Oct-4B isoform is differentially expressed in breast cancer cells: hypermethylation of regulatory elements of Oct-4A suggests an alternative promoter and transcriptional start site for Oct-4B transcription

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Synopsis
The human Oct-4 gene has three isoforms, Oct-4A, Oct-4B and Oct-4B1, which are thought to be derived from alternative splicing. It remains controversial whether the Oct-4 gene is expressed in cancer cells. Expression of Oct-4A is regulated by two elements, the PE (proximal enhancer) and DE (distal enhancer), but the expression and regulation of Oct-4B are not well known. Here, we firstly report that Oct-4B is expressed at low levels in MCF-7 cells, while the Oct-4A gene is inactivated. By analysing the function of different promoter constructs and the DNA methylation status of three regulatory regions, we demonstrate that the Oct-4A gene in MCF-7 cells is repressed by epigenetic control rather than transcriptional control. In addition, we speculate that the transcription of Oct-4B in MCF-7 cells is differentially regulated by additional regulatory elements. This work will enhance the understanding of Oct-4 gene in differential regulation.

Key words: alternative transcription, cis-regulatory element, DNA methylation, epigenetics, MCF-7 cells, Oct-4

INTRODUCTION
The transcription factor Oct-4 is specifically expressed in ICM (inner cell mass) and PGCs (primordial germ cells), playing a critical role in early embryonic development [1]. In ES cells (embryonic stem cells), Oct-4 is considered to be a key factor in maintaining the pluripotency of stem cells [1,2]. Human Oct-4 has three isoforms, Oct-4A (traditional Oct-4) [3], Oct-4B [3] and a novel isoform, Oct-4B1 [4]. These isoforms share the same gene locus and are thought to be generated from alternative splicing [3,4]. The transcriptional regulatory elements of Oct-4A consist of a TATA-less minimal PP (proximal promoter) and two upstream enhancer elements, the PE (proximal enhancer) and DE (distal enhancer) [5]. Several transcriptional activators and repressors have been shown to bind to these regulatory elements and regulate Oct-4 expression [6–9].

DNA methylation is another important mechanism controlling gene expression. Methylation of the regulatory regions of the Oct-4 gene has been linked to transcriptional silencing of Oct-4 during ES and EC cell (embryonal carcinoma cell) differentiation as well as during embryonic development [10–12]. Silencing of Oct-4 has been implicated in gestational trophoblastic disease [13]. In cancer cells, methylation of the Oct-4 gene has not been well studied [14]. The DE is reported to be hypermethylated in MCF-7 cells [14], but it is unknown whether other regulatory regions, such as the PE and ExonI, are fully methylated in MCF-7 cells.

Oct-4A and Oct-4B are composed of five exons and four exons respectively which encode 360 and 265 amino acids respectively. They are identical in approx. 850 bp of 3′-terminal mRNA and 225 C-terminal amino acids [3,15]. The novel isoform, Oct-4B1, shares the same mRNA initiation sequence with Oct-4B, but encodes a potentially truncated 115-amino acid peptide [4,16].
In addition to these isoforms, there are at least six pseudogenes of the Oct-4 gene. These pseudogenes often account for the false identification of Oct-4 gene expression, especially in MCF-7 cells [15,17–20]. To date, Oct-4B gene expression and the mechanisms of its regulation have not been investigated.

To understand the role of the Oct-4 gene in influencing cellular function, it is necessary to explore the expression pattern of different isoforms of Oct-4 in cancer cells. In this report, we confirm that the Oct-4B isoform is expressed in the breast cancer cell line, MCF-7, but not in breast adenofibroma (benign tumour) or ES cells. In addition, we demonstrate that the CpG dinucleotides in the DE, PE and ExonI of the Oct-4 gene are hypermethylated in the breast cancer cells, MCF-7 cells. We propose that the Oct-4B isoform is activated in MCF-7 cells by distinct regulatory elements and an alternative TSS (transcriptional start site).

