: 32	2333 (exon 11)	1550	56	100
SEQ ID NO: 35	985 (exon 4)	SEQ ID NO: 32	2333 (exon 11)	1348	57	90
SEQ ID NO: 36	1280 (exon 4)	SEQ ID NO: 32	2333 (exon 11)	1053	54	80
SEQ ID NO: 37	1378 (exon 4)	SEQ ID NO: 32	2333 (exon 11)	955	54	70
SEQ ID NO: 38	1441 (exon 6)	SEQ ID NO: 32	2333 (exon 11)	892	56	60

Determination of BARD1 cDNA 5' Ends in Ovarian Cancer

[0159] GeneRacer™ Kit (invitrogen) was used to amplify 5' cDNA end for RNA of ovarian cancer patient and Hela cells. Total RNA (4.5 μg) ovarian cancer and Hela cells were used. Then treated the total RNA with calf intestinal phosphatise (CIP) to dephosphorylate non-mRNA or truncated mRNA. Remove the mRNA 5' cap structure and ligate the RNA oligo to decapped mRNA. Then reverse transcribing was performed to get the cDNA. In order to amplify the 5' cDNA end, first PCR was performed with 5' race primer of SEQ ID NO: 26 (5'-CGACTGGAGCACGAGGACACTGA-3') and reverse primer in exon 11 of SEQ ID NO: 27 (5'-GTTGCCAAAGCTGTTTG-3). 5' nested PCR was per-

formed with 5' nested primer of SEQ ID NO: 28 (5'-GGACACTGACATGGACTGAAGGAGTA-3') and reverse primer in exon 6 of SEQ ID NO: 29 (5'-TTTTGATACCCG-GTGGTGTT-3'). All these procedures were performed according to the manufacturer's instructions. The PCR bands of 5' nested PCR were loaded on 1% low melting gel, cut, and purified with the QIAEX II kit (Qiagen, Hombrechtikon, Switzerland) followed by sequencing with 5' nested primer and reverse primer.

Western Blots

[0160] BARD1 antibodies H300 (sc-7372; Santa Cruz, Calif.) was used to detect the N terminus. A synthetic peptide with the sequence GLRPVDYTDDESMKSLLL (SEQ ID NO: 30) within exon 7 of BARD1 was used to generate polyclonal antibodies designated JH3 in rabbits, and was used to detect the C terminus. Protein extracts from different ovarian cancer cells lines were prepared and 40 μg of protein per lane were loaded on 10% SDS-PAGE and blotted onto nylon filters. Membranes were blocked with 5% milk powder in TBS. Antibody incubated with purified anti-Bard1 H300 and JH3 in a 1:500 dilution. Secondary anti-rabbit peroxidase-coupled antibodies were applied in a 1:10,000 dilution. Signal detection was performed with the enhanced chemiluminescence kit (Amersham, Arlington Heights, Ill.).

Immunohistochemistry

[0161] Formalin-fixed and paraffin-embedded micro tissure array were deparaffinized with xylene for 48 hours, and rehydrated through descending alcohol (100% alcohol, 95% alcohol, 70% alcohol, H_2O). The sections were boiled 5 minutes in microwave for antigen retrieval, and then blocking the endogenous peroxidase. Slides were incubated 24 hours at 4° C. in a humidifying chamber with first antibody after BSA (bovine serum albumin) blocking the nonspecific proteins. The primary antibodies used for BARD1 detection were N19 (se-7373, Santa Cruz Biotechnology) WFS described previously (Irminger-Finger et al., 1998, *The Journal of cell biology* 143, 1329-1339), and C20 (sc-7372, Santa Cruz, Calif.), which recognize N-terminal, epitope in exon 4, and C-terminal epitopes of BARD1, respectively. Secondary antibodies (goat anti-rabbit or rabbit anti-goat) conjugated with horse radish peroxidase (HRP) were applied in 1:100 dilutions at room temperature for 1 hr. Then diaminobenzidine (DAB) staining was permitted for 15 min at room temperature. Slides were counter stained with hematoxylin before dehydration and mounting.

[0162] To quantify BARD1 expressing, staining was scored by intensity and percentage of the stained cells. The value of staining intensity and positive cell percentage times together gets the final staining score.

Clinical Data

[0163] Ovarian cancer specimens were obtained from Austria. The pathological diagnosis were made by experienced pathologists and staged according to the WHO and AJCC classification. 106 cases of ovarian cancer from 32-87 year old women, were analyzed, comprising of 60 cases of serous carcinoma, 24 cases of endomeriod carcinoma, 16 cases of mucinous carcinoma, and 6 cases of clear cell carcinoma. According to TNM staging system, there were 38 cases in T1; 15 cases in T2; 53 cases in T3; 39 cases in N0, and 67 cases in N1 stage. There were 25, 26, and 55 cases of pathologic grade 1 to 3, respectively.

BARD1 Repression in NuTu Cells

[0164] NuTu cell culture—as described in literature

[0165] siRNA—standard methods

[0166] Transfection of NuTu cells—standard methods

[0167] Fluorescence microscopy—standard methods.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 38

<210> SEQ ID NO 1
<211> LENGTH: 777
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 1

```
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1               5                   10                  15

Gly Asn Glu Pro Arg Ser Ala Pro Ala Met Glu Pro Asp Gly Arg Gly
20                  25                  30

Ala Trp Ala His Ser Arg Ala Ala Leu Asp Arg Leu Glu Lys Leu Leu
35                  40                  45

Arg Cys Ser Arg Cys Thr Asn Ile Leu Arg Glu Pro Val Cys Leu Gly
50                  55                  60

Gly Cys Glu His Ile Phe Cys Ser Asn Cys Val Ser Asp Cys Ile Gly
65                  70                  75                  80

Thr Gly Cys Pro Val Cys Tyr Thr Pro Ala Trp Ile Gln Asp Leu Lys
85                  90                  95
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-continued

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Ile Asn Arg Gln Leu Asp Ser Met Ile Gln Leu Cys Ser Lys Leu Arg
100                 105                 110

Asn Leu Leu His Asp Asn Glu Leu Ser Asp Leu Lys Glu Asp Lys Pro
115                 120                 125

Arg Lys Ser Leu Phe Asn Asp Ala Gly Asn Lys Lys Asn Ser Ile Lys
130                 135                 140

Met Trp Phe Ser Pro Arg Ser Lys Lys Val Arg Tyr Val Val Ser Lys
145                 150                 155                 160

Ala Ser Val Gln Thr Gln Pro Ala Ile Lys Lys Asp Ala Ser Ala Gln
165                 170                 175

Gln Asp Ser Tyr Glu Phe Val Ser Pro Ser Pro Pro Ala Asp Val Ser
180                 185                 190

Glu Arg Ala Lys Lys Ala Ser Ala Arg Ser Gly Lys Lys Gln Lys Lys
195                 200                 205

Lys Thr Leu Ala Glu Ile Asn Gln Lys Trp Asn Leu Glu Ala Glu Lys
210                 215                 220

Glu Asp Gly Glu Phe Asp Ser Lys Glu Glu Ser Lys Gln Lys Leu Val
225                 230                 235                 240

Ser Phe Cys Ser Gln Pro Ser Val Ile Ser Ser Pro Gln Ile Asn Gly
245                 250                 255

Glu Ile Asp Leu Leu Ala Ser Gly Ser Leu Thr Glu Ser Glu Cys Phe
260                 265                 270

Gly Ser Leu Thr Glu Val Ser Leu Pro Leu Ala Glu Gln Ile Glu Ser
275                 280                 285

Pro Asp Thr Lys Ser Arg Asn Glu Val Val Thr Pro Glu Lys Val Cys
290                 295                 300

Lys Asn Tyr Leu Thr Ser Lys Lys Ser Leu Pro Leu Glu Asn Asn Gly
305                 310                 315                 320

Lys Arg Gly His His Asn Arg Leu Ser Ser Pro Ile Ser Lys Arg Cys
325                 330                 335

Arg Thr Ser Ile Leu Ser Thr Ser Gly Asp Phe Val Lys Gln Thr Val
340                 345                 350

Pro Ser Glu Asn Ile Pro Leu Pro Glu Cys Ser Ser Pro Pro Ser Cys
355                 360                 365

Lys Arg Lys Val Gly Gly Thr Ser Gly Arg Lys Asn Ser Asn Met Ser
370                 375                 380

Asp Glu Phe Ile Ser Leu Ser Pro Gly Thr Pro Pro Ser Thr Leu Ser
385                 390                 395                 400

Ser Ser Ser Tyr Arg Gln Val Met Ser Ser Pro Ser Ala Met Lys Leu
405                 410                 415

Leu Pro Asn Met Ala Val Lys Arg Asn His Arg Gly Glu Thr Leu Leu
420                 425                 430

His Ile Ala Ser Ile Lys Gly Asp Ile Pro Ser Val Glu Tyr Leu Leu
435                 440                 445

Gln Asn Gly Ser Asp Pro Asn Val Lys Asp His Ala Gly Trp Thr Pro
450                 455                 460

Leu His Glu Ala Cys Asn His Gly His Leu Lys Val Val Glu Leu Leu
465                 470                 475                 480

Leu Gln His Lys Ala Leu Val Asn Thr Thr Gly Tyr Gln Asn Asp Ser
485                 490                 495

Pro Leu His Asp Ala Ala Lys Asn Gly His Val Asp Ile Val Lys Leu
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500                 505                 510

Leu Leu Ser Tyr Gly Ala Ser Arg Asn Ala Val Asn Ile Phe Gly Leu
515                 520                 525

Arg Pro Val Asp Tyr Thr Asp Asp Glu Ser Met Lys Ser Leu Leu Leu
530                 535                 540

Leu Pro Glu Lys Asn Glu Ser Ser Ser Ala Ser His Cys Ser Val Met
545                 550                 555                 560

Asn Thr Gly Gln Arg Arg Asp Gly Pro Leu Val Leu Ile Gly Ser Gly
565                 570                 575

Leu Ser Ser Glu Gln Gln Lys Met Leu Ser Glu Leu Ala Val Ile Leu
580                 585                 590

Lys Ala Lys Lys Tyr Thr Glu Phe Asp Ser Thr Val Thr His Val Val
595                 600                 605

Val Pro Gly Asp Ala Val Gln Ser Thr Leu Lys Cys Met Leu Gly Ile
610                 615                 620

Leu Asn Gly Cys Trp Ile Leu Lys Phe Glu Trp Val Lys Ala Cys Leu
625                 630                 635                 640

Arg Arg Lys Val Cys Glu Gln Glu Glu Lys Tyr Glu Ile Pro Glu Gly
645                 650                 655

Pro Arg Arg Ser Arg Leu Asn Arg Glu Gln Leu Leu Pro Lys Leu Phe
660                 665                 670

Asp Gly Cys Tyr Phe Tyr Leu Trp Gly Thr Phe Lys His His Pro Lys
675                 680                 685

Asp Asn Leu Ile Lys Leu Val Thr Ala Gly Gly Gly Gln Ile Leu Ser
690                 695                 700

Arg Lys Pro Lys Pro Asp Ser Asp Val Thr Gln Thr Ile Asn Thr Val
705                 710                 715                 720

Ala Tyr His Ala Arg Pro Asp Ser Asp Gln Arg Phe Cys Thr Gln Tyr
725                 730                 735

Ile Ile Tyr Glu Asp Leu Cys Asn Tyr His Pro Glu Arg Val Arg Gln
740                 745                 750

Gly Lys Val Trp Lys Ala Pro Ser Ser Trp Phe Ile Asp Cys Val Met
755                 760                 765

Ser Phe Glu Leu Leu Pro Leu Asp Ser
770                 775


<210> SEQ ID NO 2
<211> LENGTH: 758
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

Met Pro Asp Asn Arg Gln Pro Arg Asn Arg Gln Pro Arg Ile Arg Ser
1               5                   10                  15

Gly Asn Glu Pro Arg Ser Ala Pro Ala Met Glu Pro Asp Gly Arg Gly
20                  25                  30

Ala Trp Ala His Ser Arg Ala Ala Leu Asp Arg Leu Glu Lys Leu Leu
35                  40                  45

Arg Cys Ser Arg Cys Asn Cys Val Ser Asp Cys Ile Gly Thr Gly Cys
50                  55                  60

Pro Val Cys Tyr Thr Pro Ala Trp Ile Gln Asp Leu Lys Ile Asn Arg
65                  70                  75                  80
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Gln Leu Asp Ser Met Ile Gln Leu Cys Ser Lys Leu Arg Asn Leu Leu
85                  90                  95

His Asp Asn Glu Leu Ser Asp Leu Lys Glu Asp Lys Pro Arg Lys Ser
100                 105                 110

Leu Phe Asn Asp Ala Gly Asn Lys Lys Asn Ser Ile Lys Met Trp Phe
115                 120                 125

Ser Pro Arg Ser Lys Lys Val Arg Tyr Val Val Ser Lys Ala Ser Val
130                 135                 140

Gln Thr Gln Pro Ala Ile Lys Lys Asp Ala Ser Ala Gln Gln Asp Ser
145                 150                 155                 160

Tyr Glu Phe Val Ser Pro Ser Pro Pro Ala Asp Val Ser Glu Arg Ala
165                 170                 175

Lys Lys Ala Ser Ala Arg Ser Gly Lys Lys Gln Lys Lys Lys Thr Leu
180                 185                 190

Ala Glu Ile Asn Gln Lys Trp Asn Leu Glu Ala Glu Lys Glu Asp Gly
195                 200                 205

Glu Phe Asp Ser Lys Glu Glu Ser Lys Gln Lys Leu Val Ser Phe Cys
210                 215                 220

Ser Gln Pro Ser Val Ile Ser Ser Pro Gln Ile Asn Gly Glu Ile Asp
225                 230                 235                 240

Leu Leu Ala Ser Gly Ser Leu Thr Glu Ser Glu Cys Phe Gly Ser Leu
245                 250                 255

Thr Glu Val Ser Leu Pro Leu Ala Glu Gln Ile Glu Ser Pro Asp Thr
260                 265                 270

Lys Ser Arg Asn Glu Val Val Thr Pro Glu Lys Val Cys Lys Asn Tyr
275                 280                 285

Leu Thr Ser Lys Lys Ser Leu Pro Leu Glu Asn Asn Gly Lys Arg Gly
290                 295                 300

His His Asn Arg Leu Ser Ser Pro Ile Ser Lys Arg Cys Arg Thr Ser
305                 310                 315                 320

Ile Leu Ser Thr Ser Gly Asp Phe Val Lys Gln Thr Val Pro Ser Glu
325                 330                 335

Asn Ile Pro Leu Pro Glu Cys Ser Ser Pro Pro Ser Cys Lys Arg Lys
340                 345                 350

Val Gly Gly Thr Ser Gly Arg Lys Asn Ser Asn Met Ser Asp Glu Phe
355                 360                 365

Ile Ser Leu Ser Pro Gly Thr Pro Pro Ser Thr Leu Ser Ser Ser Ser
370                 375                 380

Tyr Arg Gln Val Met Ser Ser Pro Ser Ala Met Lys Leu Leu Pro Asn
385                 390                 395                 400

Met Ala Val Lys Arg Asn His Arg Gly Glu Thr Leu Leu His Ile Ala
405                 410                 415

Ser Ile Lys Gly Asp Ile Pro Ser Val Glu Tyr Leu Leu Gln Asn Gly
420                 425                 430

Ser Asp Pro Asn Val Lys Asp His Ala Gly Trp Thr Pro Leu His Glu
435                 440                 445

Ala Cys Asn His Gly His Leu Lys Val Val Glu Leu Leu Leu Gln His
450                 455                 460

Lys Ala Leu Val Asn Thr Thr Gly Tyr Gln Asn Asp Ser Pro Leu His
465                 470                 475                 480

Asp Ala Ala Lys Asn Gly His Val Asp Ile Val Lys Leu Leu Leu Ser
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485 490 495

Tyr Gly Ala Ser Arg Asn Ala Val Asn Ile Phe Gly Leu Arg Pro Val
500 505 510

Asp Tyr Thr Asp Asp Glu Ser Met Lys Ser Leu Leu Leu Leu Pro Glu
515 520 525

Lys Asn Glu Ser Ser Ser Ala Ser His Cys Ser Val Met Asn Thr Gly
530 535 540

Gln Arg Arg Asp Gly Pro Leu Val Leu Ile Gly Ser Gly Leu Ser Ser
545 550 555 560

Glu Gln Gln Lys Met Leu Ser Glu Leu Ala Val Ile Leu Lys Ala Lys
565 570 575

Lys Tyr Thr Glu Phe Asp Ser Thr Val Thr His Val Val Val Pro Gly
580 585 590

Asp Ala Val Gln Ser Thr Leu Lys Cys Met Leu Gly Ile Leu Asn Gly
595 600 605

Cys Trp Ile Leu Lys Phe Glu Trp Val Lys Ala Cys Leu Arg Arg Lys
610 615 620

Val Cys Glu Gln Glu Glu Lys Tyr Glu Ile Pro Glu Gly Pro Arg Arg