**MATERIALS AND METHODS**

**Cell culture**

Four cell lines were used in the present study. The ABE-YJ cells (epithelial cells from adenofibroma tissue) and nME cells (normal mammary epithelial cells) were separated from primary breast adenofibroma (benign tumour) and adjacent normal tissue samples respectively. ABE-YJ and nME cells were cultured in DMEM-F12 (Gibco) supplemented with 10% fetal calf serum (Hyclone), 100 units/ml of penicillin, 100 μg/ml of streptomycin solution (Hyclone), 5 μg/ml of insulin (Sigma), 10 ng/ml of epidermal growth factor (Sigma) and 0.5 μg/ml of hydrocortisone (Sigma). The MCF-7 cell line was cultured in DMEM (Dulbecco’s modified Eagle’s medium; Gibco) supplemented with 10% fetal calf serum (Hyclone), 100 units/ml of penicillin, and 100 μg/ml of streptomycin solution (Hyclone). MCF-7 and hES-H1 cells were both acquired from the Stem Cell Research Center of Peking University. The identification of Oct-4 gene expression, especially in MCF-7 cells was confirmed with QIAprep Spin Miniprep kit (Qiagen). Individual clones were sequenced. Clones with at least 90% cytosine was then synthesized using the Superscript™ first-strand synthesis system (Invitrogen). A pair of primers, pO1/pO2, was used to confirm the transcription of the Oct-4 gene. Two specific primers, p4a-1/pO2 and p4b-1/pO2, were utilized to detect Oct-4A and Oct-4B mRNA. These primers are listed in Supplemental Table S1(A) at http://www.bioscirep.org/bsr/031/bsr0310109add.htm. The PCR products were sub-cloned into the pMD-18T vector (TakaRa) and ten clones from the pO1/pO2 product and six clones from the p4b-1/pO2 product were sequenced.

**Transfection and reporter assays**

Transient transfections were performed using the FuGENETM HD system. MCF-7 cells were grown to 40–50% confluence in six-well culture dishes and a 3:1 ratio of Transfection Reagent (6 μl) to DNA (2 μg) was used. After 48 h, cells were examined and photographed using a Confocal Laser Scanning Microscope (Nikon, OLS3000).

**Immunocytochemistry**

Immunocytochemistry for MCF-7, ABE-YJ and nME cells was carried out using standard techniques [14]. The polyclonal anti-human Oct-4 antibody (bs-830, Bios, Beijing, China; http://www.bios.com.cn) used in this assay binds to the homologous domain of Oct-4 and therefore recognizes both Oct-4A and Oct-4B. PI (propidium iodide) was used to stain the nucleus of cells. The fluorescent images were photographed using a Confocal Laser Scanning Microscope (Nikon, OLS3000).

**Western blotting analysis**

The protein extracts of cultured cells were measured by the BCA (bicinchoninic acid) protein assay kit (Pierce). Western blotting was performed using routine methodology. About 50 μg of total protein from each sample was separated on a 12% SDS-polyacrylamide gel. A mAb (monoclonal antibody) (MAB4401, Chemicon) was used to detect Oct-4A, and a polyclonal antibody (P100956_T100, A VIV A) was used to detect both Oct-4A and Oct-4B. HRP (horseradish peroxidase)-conjugated anti-goat and anti-mouse IgG (Santa Cruz) were used as secondary antibodies respectively. An ECL (enhanced chemiluminescence) Western blotting kit (Pierce) was used for chemoluminescence detection.

**BS-PCR (bisulfite sequencing–PCR) analysis**

BS-PCR was performed as previously described [12–14]. The primers and annealing temperatures are indicated in Supplementary Table S1(B). PCR products were sub-cloned into the pMD-19T vector (Takara), and recombinant plasmids were purified with QIAprep Spin Miniprep kit (Qiagen). Individual clones were subsequently sequenced. Clones with at least 90% cytosine conversion were accepted, and all possible clonalities were excluded based on the criteria from the BiQ Analyzer software (http://www.mpg.de).
Oct-4B isoform expressed in breast cancer cells

RESULTS

The DE and PE can function independently in driving GFP expression in MCF-7 cells
To examine whether Oct-4 is expressed in MCF-7 cells, and to determine the expression pattern of the gene, a GFP reporter under control of the 3.5 kb regulatory region upstream of the bovine Oct-4 gene (pboct4(p)-EGFP) (Figure 1A) was introduced into cells and GFP expression examined using confocal microscopy. The pboct4(p)-EGFP construct has been shown to direct GFP gene expression specifically in the ICM in blastocysts of mouse, pig, and rabbit embryos, similarly to endogenous Oct-4 gene expression [21]. Low but significant levels of GFP expression were detected in the transfected cells (Figure 2A), indicating that MCF-7 cells have the factors, such as transcriptional activators and co-activators, to direct exogenous Oct-4 expression.