625 630 635 640

Ser Arg Leu Asn Arg Glu Gln Leu Leu Pro Lys Leu Phe Asp Gly Cys
645 650 655

Tyr Phe Tyr Leu Trp Gly Thr Phe Lys His His Pro Lys Asp Asn Leu
660 665 670

Ile Lys Leu Val Thr Ala Gly Gly Gly Gln Ile Leu Ser Arg Lys Pro
675 680 685

Lys Pro Asp Ser Asp Val Thr Gln Thr Ile Asn Thr Val Ala Tyr His
690 695 700

Ala Arg Pro Asp Ser Asp Gln Arg Phe Cys Thr Gln Tyr Ile Ile Tyr
705 710 715 720

Glu Asp Leu Cys Asn Tyr His Pro Glu Arg Val Arg Gln Gly Lys Val
725 730 735

Trp Lys Ala Pro Ser Ser Trp Phe Ile Asp Cys Val Met Ser Phe Glu
740 745 750

Leu Leu Pro Leu Asp Ser
755

<210> SEQ ID NO 3
<211> LENGTH: 680
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 3

Met Val Ala Val Pro Gly Pro Thr Val Ala Pro Arg Ser Thr Ala Trp
1 5 10 15

Arg Ser Cys Cys Ala Ala Arg Val Asp Leu Lys Glu Asp Lys Pro Arg
20 25 30

Lys Ser Leu Phe Asn Asp Ala Gly Asn Lys Lys Asn Ser Ile Lys Met
35 40 45

Trp Phe Ser Pro Arg Ser Lys Lys Val Arg Tyr Val Val Ser Lys Ala
50 55 60

Ser Val Gln Thr Gln Pro Ala Ile Lys Lys Asp Ala Ser Ala Gln Gln
65 70 75 80

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Asp Ser Tyr Glu Phe Val Ser Pro Ser Pro Pro Ala Asp Val Ser Glu
85                  90                  95

Arg Ala Lys Lys Ala Ser Ala Arg Ser Gly Lys Lys Gln Lys Lys Lys
100                 105                 110

Thr Leu Ala Glu Ile Asn Gln Lys Trp Asn Leu Glu Ala Glu Lys Glu
115                 120                 125

Asp Gly Glu Phe Asp Ser Lys Glu Glu Ser Lys Gln Lys Leu Val Ser
130                 135                 140

Phe Cys Ser Gln Pro Ser Val Ile Ser Ser Pro Gln Ile Asn Gly Glu
145                 150                 155                 160

Ile Asp Leu Leu Ala Ser Gly Ser Leu Thr Glu Ser Glu Cys Phe Gly
165                 170                 175

Ser Leu Thr Glu Val Ser Leu Pro Leu Ala Glu Gln Ile Glu Ser Pro
180                 185                 190

Asp Thr Lys Ser Arg Asn Glu Val Val Thr Pro Glu Lys Val Cys Lys
195                 200                 205

Asn Tyr Leu Thr Ser Lys Lys Ser Leu Pro Leu Glu Asn Asn Gly Lys
210                 215                 220

Arg Gly His His Asn Arg Leu Ser Ser Pro Ile Ser Lys Arg Cys Arg
225                 230                 235                 240

Thr Ser Ile Leu Ser Thr Ser Gly Asp Phe Val Lys Gln Thr Val Pro
245                 250                 255

Ser Glu Asn Ile Pro Leu Pro Glu Cys Ser Ser Pro Pro Ser Cys Lys
260                 265                 270

Arg Lys Val Gly Gly Thr Ser Gly Arg Lys Asn Ser Asn Met Ser Asp
275                 280                 285

Glu Phe Ile Ser Leu Ser Pro Gly Thr Pro Pro Ser Thr Leu Ser Ser
290                 295                 300

Ser Ser Tyr Arg Gln Val Met Ser Ser Pro Ser Ala Met Lys Leu Leu
305                 310                 315                 320

Pro Asn Met Ala Val Lys Arg Asn His Arg Gly Glu Thr Leu Leu His
325                 330                 335

Ile Ala Ser Ile Lys Gly Asp Ile Pro Ser Val Glu Tyr Leu Leu Gln
340                 345                 350

Asn Gly Ser Asp Pro Asn Val Lys Asp His Ala Gly Trp Thr Pro Leu
355                 360                 365

His Glu Ala Cys Asn His Gly His Leu Lys Val Val Glu Leu Leu Leu
370                 375                 380

Gln His Lys Ala Leu Val Asn Thr Thr Gly Tyr Gln Asn Asp Ser Pro
385                 390                 395                 400

Leu His Asp Ala Ala Lys Asn Gly His Val Asp Ile Val Lys Leu Leu
405                 410                 415

Leu Ser Tyr Gly Ala Ser Arg Asn Ala Val Asn Ile Phe Gly Leu Arg
420                 425                 430

Pro Val Asp Tyr Thr Asp Asp Glu Ser Met Lys Ser Leu Leu Leu Leu
435                 440                 445

Pro Glu Lys Asn Glu Ser Ser Ser Ala Ser His Cys Ser Val Met Asn
450                 455                 460

Thr Gly Gln Arg Arg Asp Gly Pro Leu Val Leu Ile Gly Ser Gly Leu
465                 470                 475                 480

Ser Ser Glu Gln Gln Lys Met Leu Ser Glu Leu Ala Val Ile Leu Lys
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485                 490                 495

Ala Lys Lys Tyr Thr Glu Phe Asp Ser Thr Val Thr His Val Val Val
500                 505                 510

Pro Gly Asp Ala Val Gln Ser Thr Leu Lys Cys Met Leu Gly Ile Leu
515                 520                 525

Asn Gly Cys Trp Ile Leu Lys Phe Glu Trp Val Lys Ala Cys Leu Arg
530                 535                 540

Arg Lys Val Cys Glu Gln Glu Glu Lys Tyr Glu Ile Pro Glu Gly Pro
545                 550                 555                 560

Arg Arg Ser Arg Leu Asn Arg Glu Gln Leu Leu Pro Lys Leu Phe Asp
565                 570                 575

Gly Cys Tyr Phe Tyr Leu Trp Gly Thr Phe Lys His His Pro Lys Asp
580                 585                 590

Asn Leu Ile Lys Leu Val Thr Ala Gly Gly Gly Gln Ile Leu Ser Arg
595                 600                 605

Lys Pro Lys Pro Asp Ser Asp Val Thr Gln Thr Ile Asn Thr Val Ala
610                 615                 620

Tyr His Ala Arg Pro Asp Ser Asp Gln Arg Phe Cys Thr Gln Tyr Ile
625                 630                 635                 640

Ile Tyr Glu Asp Leu Cys Asn Tyr His Pro Glu Arg Val Arg Gln Gly
645                 650                 655

Lys Val Trp Lys Ala Pro Ser Ser Trp Phe Ile Asp Cys Val Met Ser
660                 665                 670

Phe Glu Leu Leu Pro Leu Asp Ser
675                 680


<210> SEQ ID NO 4
<211> LENGTH: 732
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

Ala Ser Leu Trp Phe Pro Glu Ala Ser Leu Leu Pro Ala Leu Arg Gly
1               5                   10                  15

Ala Phe His Pro Lys Ala Gly Arg Cys Arg Ile Ile Gly Ser Arg Gly
20                  25                  30

Thr Gly Ser Arg Gly Ser Ala Pro Gly Thr Ser Leu Val Pro Arg Pro
35                  40                  45

Pro Trp Asn Arg Met Val Ala Val Pro Gly Pro Thr Val Ala Pro Arg
50                  55                  60

Ser Thr Ala Trp Arg Ser Cys Cys Ala Ala Arg Val Asp Leu Lys Glu
65                  70                  75                  80

Asp Lys Pro Arg Lys Ser Leu Phe Asn Asp Ala Gly Asn Lys Lys Asn
85                  90                  95

Ser Ile Lys Met Trp Phe Ser Pro Arg Ser Lys Lys Val Arg Tyr Val
100                 105                 110

Val Ser Lys Ala Ser Val Gln Thr Gln Pro Ala Ile Lys Lys Asp Ala
115                 120                 125

Ser Ala Gln Gln Asp Ser Tyr Glu Phe Val Ser Pro Ser Pro Pro Ala
130                 135                 140

Asp Val Ser Glu Arg Ala Lys Lys Ala Ser Ala Arg Ser Gly Lys Lys
145                 150                 155                 160
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Gln Lys Lys Lys Thr Leu Ala Glu Ile Asn Gln Lys Trp Asn Leu Glu
165                 170                 175

Ala Glu Lys Glu Asp Gly Glu Phe Asp Ser Lys Glu Glu Ser Lys Gln
180                 185                 190

Lys Leu Val Ser Phe Cys Ser Gln Pro Ser Val Ile Ser Ser Pro Gln
195                 200                 205

Ile Asn Gly Glu Ile Asp Leu Leu Ala Ser Gly Ser Leu Thr Glu Ser
210                 215                 220

Glu Cys Phe Gly Ser Leu Thr Glu Val Ser Leu Pro Leu Ala Glu Gln
225                 230                 235                 240

Ile Glu Ser Pro Asp Thr Lys Ser Arg Asn Glu Val Val Thr Pro Glu
245                 250                 255

Lys Val Cys Lys Asn Tyr Leu Thr Ser Lys Lys Ser Leu Pro Leu Glu
260                 265                 270

Asn Asn Gly Lys Arg Gly His His Asn Arg Leu Ser Ser Pro Ile Ser
275                 280                 285

Lys Arg Cys Arg Thr Ser Ile Leu Ser Thr Ser Gly Asp Phe Val Lys
290                 295                 300

Gln Thr Val Pro Ser Glu Asn Ile Pro Leu Pro Glu Cys Ser Ser Pro
305                 310                 315                 320

Pro Ser Cys Lys Arg Lys Val Gly Gly Thr Ser Gly Arg Lys Asn Ser
325                 330                 335

Asn Met Ser Asp Glu Phe Ile Ser Leu Ser Pro Gly Thr Pro Pro Ser
340                 345                 350

Thr Leu Ser Ser Ser Ser Tyr Arg Gln Val Met Ser Ser Pro Ser Ala
355                 360                 365

Met Lys Leu Leu Pro Asn Met Ala Val Lys Arg Asn His Arg Gly Glu
370                 375                 380

Thr Leu Leu His Ile Ala Ser Ile Lys Gly Asp Ile Pro Ser Val Glu
385                 390                 395                 400

Tyr Leu Leu Gln Asn Gly Ser Asp Pro Asn Val Lys Asp His Ala Gly
405                 410                 415

Trp Thr Pro Leu His Glu Ala Cys Asn His Gly His Leu Lys Val Val
420                 425                 430

Glu Leu Leu Leu Gln His Lys Ala Leu Val Asn Thr Thr Gly Tyr Gln
435                 440                 445

Asn Asp Ser Pro Leu His Asp Ala Ala Lys Asn Gly His Val Asp Ile
450                 455                 460

Val Lys Leu Leu Leu Ser Tyr Gly Ala Ser Arg Asn Ala Val Asn Ile
465                 470                 475                 480

Phe Gly Leu Arg Pro Val Asp Tyr Thr Asp Asp Glu Ser Met Lys Ser
485                 490                 495

Leu Leu Leu Leu Pro Glu Lys Asn Glu Ser Ser Ser Ala Ser His Cys
500                 505                 510

Ser Val Met Asn Thr Gly Gln Arg Arg Asp Gly Pro Leu Val Leu Ile
515                 520                 525

Gly Ser Gly Leu Ser Ser Glu Gln Gln Lys Met Leu Ser Glu Leu Ala
530                 535                 540

Val Ile Leu Lys Ala Lys Lys Tyr Thr Glu Phe Asp Ser Thr Val Thr
545                 550                 555                 560

His Val Val Val Pro Gly Asp Ala Val Gln Ser Thr Leu Lys Cys Met
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565                 570                 575

Leu Gly Ile Leu Asn Gly Cys Trp Ile Leu Lys Phe Glu Trp Val Lys
580                 585                 590

Ala Cys Leu Arg Arg Lys Val Cys Glu Gln Glu Glu Lys Tyr Glu Ile
595                 600                 605

Pro Glu Gly Pro Arg Arg Ser Arg Leu Asn Arg Glu Gln Leu Leu Pro
610                 615                 620

Lys Leu Phe Asp Gly Cys Tyr Phe Tyr Leu Trp Gly Thr Phe Lys His
625                 630                 635                 640

His Pro Lys Asp Asn Leu Ile Lys Leu Val Thr Ala Gly Gly Gly Gln
645                 650                 655

Ile Leu Ser Arg Lys Pro Lys Pro Asp Ser Asp Val Thr Gln Thr Ile
660                 665                 670

Asn Thr Val Ala Tyr His Ala Arg Pro Asp Ser Asp Gln Arg Phe Cys
675                 680                 685

Thr Gln Tyr Ile Ile Tyr Glu Asp Leu Cys Asn Tyr His Pro Glu Arg
690                 695                 700

Val Arg Gln Gly Lys Val Trp Lys Ala Pro Ser Ser Trp Phe Ile Asp
705                 710                 715                 720

Cys Val Met Ser Phe Glu Leu Leu Pro Leu Asp Ser
725                 730


<210> SEQ ID NO 5
<211> LENGTH: 127
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 5

Met Pro Asp Asn Arg Gln Pro Arg Asn Arg Gln Pro Arg Ile Arg Ser
1               5                   10                  15

Gly Asn Glu Pro Arg Ser Ala Pro Ala Met Glu Pro Asp Gly Arg Gly
20                  25                  30

Ala Trp Ala His Ser Arg Ala Ala Leu Asp Arg Leu Glu Lys Leu Leu
35                  40                  45

Arg Cys Ser Arg Cys Thr Asn Ile Leu Arg Glu Pro Val Cys Leu Gly
50                  55                  60

Gly Cys Glu His Ile Phe Cys Ser Asn Cys Val Ser Asp Cys Ile Gly
65                  70                  75                  80

Thr Gly Cys Pro Val Cys Tyr Thr Pro Ala Trp Ile Gln Asp Leu Lys
85                  90                  95

Ile Asn Arg Gln Leu Asp Ser Met Ile Gln Leu Cys Ser Lys Leu Arg
100                 105                 110

Asn Leu Leu His Asp Asn Glu Leu Ser Gly Arg His Thr Phe Cys
115                 120                 125


<210> SEQ ID NO 6
<211> LENGTH: 326
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

Met Pro Asp Asn Arg Gln Pro Arg Asn Arg Gln Pro Arg Ile Arg Ser
1               5                   10                  15

Gly Asn Glu Pro Arg Ser Ala Ser Ala Met Glu Pro Asp Gly Arg Gly
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20                  25                  30

Ala Trp Ala His Ser Arg Ala Ala Leu Asp Arg Leu Glu Lys Leu Leu
35                  40                  45

Arg Cys Ser Arg Cys Thr Asn Ile Leu Arg Glu Pro Val Cys Leu Gly
50                  55                  60

Gly Cys Glu His Ile Phe Cys Ser Asn Ile Phe Gly Leu Arg Pro Val
65                  70                  75                  80

Asp Tyr Thr Asp Asp Glu Ser Met Lys Ser Leu Leu Leu Leu Pro Glu
85                  90                  95

Lys Asn Glu Ser Ser Ser Ala Ser His Cys Ser Val Met Asn Thr Gly
100                 105                 110

Gln Arg Arg Asp Gly Pro Leu Val Leu Ile Gly Ser Gly Leu Ser Ser
115                 120                 125

Glu Gln Gln Lys Met Leu Ser Glu Leu Ala Val Ile Leu Lys Ala Lys
130                 135                 140

Lys Tyr Thr Glu Phe Asp Ser Thr Val Thr His Val Val Val Pro Gly
145                 150                 155                 160

Asp Ala Val Gln Ser Thr Leu Lys Cys Met Leu Gly Ile Leu Asn Gly
165                 170                 175

Cys Trp Ile Leu Lys Phe Glu Trp Val Lys Ala Cys Leu Arg Arg Lys
180                 185                 190

Val Cys Glu Gln Glu Glu Lys Tyr Glu Ile Pro Glu Gly Pro Arg Arg
195                 200                 205

Ser Arg Leu Asn Arg Glu Gln Leu Leu Pro Lys Leu Phe Asp Gly Cys
210                 215                 220

Tyr Phe Tyr Leu Trp Gly Thr Phe Lys His His Pro Lys Asp Asn Leu
225                 230                 235                 240

Ile Lys Leu Val Thr Ala Gly Gly Gly Gln Ile Leu Ser Arg Lys Pro
245                 250                 255

Lys Pro Asp Ser Asp Val Thr Gln Thr Ile Asn Thr Val Ala Tyr His
260                 265                 270

Ala Arg Pro Asp Ser Asp Gln Arg Phe Cys Thr Gln Tyr Ile Ile Tyr
275                 280                 285

Glu Asp Leu Cys Asn Tyr His Pro Glu Arg Val Arg Gln Gly Lys Val
290                 295                 300

Trp Lys Ala Pro Ser Ser Trp Phe Ile Asp Cys Val Met Ser Phe Glu
305                 310                 315                 320

Leu Leu Pro Leu Asp Ser
325


<210> SEQ ID NO 7
<211> LENGTH: 263
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 7

Met Pro Asp Asn Arg Gln Pro Arg Asn Arg Gln Pro Arg Ile Arg Ser
1               5                   10                  15

Gly Asn Glu Pro Arg Ser Ala Ser Ala Met Glu Pro Asp Gly Arg Gly
20                  25                  30

Ala Trp Ala His Ser Arg Ala Ala Leu Asp Arg Leu Glu Lys Leu Leu
35                  40                  45
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Arg Cys Ser Arg Cys Thr Asn Ile Leu Arg Glu Pro Val Cys Leu Gly
50                  55                  60

Gly Cys Glu His Ile Phe Cys Ser Asn Cys Val Ser Asp Cys Ile Gly
65                  70                  75                  80

Thr Gly Cys Pro Val Cys Tyr Thr Pro Ala Trp Ile Gln Asp Leu Lys
85                  90                  95

Ile Asn Arg Gln Leu Asp Ser Met Ile Gln Leu Cys Ser Lys Arg Asn
100                 105                 110

Leu Leu His Asp Asn Glu Leu Ser Gly Val Lys Ala Cys Leu Arg Arg
115                 120                 125

Lys Val Cys Glu Gln Glu Glu Lys Tyr Glu Ile Pro Glu Gly Pro Arg
130                 135                 140

Arg Ser Arg Leu Asn Arg Glu Gln Leu Leu Pro Lys Leu Phe Asp Gly
145                 150                 155                 160

Cys Tyr Phe Tyr Leu Trp Gly Thr Phe Lys His His Pro Lys Asp Asn
165                 170                 175

Leu Ile Lys Leu Val Thr Ala Gly Gly Gly Gln Ile Leu Ser Arg Lys
180                 185                 190

Pro Lys Pro Asp Ser Asp Val Thr Gln Thr Ile Asn Thr Val Ala Tyr
195                 200                 205

His Ala Arg Pro Asp Ser Asp Gln Arg Phe Cys Thr Gln Tyr Ile Ile
210                 215                 220

Tyr Glu Asp Leu Cys Asn Tyr His Pro Glu Arg Val Arg Gln Gly Lys
225                 230                 235                 240

Val Trp Lys Ala Pro Ser Ser Trp Phe Ile Asp Cys Val Met Ser Phe
245                 250                 255

Glu Leu Leu Pro Leu Asp Ser
260


<210> SEQ ID NO 8
<211> LENGTH: 167
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

Met Val Ala Val Pro Gly Pro Thr Val Ala Pro Arg Ser Thr Ala Trp
1               5                   10                  15

Arg Ser Cys Cys Ala Ala Arg Val Gly Val Lys Ala Cys Leu Arg Arg
20                  25                  30

Lys Val Cys Glu Gln Glu Glu Lys Tyr Glu Ile Pro Glu Gly Pro Arg
35                  40                  45

Arg Ser Arg Leu Asn Arg Glu Gln Leu Leu Pro Lys Leu Phe Asp Gly
50                  55                  60

Cys Tyr Phe Tyr Leu Trp Gly Thr Phe Glu His His Pro Lys Asp Asn
65                  70                  75                  80

Leu Ile Lys Leu Val Thr Ala Gly Gly Gly Gln Ile Leu Ser Arg Lys
85                  90                  95

Pro Lys Pro Asp Ser Asp Val Thr Gln Thr Ile Asn Thr Val Ala Tyr
100                 105                 110

His Ala Arg Pro Asp Ser Asp Gln Arg Phe Cys Thr Gln Tyr Ile Ile
115                 120                 125

Tyr Glu Asp Leu Cys Asn Tyr His Pro Glu Arg Val Arg Gln Gly Lys
130                 135                 140
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Val Trp Lys Ala Pro Ser Ser Trp Phe Ile Asp Cys Val Met Ser Phe
145                 150                 155                 160

Glu Leu Leu Pro Leu Asp Ser
165


<210> SEQ ID NO 9
<211> LENGTH: 219
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 9

Ala Ser Leu Trp Phe Pro Glu Ala Ser Leu Leu Pro Ala Leu Arg Gly
1               5                   10                  15

Ala Phe His Pro Lys Ala Gly Arg Cys Arg Ile Ile Gly Ser Arg Gly
20                  25                  30

Thr Gly Ser Arg Gly Ser Ala Pro Gly Thr Ser Leu Val Pro Arg Pro
35                  40                  45

Pro Trp Asn Arg Met Val Ala Val Pro Gly Pro Thr Val Ala Pro Arg
50                  55                  60

Ser Thr Ala Trp Arg Ser Cys Cys Ala Ala Arg Val Gly Val Lys Ala
65                  70                  75                  80

Cys Leu Arg Arg Lys Val Cys Glu Gln Glu Glu Lys Tyr Glu Ile Pro
85                  90                  95

Glu Gly Pro Arg Arg Ser Arg Leu Asn Arg Glu Gln Leu Leu Pro Lys
100                 105                 110

Leu Phe Asp Gly Cys Tyr Phe Tyr Leu Trp Gly Thr Phe Glu His His
115                 120                 125

Pro Lys Asp Asn Leu Ile Lys Leu Val Thr Ala Gly Gly Gly Gln Ile
130                 135                 140

Leu Ser Arg Lys Pro Lys Pro Asp Ser Asp Val Thr Gln Thr Ile Asn
145                 150                 155                 160

Thr Val Ala Tyr His Ala Arg Pro Asp Ser Asp Gln Arg Phe Cys Thr
165                 170                 175

Gln Tyr Ile Ile Tyr Glu Asp Leu Cys Asn Tyr His Pro Glu Arg Val
180                 185                 190

Arg Gln Gly Lys Val Trp Lys Ala Pro Ser Ser Trp Phe Ile Asp Cys
195                 200                 205

Val Met Ser Phe Glu Leu Leu Pro Leu Asp Ser
210                 215


<210> SEQ ID NO 10
<211> LENGTH: 624
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 10

Val Arg Tyr Val Val Ser Lys Ala Ser Val Gln Thr Gln Pro Ala Ile
1               5                   10                  15

Lys Lys Asp Ala Ser Ala Gln Gln Asp Ser Tyr Glu Phe Val Ser Pro
20                  25                  30

Ser Pro Pro Ala Asp Val Ser Glu Arg Ala Lys Lys Ala Ser Ala Arg
35                  40                  45

Ser Gly Lys Lys Gln Lys Lys Lys Thr Leu Ala Glu Ile Asn Gln Lys
50                  55                  60
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Trp Asn Leu Glu Ala Glu Lys Glu Asp Gly Glu Phe Asp Ser Lys Glu
65                  70                  75                  80

Glu Ser Lys Gln Lys Leu Val Ser Phe Cys Ser Gln Pro Ser Val Ile
85                  90                  95

Ser Ser Pro Gln Ile Asn Gly Glu Ile Asp Leu Leu Ala Ser Gly Ser
100                 105                 110