Next, to identify the elements that are required for Oct-4 expression in MCF-7 cells, the vectors that had deletions in the PE (Figure 1B), DE (Figure 1C) and both (Figure 1D) were examined for GFP expression. Similarly to the parental pboct4(p)-EGFP construct, deletions in either the PE or DE alone displayed low-level expression of the Oct-4 gene (Figures 2B and 2C). In addition, no fluorescence signal was observed in pbEGFP-transfected cells (Figure 2D). These data suggest that the DE and PE play redundant roles in Oct-4 expression.

Figure 1 The schematic representation of the different constructs
(A) The vector pboct4(p)-EGFP contained a 3.5 kb regulatory region upstream of the bovine Oct-4 to drive GFP expression, including PP, DE and PE. (B) The vector pboct4(de)-EGFP deleted the PE, but contained DE and PP. (C) The vector pboct4(pe)-EGFP deleted the DE, but contained PE and PP. (D) The vector pbEGFP deleted both DE and PE, and contained PP only.

Figure 2 The function of different constructs of bOct-4 upstream cis elements in MCF-7 cells
MCF-7 cells were (A) transfected with pboct4(p)-EGFP(vector). (B) Transfected with pboct4(de)-EGFP(vector). (C) Transfected with pboct4(pe)-EGFP(vector). (D) Transfected with pbEGFP(vector). Phase–contrast (panel 1), fluorescence (panel 3) and co-localization views (panel 2) are shown. Scale bars: 60.00 μm.

Figure 3 RT–PCR analysis of Oct-4 expression in hES-H1, MCF-7, ABE-YJ and nME cells
NT is the no-template control, RT is the No-RT negative control of MCF-7 cells. (A) pO1 and pO2 were used to detect Oct-4 isoforms. The specific band was amplified in hES-H1 and MCF-7 cells. (B) p4a-1 and pO2 were used to detect Oct-4A, and the specific amplified band was seen in hES-H1 cells only. (C) p4b-1 and pO2 were used to detect Oct-4B. The amplified band was restricted to MCF-7 cells. (D) β-Actin was used as a positive control.

Endogenous Oct-4B is transcribed in MCF-7 cells
To explore whether or not the endogenous Oct-4 gene is actually transcribed in MCF-7 cells, a pair of primers, pO1/pO2, was designed to recognize Oct-4A, Oct-4B and Oct-4 pseudogene mRNA. The primers generate a 393 bp RT–PCR product. Analysis of the RT–PCR product showed a specific band for Oct-4 that was present in both hES-H1 and MCF-7 cells (Figure 3A).
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Figure 4 Analysis of Oct-4 expression by immunofluorescence in cell lines

MCF-7 cells (A–C) show cytoplasmic staining of Oct-4 (in green). ABE-YJ (D–F) and nME (G–I) cells show negative staining. The negative control of MCF-7 cells without primary antibody (J–L) does not show non-specific bindings. (C), (F), (I) and (K) show Oct-4 co-localization with PI red nuclear stain. (B), (E), (H) and (L) show PI nucleus staining. Scale bars: (A–C) and (J–L), 10.00 μm, (D–F) and (G–I), 20.00 μm.

The RT–PCR products were then cloned and sequenced. The results showed that the sequences from ten recombinants were entirely the same, and were identical with Oct-4A (GenBank® accession number NM_002701) and Oct-4B (GenBank® accession number NM_203289) mRNAs, but not to the pseudogene sequences as assessed by sequence alignment (Supplementary Figure S1 at http://www.bioscirep.org/bsr/031/bsr0310109add.htm). This result suggests that both the Oct-4A and Oct-4B isoforms are transcribed in MCF-7 cells.

To further address which isoform was expressed in MCF-7 cells, the primers p4a-1/pO2 (specific for Oct-4A) and p4b-1/pO2 (specific for Oct-4B) were used to detect Oct-4A and Oct-4B respectively. As expected, Oct-4A mRNA could only be detected in hES-H1 cells (Figure 3B), while Oct-4B was restricted to MCF-7 cells (Figure 3C). The p4b-1/pO2 PCR products were identified by sequencing (Supplementary Figure S2 at http://www.bioscirep.org/bsr/031/bsr0310109add.htm).

Oct-4B protein is localized to the cytosol and expressed at a low level in MCF-7 cells

To explore the expression of Oct-4B at the protein level, immunocytochemistry was performed in MCF-7 cells, as well as in human breast adenofibroma tissue cells (ABE-YJ cells) and nME cells. As shown, Oct-4B was detected in the cytoplasm of MCF-7 cells and did not overlap with PI staining, which identifies the nucleus of cells (Figures 4A–4C). In contrast, Oct-4B was not detected in either ABE-YJ or nME cells (Figures 4D and 4G). The data reveal that Oct-4B specifically localizes to the cytoplasm of MCF-7 cells.