Leu Thr Glu Ser Glu Cys Phe Gly Ser Leu Thr Glu Val Ser Leu Pro
115                 120                 125

Leu Ala Glu Gln Ile Glu Ser Pro Asp Thr Lys Ser Arg Asn Glu Val
130                 135                 140

Val Thr Pro Glu Lys Val Cys Lys Asn Tyr Leu Thr Ser Lys Lys Ser
145                 150                 155                 160

Leu Pro Leu Glu Asn Asn Gly Lys Arg Gly His His Asn Arg Leu Ser
165                 170                 175

Ser Pro Ile Ser Lys Arg Cys Arg Thr Ser Ile Leu Ser Thr Ser Gly
180                 185                 190

Asp Phe Val Lys Gln Thr Val Pro Ser Glu Asn Ile Pro Leu Pro Glu
195                 200                 205

Cys Ser Ser Pro Pro Ser Cys Lys Arg Lys Val Gly Gly Thr Ser Gly
210                 215                 220

Arg Lys Asn Ser Asn Met Ser Asp Glu Phe Ile Ser Leu Ser Pro Gly
225                 230                 235                 240

Thr Pro Pro Ser Thr Leu Ser Ser Ser Ser Tyr Arg Gln Val Met Ser
245                 250                 255

Ser Pro Ser Ala Met Lys Leu Leu Pro Asn Met Ala Val Lys Arg Asn
260                 265                 270

His Arg Gly Glu Thr Leu Leu His Ile Ala Ser Ile Lys Gly Asp Ile
275                 280                 285

Pro Ser Val Glu Tyr Leu Leu Gln Asn Gly Ser Asp Pro Asn Val Lys
290                 295                 300

Asp His Ala Gly Trp Thr Pro Leu His Glu Ala Cys Asn His Gly His
305                 310                 315                 320

Leu Lys Val Val Glu Leu Leu Leu Gln His Lys Ala Leu Val Asn Thr
325                 330                 335

Thr Gly Tyr Gln Asn Asp Ser Pro Leu His Asp Ala Ala Lys Asn Gly
340                 345                 350

His Val Asp Ile Val Lys Leu Leu Leu Ser Tyr Gly Ala Ser Arg Asn
355                 360                 365

Ala Val Asn Ile Phe Gly Leu Arg Pro Val Asp Tyr Thr Asp Asp Glu
370                 375                 380

Ser Met Lys Ser Leu Leu Leu Leu Pro Glu Lys Asn Glu Ser Ser Ser
385                 390                 395                 400

Ala Ser His Cys Ser Val Met Asn Thr Gly Gln Arg Arg Asp Gly Pro
405                 410                 415

Leu Val Leu Ile Gly Ser Gly Leu Ser Ser Glu Gln Gln Lys Met Leu
420                 425                 430

Ser Glu Leu Ala Val Ile Leu Lys Ala Lys Lys Tyr Thr Glu Phe Asp
435                 440                 445

Ser Thr Val Thr His Val Val Val Pro Gly Asp Ala Val Gln Ser Thr
450                 455                 460
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Leu Lys Cys Met Leu Gly Ile Leu Asn Gly Cys Trp Ile Leu Lys Phe
465                 470                 475                 480

Glu Trp Val Lys Ala Cys Leu Arg Arg Lys Val Cys Glu Gln Glu Glu
485                 490                 495

Lys Tyr Glu Ile Pro Glu Gly Pro Arg Arg Ser Arg Leu Asn Arg Glu
500                 505                 510

Gln Leu Leu Pro Lys Leu Phe Asp Gly Cys Tyr Phe Tyr Leu Trp Gly
515                 520                 525

Thr Phe Lys His His Pro Lys Asp Asn Leu Ile Lys Leu Val Thr Ala
530                 535                 540

Gly Gly Gly Gln Ile Leu Ser Arg Lys Pro Lys Pro Asp Ser Asp Val
545                 550                 555                 560

Thr Gln Thr Ile Asn Thr Val Ala Tyr His Ala Arg Pro Asp Ser Asp
565                 570                 575

Gln Arg Phe Cys Thr Gln Tyr Ile Ile Tyr Glu Asp Leu Cys Asn Tyr
580                 585                 590

His Pro Glu Arg Val Arg Gln Gly Lys Val Trp Lys Ala Pro Ser Ser
595                 600                 605

Trp Phe Ile Asp Cys Val Met Ser Phe Glu Leu Leu Pro Leu Asp Ser
610                 615                 620


<210> SEQ ID NO 11
<211> LENGTH: 449
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11

Ser Ser Pro Ile Ser Lys Arg Cys Arg Thr Ser Ile Leu Ser Thr Ser
1               5                  10                  15

Gly Asp Phe Val Lys Gln Thr Val Pro Ser Glu Asn Ile Pro Leu Pro
20                  25                  30

Glu Cys Ser Ser Pro Pro Ser Cys Lys Arg Lys Val Gly Gly Thr Ser
35                  40                  45

Gly Arg Lys Asn Ser Asn Met Ser Asp Glu Phe Ile Ser Leu Ser Pro
50                  55                  60

Gly Thr Pro Pro Ser Thr Leu Ser Ser Ser Ser Tyr Arg Gln Val Met
65                  70                  75                  80

Ser Ser Pro Ser Ala Met Lys Leu Leu Pro Asn Met Ala Val Lys Arg
85                  90                  95

Asn His Arg Gly Glu Thr Leu Leu His Ile Ala Ser Ile Lys Gly Asp
100                 105                 110

Ile Pro Ser Val Glu Tyr Leu Leu Gln Asn Gly Ser Asp Pro Asn Val
115                 120                 125

Lys Asp His Ala Gly Trp Thr Pro Leu His Glu Ala Cys Asn His Gly
130                 135                 140

His Leu Lys Val Val Glu Leu Leu Leu Gln His Lys Ala Leu Val Asn
145                 150                 155                 160

Thr Thr Gly Tyr Gln Asn Asp Ser Pro Leu His Asp Ala Ala Lys Asn
165                 170                 175

Gly His Val Asp Ile Val Lys Leu Leu Leu Ser Tyr Gly Ala Ser Arg
180                 185                 190

Asn Ala Val Asn Ile Phe Gly Leu Arg Pro Val Asp Tyr Thr Asp Asp
195                 200                 205
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Glu Ser Met Lys Ser Leu Leu Leu Leu Pro Glu Lys Asn Glu Ser Ser
210                 215                 220

Ser Ala Ser His Cys Ser Val Met Asn Thr Gly Gln Arg Arg Asp Gly
225                 230                 235                 240

Pro Leu Val Leu Ile Gly Ser Gly Leu Ser Ser Glu Gln Gln Lys Met
245                 250                 255

Leu Ser Glu Leu Ala Val Ile Leu Lys Ala Lys Lys Tyr Thr Glu Phe
260                 265                 270

Asp Ser Thr Val Thr His Val Val Val Pro Gly Asp Ala Val Gln Ser
275                 280                 285

Thr Leu Lys Cys Met Leu Gly Ile Leu Asn Gly Cys Trp Ile Leu Lys
290                 295                 300

Phe Glu Trp Val Lys Ala Cys Leu Arg Arg Lys Val Cys Glu Gln Glu
305                 310                 315                 320

Glu Lys Tyr Glu Ile Pro Glu Gly Pro Arg Arg Ser Arg Leu Asn Arg
325                 330                 335

Glu Gln Leu Leu Pro Lys Leu Phe Asp Gly Cys Tyr Phe Tyr Leu Trp
340                 345                 350

Gly Thr Phe Lys His His Pro Lys Asp Asn Leu Ile Lys Leu Val Thr
355                 360                 365

Ala Gly Gly Gly Gln Ile Leu Ser Arg Lys Pro Lys Pro Asp Ser Asp
370                 375                 380

Val Thr Gln Thr Ile Asn Thr Val Ala Tyr His Ala Arg Pro Asp Ser
385                 390                 395                 400

Asp Gln Arg Phe Cys Thr Gln Tyr Ile Ile Tyr Glu Asp Leu Cys Asn
405                 410                 415

Tyr His Pro Glu Arg Val Arg Gln Gly Lys Val Trp Lys Ala Pro Ser
420                 425                 430

Ser Trp Phe Ile Asp Cys Val Met Ser Phe Glu Leu Leu Pro Leu Asp
435                 440                 445

Ser
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<210> SEQ ID NO 12
<211> LENGTH: 2530
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

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cgaaggcggg acgatgccgg ataatcggca gccgaggaac cggcagccga ggatccgctc    120

cgggaacgag cctcgttccg cgcccgccat ggaaccggat ggtcgcggtg cctgggccca    180

cagtcgcgcc gcgctcgacc gcctggagaa gctgctgcgc tgctcgcgtt gtactaacat    240

tctgagagag cctgtgtgtt taggaggatg tgagcacatc ttctgtagta attgtgtaag    300

tgactgcatt ggaactggat gtccagtgtg ttacaccccg gcctggatac aagacttgaa    360

gataaataga caactggaca gcatgattca actttgtagt aagcttcgaa atttgctaca    420

tgacaatgag ctgtcagatt tgaaagaaga taaacctagg aaaagtttgt ttaatgatgc    480

aggaaacaag aagaattcaa ttaaaatgtg gtttagccct cgaagtaaga aagtcagata    540

tgttgtgagt aaagcttcag tgcaaaccca gcctgcaata aaaaaagatg caagtgctca    600
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gcaagactca tatgaatttg tttccccaag tcctcctgca gatgtttctg agagggctaa 660

aaaggcttct gcaagatctg gaaaaaagca aaaaaagaaa actttagctg aaatcaacca 720

aaaatggaat ttagaggcag aaaaagaaga tggtgaattt gactccaaag aggaatctaa 780

gcaaaagctg gtatccttct gtagccaacc atctgttatc tccagtcctc agataaatgg 840

tgaaatagac ttactagcaa gtggctcctt gacagaatct gaatgttttg gaagtttaac 900

tgaagtctct ttaccattgg ctgagcaaat agagtctcca gacactaaga gcaggaatga 960

agtagtgact cctgagaagg tctgcaaaaa ttatcttaca tctaagaaat ctttgccatt 1020

agaaaataat ggaaaacgtg gccatcacaa tagactttcc agtcccattt ctaagagatg 1080

tagaaccagc attctgagca ccagtggaga ttttgttaag caaaccgtgc cctcagaaaa 1140

tataccattg cctgaatgtt cttcaccacc ttcatgcaaa cgtaaagttg gtggtacatc 1200

agggaggaaa aacagtaaca tgtccgatga attcattagt ctttcaccag gtacaccacc 1260

ttctacatta agtagttcaa gttacaggca agtgatgtct agtccctcag caatgaagct 1320

gttgcccaat atggctgtga aaagaaatca tagaggagag actttgctcc atattgcttc 1380

tattaagggc gacatacctt ctgttgaata ccttttacaa aatggaagtg atccaaatgt 1440

taaagaccat gctggatgga caccattgca tgaagcttgc aatcatgggc acctgaaggt 1500

agtggaatta ttgctccagc ataaggcatt ggtgaacacc accgggtatc aaaatgactc 1560

accacttcac gatgcagcca agaatgggca cgtggatata gtcaagctgt tactttccta 1620

tggagcctcc agaaatgctg ttaatatatt tggtctgcgg cctgtcgatt atacagatga 1680

tgaaagtatg aaatcgctat tgctgctacc agagaagaat gaatcatcct cagctagcca 1740

ctgctcagta atgaacactg ggcagcgtag ggatggacct cttgtactta taggcagtgg 1800

gctgtcttca gaacaacaga aaatgctcag tgagcttgca gtaattctta aggctaaaaa 1860

atatactgag tttgacagta cagtaactca tgttgttgtt cctggtgatg cagttcaaag 1920

taccttgaag tgtatgcttg ggattctcaa tggatgctgg attctaaaat ttgaatgggt 1980

aaaagcatgt ctacgaagaa aagtatgtga acaggaagaa aagtatgaaa ttcctgaagg 2040

tccacgcaga agcaggctca acagagaaca gctgttgcca aagctgtttg atggatgcta 2100

cttctatttg tggggaacct tcaaacacca tccaaaggac aaccttatta agctcgtcac 2160

tgcaggtggg ggccagatcc tcagtagaaa gcccaagcca gacagtgacg tgactcagac 2220

catcaataca gtcgcatacc atgcgagacc cgattctgat cagcgcttct gcacacagta 2280

tatcatctat gaagatttgt gtaattatca cccagagagg gttcggcagg gcaaagtctg 2340

gaaggctcct tcgagctggt ttatagactg tgtgatgtcc tttgagttgc ttcctcttga 2400

cagctgaata ttataccaga tgaacatttc aaattgaatt tgcacggttt gtgagagccc 2460

agtcattgta ctgtttttaa tgttcacatt tttacaaata ggtagagtca ttcatatttg 2520

tctttgaatc 2530

<210> SEQ ID NO 13
<211> LENGTH: 2473
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: /note="synthetic construct"

<400> SEQUENCE: 13

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cgaaggcggg acgatgccgg ataatcggca gccgaggaac cggcagccga ggatccgctc    120
cgggaacgag cctcgttccg cgcccgccat ggaaccggat ggtcgcggtg cctgggccca    180
cagtcgcgcc gcgctcgacc gcctggagaa gctgctgcgc tgctcgcgtt gtaattgtgt    240
aagtgactgc attggaactg gatgtccagt gtgttacacc ccggcctgga tacaagactt    300
gaagataaat agacaactgg acagcatgat tcaactttgt agtaagcttc gaaatttgct    360
acatgacaat gagctgtcag atttgaaaga agataaacct aggaaaagtt tgtttaatga    420
tgcaggaaac aagaagaatt caattaaaat gtggtttagc cctcgaagta agaaagtcag    480
atatgttgtg agtaaagctt cagtgcaaac ccagcctgca ataaaaaaag atgcaagtgc    540
tcagcaagac tcatatgaat ttgtttcccc aagtcctcct gcagatgttt ctgagagggc    600
taaaaaggct tctgcaagat ctggaaaaaa gcaaaaaaag aaaactttag ctgaaatcaa    660
ccaaaaatgg aatttagagg cagaaaaaga agatggtgaa tttgactcca aagaggaatc    720
taagcaaaag ctggtatcct tctgtagcca accatctgtt atctccagtc ctcagataaa    780
tggtgaaata gacttactag caagtggctc cttgacagaa tctgaatgtt ttggaagttt    840
aactgaagtc tctttaccat tggctgagca aatagagtct ccagacacta agagcaggaa    900
tgaagtagtg actcctgaga aggtctgcaa aaattatctt acatctaaga aatctttgcc    960
attagaaaat aatggaaaac gtggccatca caatagactt tccagtccca tttctaagag   1020
atgtagaacc agcattctga gcaccagtgg agattttgtt aagcaaaccg tgccctcaga   1080
aaatatacca ttgcctgaat gttcttcacc accttcatgc aaacgtaaag ttggtggtac   1140
atcagggagg aaaaacagta acatgtccga tgaattcatt agtctttcac caggtacacc   1200
accttctaca ttaagtagtt caagttacag gcaagtgatg tctagtccct cagcaatgaa   1260
gctgttgccc aatatggctg tgaaaagaaa tcatagagga gagactttgc tccatattgc   1320
ttctattaag ggcgacatac cttctgttga ataccttta caaaatggaa gtgatccaaa   1380
tgttaaagac catgctggat ggacaccatt gcatgaagct tgcaatcatg ggcacctgaa   1440
ggtagtggaa ttattgctcc agcataaggc attggtgaac accaccgggt atcaaaatga   1500
ctcaccactt cacgatgcag ccaagaatgg gcacgtggat atagtcaagc tgttactttc   1560
ctatggagcc tccagaaatg ctgttaatat atttggtctg cggcctgtcg attatacaga   1620
tgatgaaagt atgaaatcgc tattgctgct accagagaag aatgaatcat cctcagctag   1680
ccactgctca gtaatgaaca ctgggcagcg tagggatgga cctcttgtac ttataggcag   1740
tgggctgtct tcagaacaac agaaaatgct cagtgagctt gcagtaattc ttaaggctaa   1800
aaaatatact gagtttgaca gtacagtaac tcatgttgtt gttcctggtg atgcagttca   1860
aagtaccttg aagtgtatgc ttgggattct caatggatgc tggattctaa aatttgaatg   1920
ggtaaaagca tgtctacgaa gaaaagtatg tgaacaggaa gaaaagtatg aaattcctga   1980
aggtccacgc agaagcaggc tcaacagaga acagctgttg ccaaagctgt ttgatggatg   2040
ctacttctat ttgtggggaa ccttcaaaca ccatccaaag gacaacctta ttaagctcgt   2100
cactgcaggt gggggccaga tcctcagtag aaagcccaag ccagacagtg acgtgactca   2160
gaccatcaat acagtcgcat accatgcgag acccgattct gatcagcgct tctgcacaca   2220
gtatatcatc tatgaagatt tgtgtaatta tcacccagag agggttcggc agggcaaagt   2280
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ctggaaggct ccttcgagct ggtttataga ctgtgtgatg tcctttgagt tgcttcctct   2340

tgacagctga atattatacc agatgaacat ttcaaattga atttgcacgg tttgtgagag   2400

cccagtcatt gtactgtttt taatgttcac atttttacaa ataggtagag tcattcatat   2460

ttgtctttga atc                                                     2473


<210> SEQ ID NO 14
<211> LENGTH: 2324
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: /note="synthetic construct"

<400> SEQUENCE: 14

cagcttccct gtggtttccc gaggcttcct tgcttcccgc tctgcgagga gcctttcatc     60

cgaaggcggg acgatgccgg ataatcggca gccgaggaac cggcagccga ggatccgctc    120

cgggaacgag cctcgttccg cgcccgccat ggaaccggat ggtcgcggtg cctgggccca    180

cagtcgcgcc gcgctcgacc gcctggagaa gctgctgcgc tgctcgcgtt gatttgaaag    240

aagataaacc taggaaaagt ttgtttaatg atgcaggaaa caagaagaat tcaattaaaa    300

tgtggtttag ccctcgaagt aagaaagtca gatatgttgt gagtaaagct tcagtgcaaa    360

cccagcctgc aataaaaaaa gatgcaagtg ctcagcaaga ctcatatgaa tttgtttccc    420

caagtcctcc tgcagatgtt tctgagaggg ctaaaaaggc ttctgcaaga tctggaaaaa    480

agcaaaaaaa gaaaacttta gctgaaatca accaaaaatg gaatttagag gcagaaaaag    540

aagatggtga atttgactcc aaagaggaat ctaagcaaaa gctggtatcc ttctgtagcc    600

aaccatctgt tatctccagt cctcagataa atggtgaaat agacttacta gcaagtggct    660

ccttgacaga atctgaatgt tttggaagtt taactgaagt ctctttacca ttggctgagc    720

aaatagagtc tccagacact aagagcagga atgaagtagt gactcctgag aaggtctgca    780

aaaattatct tacatctaag aaatctttgc cattagaaaa taatggaaaa cgtggccatc    840

acaatagact ttccagtccc atttctaaga gatgtagaac cagcattctg agcaccagtg    900

gagattttgt taagcaaacc gtgccctcag aaaatatacc attgcctgaa tgttcttcac    960

caccttcatg caaacgtaaa gttggtggta catcagggag gaaaaacagt aacatgtccg   1020

atgaattcat tagtctttca ccaggtacac caccttctac attaagtagt tcaagttaca   1080

ggcaagtgat gtctagtccc tcagcaatga agctgttgcc caatatggct gtgaaaagaa   1140

atcatagagg agagactttg ctccatattg cttctattaa gggcgacata ccttctgttg   1200

aataccttt acaaaatgga agtgatccaa atgttaaaga ccatgctgga tggacaccat   1260

tgcatgaagc ttgcaatcat gggcacctga aggtagtgga attattgctc cagcataagg   1320

cattggtgaa caccaccggg tatcaaaatg actcaccact tcacgatgca gccaagaatg   1380

ggcacgtgga tatagtcaag ctgttacttt cctatggagc ctccagaaat gctgttaata   1440

tatttggtct gcggcctgtc gattatacag atgatgaaag tatgaaatcg ctattgctgc   1500

taccagagaa gaatgaatca tcctcagcta gccactgctc agtaatgaac actgggcagc   1560

gtagggatgg acctcttgta cttataggca gtgggctgtc ttcagaacaa cagaaaatgc   1620

tcagtgagct tgcagtaatt cttaaggcta aaaaatatac tgagtttgac agtacagtaa   1680

ctcatgttgt tgttcctggt gatgcagttc aaagtacctt gaagtgtatg cttgggattc   1740
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tcaatggatg ctggattcta aaatttgaat gggtaaaagc atgtctacga agaaaagtat 1800

gtgaacagga agaaaagtat gaaattcctg aaggtccacg cagaagcagg ctcaacagag 1860

aacagctgtt gccaaagctg tttgatggat gctacttcta tttgtgggga accttcaaac 1920

accatccaaa ggacaacctt attaagctcg tcactgcagg tgggggccag atcctcagta 1980

gaaagcccaa gccagacagt gacgtgactc agaccatcaa tacagtcgca taccatgcga 2040

gacccgattc tgatcagcgc ttctgcacac agtatatcat ctatgaagat ttgtgtaatt 2100

atcacccaga gagggttcgg cagggcaaag tctggaaggc tccttcgagc tggtttatag 2160

actgtgtgat gtcctttgag ttgcttcctc ttgacagctg aatattatac cagatgaaca 2220

tttcaaattg aatttgcacg gtttgtgaga gcccagtcat tgtactgttt ttaatgttca 2280

cattttaca aataggtaga gtcattcata tttgtctttg aatc 2324

<210> SEQ ID NO 15
<211> LENGTH: 1456
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: /note="synthetic construct"

<400> SEQUENCE: 15

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cgggaacgag cctcgttccg cgcccgccat ggaaccggat ggtcgcggtg cctgggccca 180

cagtcgcgcc gcgctcgacc gcctggagaa gctgctgcgc tgctcgcgtt gtactaacat 240

tctgagagag cctgtgtgtt taggaggatg tgagcacatc ttctgtagta attgtgtaag 300

tgactgcatt ggaactggat gtccagcgtg ttacaccccg gcctggatac aagacttgaa 360

gataaataga caactggaca gcatgattca actttgtagt aagcttcgaa atttgctaca 420

tgacaatgag ctgtcagggc gacatacctt ctgttgaata ccttttacaa aatggaagtg 480

atccaaatgt taaagaccat gctggatgga caccattgca tgaagcttgc aatcatgggc 540

acctggaggt agtggaatta ttgctccagc ataaggcatt ggtgaacacc accgggtatc 600

aaaatgactc accacttcac gatgcagcca agaatgggca catggatata gtcaagctgt 660

tactttccta tggagcctcc agaaatgctg ttaatatatt tggtctgcgg cctgtcgatt 720

atacagatga tgaaagtatg aaatcgctat tgctgctacc agagaagaat gaatcatcct 780

cagctagcca ctgctcagta atgaacactg ggcagcgtag ggatggacct cttgtactta 840

taggcagtgg gctgtcttca gaacaacaga aaatgctcag tgagcttgca gtaattctta 900

aggctaaaaa atatactgag tttgacagta cagtaactca tgttgttgtt cctggtgatg 960

cagttcaaag taccttgaag tgtatgcttg ggattctcaa tggatgctgg attctaaaat 1020

ttgaatgggt aaaagcatgt ctacgaagaa aagtatgtga acaggaagaa aagtatgaaa 1080

ttcctgaagg tccacgcaga agcaggctca acagagaaca gctgttgcca aagctgtttg 1140

atggatgcta cttctatttg tggggaacct tcaaacacca tccaaaggac aaccttatta 1200

agctcgtcac tgcaggtggg ggccagatcc tcagtagaaa gcccaagcca gacagtgacg 1260

tgactcagac catcaataca gtcgcatacc atgcgagacc cgattctgat cagcgcttct 1320

gcacacagta tatcatctat gaagatttgt gtaattatca cccagagagg gttcggcagg 1380

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gcaaagtctg gaaggctcct tcgagctggt ttatagactg tgtgatgtcc tttgagttgc 1440

ttcctcttga cagctg 1456

<210> SEQ ID NO 16
<211> LENGTH: 1010
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: /note="synthetic construct"

<400> SEQUENCE: 16

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gccgaggatc cgctccggga acgagcctcg ttccgcgtcc gccatggaac cggatggtcg 120

cggtgcctgg gcccacagtc gcgccgcgct cgaccgcctg gagaagctgc tgcgctgctc 180

gcgttgtact aacattctga gagagcctgt gtgtttagga ggatgtgagc acatcttctg 240

tagtaatata tttggtctgc ggcctgtcga ttatacagat gatgaaagta tgaaatcgct 300

attgctgcta ccagagaaga atgaatcatc ctcagctagc cactgctcag taatgaacac 360

tgggcagcgt agggatggac ctcttgtact tataggcagt gggctgtctt cagaacaaca 420

gaaaatgctc agtgagcttg cagtaattct taaggctaaa aaatatactg agtttgacag 480

tacagtaact catgttgttg ttcctggtga tgcagttcaa agtaccttga agtgtatgct 540

tgggattctc aatggatgct ggattctaaa atttgaatgg gtaaaagcat gtctacgaag 600

aaaagtatgt gaacaggaag aaaagtatga aattcctgaa ggtccacgca gaagcaggct 660

caacagagaa cagctgttgc caaagctgtt tgatggatgc tacttctatt tgtggggaac 720

cttcaaacac catccaaagg acaaccttat taagctcgtc actgcaggtg ggggccagat 780

cctcagtaga aagcccaagc cagacagtga cgtgactcag accatcaata cagtcgcata 840

ccatgcgaga cccgattctg atcagcgctt ctgcacacag tatatcatct atgaagattt 900

gtgtaattat cacccagaga gggttcggca gggcaaagtc tggaaggctc cttcgagctg 960

gtttatagac tgtgtgatgt cctttgagtt gcttcctctt gacagctgaa 1010

<210> SEQ ID NO 17
<211> LENGTH: 780
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 17

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cggtgcctgg gcccacagtc gcgccgcgct cgaccgcctg gagaagctgc tgcgctgctc 180

gcgttgtact aacattctga gagagcctgt gtgtttagga ggatgtgagc acatcttctg 240

tagtaattgt gtaagtgact gcattggaac tggatgtcca gtgtgttaca ccccggcctg 300

gatacaagac ttgaagataa atagacaact ggacagcatg attcaacttt gtagtaagct 360

tcgaaatttg ctacatgaca atgagctgtc aggggtaaaa gcatgtctac gaagaaaagt 420