Western-blot analysis was also performed using two Oct-4 antibodies. The mAb (MAB4401) specifically recognizes Oct-4A, while the polyclonal antibody (P100956_T100) recognizes both Oct-4A and Oct-4B. The results showed a band for Oct-4A at ~45 kDa, and the protein was specifically detected by the mAb in hES-H1 but not in MCF-7 cells (Figure 5A). However, a weak ~35 kDa band corresponding to the Oct-4B polypeptide was detected only in MCF-7 cells by the polyclonal antibody (Figure 5C). Neither Oct-4A nor Oct-4B was detected in ABE-YJ or nME cells (Figure 5).

The 5' regulatory region of the Oct-4 gene in MCF-7 cells exhibits high DNA methylation

To understand the relationship between the differential expression pattern of Oct-4 isoforms and DNA methylation, and to identify
which cis element plays the crucial role in regulating Oct-4B expression, BS analysis was performed to determine the DNA methylation status of the 5′ regulatory regions in MCF-7, as well as ABE-YJ (benign tumour) and hES-H1 cells. Three pairs of primers were designed to detect the CpG sites within the DE, PE and Oct-4A ExonI regions (NC_000006, 6338 nt). The methylation status of 23 CpGs (5 in DE, 5 in PE, 13 in ExonI) were investigated (Figure 6). As shown, almost all of the sites were hypomethylated in the undifferentiated hES-H1 cells, but hypermethylated (99% of DE, 99% of PE and 98.5% ExonI) in MCF-7 cells.

Interestingly, the same CpG regions in ABE-YJ (benign tumour) cells exhibited a different methylation pattern. Here, 92% and 70% of the DE element and ExonI region were methylated respectively while the PE element was unmethylated (Figure 6). It is known that cells within the tumour population often exhibit functional heterogeneity, showing distinct proliferative and differentiation capacities [22]. The ABE-YJ cells, isolated from primary benign breast adenofibroma tissue, may consist of a heterogeneous cell population that results in a variation in methylation status of the Oct-4 promoter.

There are many contradictory results on Oct-4 expression in breast cancer cells [14,23–29], and less data are available on endogenous Oct-4B expression. To determine which isoform has actually been identified in MCF-7 cells, we carefully reviewed the conclusions from previous studies analysing Oct-4 expression by assessing the methods (primers or antibodies) used by the authors [14,23–29]. Most of these studies showed that Oct-4A and Oct-4B expression were indistinguishable in MCF-7 cells (Supplementary Table S2 at http://www.bioscirep.org/bsr/031/bsr0310109add.htm). Recently, Oct-4A was suggested to be absent in somatic cells, including MCF-7 cells [14], while very low levels of Oct-4B expression were demonstrated in MCF-7 cells, similar to that observed in other cell lines [27]. In the present study, we demonstrate with unambiguous evidence that Oct-4B is expressed in MCF-7 cells, while Oct-4A is silenced. Furthermore, we show that Oct-4 pseudogenes are most likely not transcribed. The weak signal observed in the Western-blot analysis might account for the negative results in Oct-4B expression demonstrated in previous reports [27].
DE and PE elements have a redundant function in the regulation of Oct-4 gene expression and might share a common transcriptional binding site recognized by the same regulatory factors. Therefore, the silencing of endogenous Oct-4A in MCF-7 cells is most likely due to the lack of binding of transcriptional activators to the endogenous Oct-4 promoter. The inability of regulatory factors to bind to the promoter region of Oct-4 is probably caused by epigenetic rather than genetic control. As expected, further investigation of DNA methylation showed hypermethylation of both DE and PE, as well as ExonI, of Oct-4A in MCF-7 cells. Because the results in undifferentiated hES-H1 and NT2 (human embryonal carcinoma cell line, NT2/D1) cells show that both the DE and PE are not highly methylated while Oct-4A is expressed [12,31], we presume that DNA methylation of regulatory regions contributes to Oct-4A silencing in MCF-7 cells.

The interesting question raised by the present study is how the Oct-4B is expressed. It was proposed that Oct-4B was derived from alternative splicing [3]; however, as mentioned above, hypermethylation of regulatory regions suppresses the transcription of the Oct-4A gene. Thus, the premature mRNA of Oct-4 should not be transcribed. Therefore, we think that Oct-4B is not derived from alternative splicing, and that other distinct regulatory elements and a unique TSS might be involved in regulating Oct-4B expression. This unknown TSS is probably located in Intron1 of Oct-4A, since the transcription of Oct-4B begins at Intron1 of the Oct-4 gene, and ExonI of Oct-4A was hypermethylated (Figures 6 and 7). Thus, we further speculate that various isoforms of Oct-4 are transcribed from different TSSs, which further influence the different splicing patterns by interacting with lineage-specific transcriptional activators and splicing factors.