atgtgaacag gaagaaaagt atgaaattcc tgaaggtcca cgcagaagca ggctcaacag 480

agaacagctg ttgccaaagc tgtttgatgg atgctacttc tatttgtggg gaaccttcaa 540

acaccatcca aaggacaacc ttattaagct cgtcactgca ggtgggggcc agatcctcag 600

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gagacccgat tctgatcagc gcttctgcac acagtatatc atctatgaag atttgtgtaa 720

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<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
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gataatcggc agccgaggaa ccggcagccg aggatccgct ccgggaacga gcctcgttcc 180

gcgcccgcca tggaaccgga tggtcgcggt gcctgggccc acagtcgcgc cgcgctcgac 240

cgcctggaga agctgctgcg ctgctcgcgt tggggtaaaa gcatgtctac gaagaaaagt 300

atgtgaacag gaagaaaagt atgaaattcc tgaaggtcca cgcagaagca ggctcaacag 360

agaacagctg ttgccaaagc tgtttgatgg atgctacttc tatttgtggg gaaccttcga 420

acaccatcca aaggacaacc ttattaagct cgtcactgca ggtgggggcc agatcctcag 480

tagaaagccc aagccagaca gtgacgtgac tcagaccatc aatacagtcg cataccatgc 540

gagacccgat tctgatcagc gcttctgcac acagtatatc atctatgaag atttgtgtaa 600

ttatcaccca gagagggttc ggcagggcaa agtctggaag gctccttcga gctggtttat 660

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<210> SEQ ID NO 19
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<212> TYPE: DNA
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aaatcaacca aaaatggaat ttagaggcag aaaaagaaga tggtgaattt gactccaaag 240

aggaatctaa gcaaaagctg gtatccttct gtagccaacc atctgttatc tccagtcctc 300

agataaatgg tgaaatagac ttactagcaa gtggctcctt gacagaatct gaatgttttg 360

gaagtttaac tgaagtctct ttaccattgg ctgagcaaat agagtctcca gacactaaga 420

gcaggaatga agtagtgact cctgagaagg tctgcaaaaa ttatcttaca tctaagaaat 480

ctttgccatt agaaaataat ggaaaacgtg gccatcacaa tagactttcc agtcccattt 540

ctaagagatg tagaaccagc attctgagca ccagtggaga ttttgttaag caaaccgtgc 600

cctcagaaaa tataccattg cctgaatgtt cttcaccacc ttcatgcaaa cgtaaagttg 660

gtggtacatc agggaggaaa aacagtaaca tgtccgatga attcattagt ctttcaccag 720

gtacaccacc ttctacatta agtagttcaa gttacaggca agtgatgtct agtccctcag 780

caatgaagct gttgcccaat atggctgtga aaagaaatca tagaggagag actttgctcc 840

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tactttccta tggagcctcc agaaatgctg ttaatatatt tggtctgcgg cctgtcgatt 1140

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cagctagcca ctgctcagta atgaacactg ggcagcgtag ggatggacct cttgtactta 1260

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atggatgcta cttctatttg tggggaacct tcaaacacca tccaaaggac aaccttatta 1620

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tgactcagac catcaataca gtcgcatacc atgcgagacc cgattctgat cagcgcttct 1740

gcacacagta tatcatctat gaagatttgt gtaattatca cccagagagg gttcggcagg 1800

gcaaagtctg gaaggctcct tcgagctggt ttatagactg tgtgatgtcc tttgagttgc 1860

ttcctcttga cagctgaata ttataccaga tgaacatttc aaattgaatt tgcacggttt 1920

gtgagagccc agtcattgta ctgtttttaa tgttcacatt tttacaaata ggtagagtca 1980

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<211> LENGTH: 1475
<212> TYPE: DNA
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<400> SEQUENCE: 20

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gcaaacgtaa agttggtggt acatcaggga ggaaaaacag taacatgtcc gatgaattca 180

ttagtctttc accaggtaca ccaccttcta cattaagtag ttcaagttac aggcaagtga 240

tgtctagtcc ctcagcaatg aagctgttgc ccaatatggc tgtgaaaaga aatcatagag 300

gagagacttt gctccatatt gcttctatta agggcgacat accttctgtt gaataccttt 360

tacaaaatgg aagtgatcca aatgttaaag accatgctgg atggacacca ttgcatgaag 420

cttgcaatca tgggcacctg aaggtagtgg aattattgct ccagcataag gcattggtga 480

acaccaccgg gtatcaaaat gactcaccac ttcacgatgc agccaagaat gggcacgtgg 540

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tgcggcctgt cgattataca gatgatgaaa gtatgaaatc gctattgctg ctaccagaga 660

agaatgaatc atcctcagct agccactgct cagtaatgaa cactgggcag cgtagggatg 720

gacctcttgt acttataggc agtgggctgt cttcagaaca acagaaaatg ctcagtgagc 780

ttgcagtaat tcttaaggct aaaaaatata ctgagtttga cagtacagta actcatgttg 840

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aagaaaagta tgaaattcct gaaggtccac gcagaagcag gctcaacaga gaacagctgt 1020

tgccaaagct gtttgatgga tgctacttct atttgtgggg aaccttcaaa caccatccaa 1080

aggacaacct tattaagctc gtcactgcag gtgggggcca gatcctcagt agaaagccca 1140

agccagacag tgacgtgact cagaccatca atacagtcgc ataccatgcg agacccgatt 1200

ctgatcagcg cttctgcaca cagtatatca tctatgaaga tttgtgtaat tatcacccag 1260

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tgtcctttga gttgcttcct cttgacagct gaatattata ccagatgaac atttcaaatt 1380

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<210> SEQ ID NO 21
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: /note="synthetic construct"

<400> SEQUENCE: 21

Met Val Ala Val Pro Gly Pro Thr Val Ala Pro Arg Ser Thr Ala Trp
1 5 10 15

Arg Ser Cys Cys Ala Ala Arg Val
20

<210> SEQ ID NO 22
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: /note="synthetic construct"

<400> SEQUENCE: 22

Gly Arg His Thr Phe Cys
1 5

<210> SEQ ID NO 23
<211> LENGTH: 1168
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 23

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tcatgggcac ctgaaggtag tggaattatt gctccagcat aaggcattgg tgaacaccac 180

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tgtacttata ggcagtgggc tgtcttcaga acaacagaaa atgctcagtg agcttgcagt 480

aattcttaag gctaaaaaat atactgagtt tgacagtaca gtaactcatg ttgttgttcc 540

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gctgtttgat ggatgctact tctatttgtg gggaaccttc aaacaccatc caaaggacaa 780

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gcgcttctgc acacagtata tcatctatga agatttgtgt aattatcacc cagagagggt 960

tcggcagggc aaagtctgga aggctccttc gagctggttt atagactgtg tgatgtcctt 1020

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<210> SEQ ID NO 24
<211> LENGTH: 347
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 24

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1 5 10 15

Leu Leu Gln Asn Gly Ser Asp Pro Asn Val Lys Asp His Ala Gly Trp
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Thr Pro Leu His Glu Ala Cys Asn His Gly His Leu Lys Val Val Glu
35 40 45

Leu Leu Leu Gln His Lys Ala Leu Val Asn Thr Thr Gly Tyr Gln Asn
50 55 60

Asp Ser Pro Leu His Asp Ala Ala Lys Asn Gly His Val Asp Ile Val
65 70 75 80

Lys Leu Leu Leu Ser Tyr Gly Ala Ser Arg Asn Ala Val Asn Ile Phe
85 90 95

Gly Leu Arg Pro Val Asp Tyr Thr Asp Asp Glu Ser Met Lys Ser Leu
100 105 110

Leu Leu Leu Pro Glu Lys Asn Glu Ser Ser Ser Ala Ser His Cys Ser
115 120 125

Val Met Asn Thr Gly Gln Arg Arg Asp Gly Pro Leu Val Leu Ile Gly
130 135 140

Ser Gly Leu Ser Ser Glu Gln Gln Lys Met Leu Ser Glu Leu Ala Val
145 150 155 160

Ile Leu Lys Ala Lys Lys Tyr Thr Glu Phe Asp Ser Thr Val Thr His
165 170 175

Val Val Val Pro Gly Asp Ala Val Gln Ser Thr Leu Lys Cys Met Leu
180 185 190

Gly Ile Leu Asn Gly Cys Trp Ile Leu Lys Phe Glu Trp Val Lys Ala
195 200 205

Cys Leu Arg Arg Lys Val Cys Glu Gln Glu Glu Lys Tyr Glu Ile Pro
210 215 220

Glu Gly Pro Arg Arg Ser Arg Leu Asn Arg Glu Gln Leu Leu Pro Lys
225 230 235 240

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Leu Phe Asp Gly Cys Tyr Phe Tyr Leu Trp Gly Thr Phe Lys His His
245 250 255

Pro Lys Asp Asn Leu Ile Lys Leu Val Thr Ala Gly Gly Gly Gln Ile
260 265 270

Leu Ser Arg Lys Pro Lys Pro Asp Ser Asp Val Thr Gln Thr Ile Asn
275 280 285

Thr Val Ala Tyr His Ala Arg Pro Asp Ser Asp Gln Arg Phe Cys Thr
290 295 300

Gln Tyr Ile Ile Tyr Glu Asp Leu Cys Asn Tyr His Pro Glu Arg Val
305 310 315 320

Arg Gln Gly Lys Val Trp Lys Ala Pro Ser Ser Trp Phe Ile Asp Cys
325 330 335

Val Met Ser Phe Glu Leu Leu Pro Leu Asp Ser
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<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
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<400> SEQUENCE: 25

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Lys Lys Asn Ser Ile Lys Met Trp Phe Ser Pro Arg Ser Lys
20 25 30

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<212> TYPE: DNA
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<400> SEQUENCE: 26

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<210> SEQ ID NO 27
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: /note="synthetic construct"

<400> SEQUENCE: 27

gttgccaaag ctgtttg 17

<210> SEQ ID NO 28
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<212> TYPE: DNA
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<400> SEQUENCE: 28

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<210> SEQ ID NO 29
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<212> TYPE: DNA
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<210> SEQ ID NO 30
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<223> OTHER INFORMATION: /note="synthetic construct"

<400> SEQUENCE: 30

Gly Leu Arg Pro Val Asp Tyr Thr Asp Asp Glu Ser Met Lys Ser Leu
1 5 10 15

Leu Leu

<210> SEQ ID NO 31
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<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
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<400> SEQUENCE: 31

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<212> TYPE: DNA
<213> ORGANISM: Artificial
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<400> SEQUENCE: 32

cagctgtcaa gaggaagcaa c 21

<210> SEQ ID NO 33
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<220> FEATURE:
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<400> SEQUENCE: 33

gtgactgcat tggaactgga 20

<210> SEQ ID NO 34
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<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
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<400> SEQUENCE: 34

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<210> SEQ ID NO 35
<211> LENGTH: 24
<212> TYPE: DNA

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<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: /note="synthetic construct"

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<210> SEQ ID NO 36
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<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: /note="synthetic construct"

<400> SEQUENCE: 36

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<210> SEQ ID NO 37
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<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: /note="synthetic construct"

<400> SEQUENCE: 37

gctggatgga caccattg 18

<210> SEQ ID NO 38
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<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: /note="synthetic construct"

<400> SEQUENCE: 38

ctccagcata aggcattggt 20

1-57. (canceled)

58. A method for detecting the presence of gynaecological cancer related proteins in a biological sample, comprising the steps of:

a) Determining one or more of the following in a sample from a female mammal:

(i) The expression level of a protein of SEQ ID NO: 1 through a detectable signal proportional to the said level of expression;

(iia) The expression level of at least one protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7; SEQ ID NO: 8 and SEQ ID NO: 9 through a detectable signal proportional to the said level of expression; and/or

(iib) The expression and/or expression level of at least one protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 10; SEQ ID NO: 11 and SEQ ID NO: 24 through a detectable signal proportional to the said level of expression; and

b) Detecting a signal indicative of a ratio lower than a 1:1 ratio between the expression level obtained under step (i) and the expression level obtained under steps (iia) and/or (iib); or detecting a signal indicative of the expression/expression level determined under step (iib).

59. The method according to claim **58**, comprising the steps of:

a) Determining one or more of the following in a sample from a female mammal:

(i) The expression level of a protein of SEQ ID NO: 1 through a detectable signal proportional to the said level of expression; and/or

(iia) The expression level of at least one protein of an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7; SEQ ID NO: 8 and SEQ ID NO: 9 through a detectable signal proportional to the said level of expression; and

b) Detecting a signal indicative of a ratio lower than a 1:1 ratio between the expression level obtained under step (i) and the expression level obtained under step (iia).

60. The method according to claim **58** comprising the steps of:

a) Determining one or more of the following in a sample from a female mammal:

(i) The expression level of a protein of SEQ ID NO: 1 through a detectable signal proportional to the said level of expression; and

(iib) The expression and/or expression level of at least one protein of an amino acid sequence selected from the group consisting of SEQ ID NO: 10; SEQ ID NO: 11 and SEQ ID NO: 24 through a detectable signal proportional to the said level of expression; and

b) Detecting a signal indicative of the expression/expression level obtained under step (iib).

61. The method according to claim **60**, wherein the signal obtained under the detection step (c) is indicative of a gynaecological cancer or a risk of developing a future gynaecological cancer in the subject.

62. The method according to claim **58**, further comprising comparing the expression levels obtained under step (i) with the expression level obtained under steps (iia) and/or (iib).

63. The method according to claim **59**, further comprising comparing the expression levels obtained under step (i) with the expression level obtained under steps (iia) and/or (iib).

64. The method according to claim **58**, wherein the biological sample is blood.

65. A method for detecting the presence of gynaecological cancer related proteins in a biological sample, comprising the steps of:

a) Reacting a sample from a female mammal with at least one antibody, a fragment thereof or a combination thereof, which is specific to a protein of SEQ ID NO: 1;

b) Reacting the said sample with at least one antibody, fragment thereof, or a combination thereof, which is specific to at least one protein comprising an amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7; SEQ ID NO: 8 and SEQ ID NO: 9;

c) Reacting the said sample with at least one antibody, a fragment thereof or a combination thereof, which is specific to at least one protein comprising an amino acid sequence selected from SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 24;

d) Detecting (a) a protein of SEQ ID NO: 1; and (b) a protein comprising an amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7; SEQ ID NO: 8 and SEQ ID NO: 9; and/or (c) a protein comprising an amino acid sequence selected from SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 24; wherein the detection is achieved through the detection of the interaction of each said antibody, fragment thereof or combination thereof, used under steps (i) and (ii) and/or (iii) with the corresponding said at least one protein, wherein the presence of the interaction correlates with the concentration of the protein in the biological sample; and

e) Detecting a signal indicative of a ratio lower than a 1:1 ratio between the said interaction detection signal obtained under step (iv) for a protein of SEQ ID NO: 1 and the said interaction detection signal obtained under step (iv) for either a protein comprising an amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7; SEQ ID NO: 8 and SEQ ID NO: 9; or for a protein comprising an amino acid sequence selected from SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 24; or detecting a signal indicative of an interaction signal detected under step (iv) for a protein comprising an amino acid sequence selected from SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 24.

66. The method according to claim **65**, wherein the signal detected under step (v) indicative of a ratio lower than a 1:1 ratio between the said interaction detection signal obtained under step (iv) for a protein of SEQ ID NO: 1 and the said interaction detection signal obtained under step (iv) for a protein comprising an amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7; SEQ ID NO: 8 and SEQ ID NO: 9, is indicative of a gynaecological cancer.

67. The method according to claim **65**, wherein the signal detected under step (v) indicative for a protein comprising an amino acid sequence selected from SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 24, is indicative of a gynaecological cancer or a risk of developing a future gynaecological cancer in the subject.

68. The method according to claim **65**, wherein the antibodies used under step (ii) is a combination of antibodies wherein the combination comprises (a) at least one antibody against exon 1 from full length BARD1 (SEQ ID NO: 12); (b) at least one antibody against one exon selected from exon 4, exon 5, exon 6, exon 7, exon 8 and exon 9 from full length BARD1 (SEQ ID NO: 12); and (c) at least one antibody against exon 10 and/or exon 11 from full length BARD 1 (SEQ ID NO: 12).

69. An isolated polypeptide comprising at least one sequence of amino acids having at least 80% identity or homology with a sequence comprising SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 24.

70. An isolated nucleic acid consisting of a nucleotide sequence encoding a polypeptide according to claim **69**.

71. An isolated nucleic acid according to claim **70**, said nucleic acid selected from the group consisting of SEQ ID NO: 13; SEQ ID NO: 14; SEQ ID NO: 15; SEQ ID NO: 16; SEQ ID NO: 17; SEQ ID NO: 18, SEQ ID NO: 19; SEQ ID NO: 20 and SEQ ID NO: 23.

72. A method of expressing a polypeptide comprising culturing a recombinant host cell expressing a nucleic acid according to claim **70** under conditions that allow for the expression of a polypeptide.

73. A probe or primer that hybridizes with a nucleic acid according to claim **70**.

74. An isolated antibody that selectively binds at least one polypeptide according to claim **69**.

75. A combination of antibodies comprising (a) at least one antibody against exon 1 from full length BARD 1 (SEQ ID NO: 12); (b) at least one antibody against one exon selected from exon 4, exon 5, exon 6, exon 7, exon 8 and exon 9 from full length BARD 1 (SEQ ID NO: 12); and (c) at least one antibody against exon 10 and/or exon 11 from full length BARD 1 (SEQ ID NO: 12).

76. A method for detecting the level of cellular expression of proteins of comprising the step of:

(i) Contacting at least one antibody according to claim **74** or a fragment thereof, or a combination of antibodies according to claim **75** with cells to be tested under appropriate conditions for binding of the said antibodies, combination thereof or combination of antibodies to at least a protein comprising SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 24 on the cells;

(ii) Determining the level of binding of said at least one antibody, combination thereof, or combination of antibodies to the cell as indicative level of expression of the said protein.

77. A recombinant expression vector comprising a nucleic acid molecule according to claim **70**, wherein the vector optionally comprises an expression control sequence, allowing expression in prokaryotic or eukaryotic host cells of the encoded polypeptide, operably linked to the nucleic acid molecule.

78. An immunoassay kit for detecting gynaecological cancer in a biological sample, the kit comprising at least one antibody according to claim **74** or a fragment thereof or a combination of antibodies according to claim **75**.

79. A method of treating a disease comprising the administration of a therapeutically effective amount of an antagonist of a polypeptide according to claim **69** in a mammal in need thereof; wherein the disease is a gynaecological cancer, including breast, ovarian, cervical and uterine cancers.

80. The method according to claim **79**, wherein the antagonist is a SiRNA and whereby tumor growth is arrested.

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专利名称(译)	带有BARD1同种型的缺失及其用途		
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摘要(译)

本发明涉及新蛋白质同种型，其用途，其制备方法，其检测方法，其抗体，其抗体组合，这些抗体的用途及其组合，以及这些同种型的拮抗剂在治疗妇科癌症中的用途。