In conclusion, we have shown that Oct-4B rather than other isoforms is differentially expressed in MCF-7 cells, which might shed light on the previous controversy. Moreover, we suggest that hypermethylation of regulatory regions contributes to Oct-4A silencing, and that expression of Oct-4B is regulated by distinct regulatory elements and a unique TSS rather than the DE and PE of Oct-4A. This study offers an insight into the significance of differential gene regulation. An interesting question remains as to whether Oct-4B plays a role in the development of cancer, which requires further research.

ACKNOWLEDGEMENTS

We strongly acknowledge the support of the Stem Cell Research Center of Peking University Health Science Center, and thank Professor Chunyan Zhou (Peking University Health Center) for providing the MCF-7 cell line, Dr Li Wang (Stem Cell Research Center of Peking University Health Science Center) for providing the hES-H1 cell line, and Dr Lei Wang (Beijing Rehabilitation Hospital) for providing the breast adenofibroma tumour and adjacent normal tissue samples.

FUNDING

This work was supported by the National Natural Science Foundation of China [grant numbers 30570947, 30370726]; and partly supported by the National High-Tech Research and Development Program of China (863 Program) [grant number 2006AA02Z113].

REFERENCES


Received 5 March 2010/22 April 2010; accepted 30 April 2010
Published as Immediate Publication 30 April 2010, doi 10.1042/BSR20100033
SUPPLEMENTARY ONLINE DATA

Oct-4B isoform is differentially expressed in breast cancer cells: hypermethylation of regulatory elements of Oct-4A suggests an alternative promoter and transcriptional start site for Oct-4B transcription

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Figure S1 The existence of Oct-4 pseudogenes was not detected in MCF-7 cells
Alignment of the 393 bp sequence (PCR product amplified by pO1 and pO2) and Oct-4 pseudogenes (except Pou5f1P2 which differs significantly from the others) showed that Oct-4 pseudogenes were not detected. Sequence differences were shaded in yellow.

Figure S2 The presence of Oct-4B was detected in MCF-7 cells
Alignment of the 564 bp sequence (PCR product amplified by p4b-1 and pO2) with Oct-4A and Oct-4B shows that Oct-4B was detected. Sequence differences are shaded in yellow.

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Figure S3  The STR typing map of MCF-7 cell lines

Cell DNA was amplified using GoldeneyeTM16A STR multiplex amplification kits, and the 16 STR loci including a sex-specific gene (amelogenin) were tested using an ABI3100 Genetic analyser. Each cell DNA pattern amplified with good results showing no more than two alleles at each gene locus and no mixed samples. Testing conclusion: STR typing of MCF-7 cells demonstrated a single type of cell and was not contaminated with other human cells.
Oct-4B isoform expressed in breast cancer cells

**Table S1** List of RT–PCR and primers and bisulfite sequencing primers

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<td>pExon2 5-ACACCTAACCCTCCAATACCTAAAC-3</td>
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**Table S2** List of published Oct4 primers and antibodies in MCF-7 cells research

The conclusion of each publication: (a) Oct-4 expression in human breast cancer cells and was repression by treating with all-trans-retinoic acid; (b) the MCF-7 cells were examined for Oct-4 mRNA and Oct-4 protein expression; (c) the Oct-4 transcriptional profile could be identified in MCF-7 cells; (d) Zangrossi et al. [8] used MCF-7 cells as a positive control, and a fluorescence signal was localized to the cytosol; (e) absence of Oct-4 expression was observed in MCF-7 cells, but qRT–PCR signals of Oct-4B were similar between nTera, HeLa and MCF-7 cells; (f) almost all of the human MCF-7 cells expressed Oct-4 at a high level; (g) three protein isoforms have been identified by expression of the exogenous OCT-4B gene in HepG2 cells, but none of them were detected in MCF-7 cells. Note: (1) Epitope: a 21-amino-acid peptide identical with a C-terminal protein of mouse Oct-4; (2) speculation by us.

<table>
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<th>Antibody</th>
<th>Recognized protein isofom</th>
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REFERENCES


Received 5 March 2010/22 April 2010; accepted 30 April 2010
Published as Immediate Publication 30 April 2010, doi 10.1042/BSR20100033