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SHORT COMMUNICATION

Discovery of a Novel Variant of OCT4, OCT4B3

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ABSTRACT

Alternative splicing is an important mechanism that regulates gene expression and function in mammals, particularly in primates and human. Large-scale studies estimates a large number of human genes undergo alternative splicing. OCT4, a crucial stem cell marker, is one of the most important stemness markers that is not excluded in this regard. Up until now, it is known that OCT4 can produce several spliced variants in different cell types. In this study, we used RT-PCR and DNA sequencing approaches to verify OCT4 variant expression. Here, we reported a novel OCT4 spliced variant which is named as OCT4B3, for the first time. This variant is very similar to the OCT4B2 transcript, but a 207-bp fragment has been lost from Exon1b. Moreover, we investigated the expression pattern of OCT4B3 in several human cancer cell lines and found its expression in two cell lines of 1321N1 and 5637. Our experiments resulted in discovery of a novel OCT4 variant of OCT4B3 that is expressed in two human 5637 and 1321N1 cell lines.

Keywords: Stem Cell, Cancer cells, OCT4 gene, OCT4B3, Alternative splicing

OCT4 gene, an important stem cell marker, takes part in stemness properties of embryonic stem (ES) and embryonic carcinoma (EC) cells (1, 2). Up until now, it is known that OCT4 pre-mRNA can generates various splice variants under different situations such as OCT4A, OCT4B, OCT4B1 and also OCT4B2 (3, 4). The alternative splicing is considered as a major mechanism for gene expression and function regulation and a source of protein diversity which resulted in expanding protein function repertoire in mammals (5-7). Therefore, this mechanism of gene regulation might play a critical role in orchestrating complex regulatory function within OCT4 gene. In this study, we designed several primers located in different sites of the OCT4 gene and investigated the expression and structure of OCT4 variants by RT-PCR and sequencing approaches.

Materials and Methodologies

Cell culture

Various human cell lines were provided by Pasteur Institute, and Avicenna Research Institute of Iran, Tehran. The human cell lines of T-cell lymphoma (Jurkat), Burkit's lymphoma (Raji), ovary adenocarcinoma (Ovcar3), glioblastoma (U87), urinary bladder carcinoma (5637), and pluripotent embryonic carcinoma (NCCIT) were cultured in RPMI-1640 medium supplemented with 10% FBS, 100 mM sodium pyruvate, penicillin (100 u/ml) and streptomycin (100 µg/ml). Cell types of breast

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adenocarcinoma (MCF-7), pluripotent embryonic carcinoma (NT2), cervix adenocarcinoma (HeLa), glioblastoma (A172), medulloblastoma (Daoy), embryonic kidney (HEK-293), hepatocellular carcinoma (HepG2), human brain astrocytoma (1321N1) and bone marrow normal fibroblast (HS5) were cultured in High Glucose Dulbecco's Modified Eagle Medium (DMEM, 4500 mg/l) supplemented with 10% FBS and sodium pyruvate and penicillin/streptomycin as described above. Y-79 cell line was cultured in RPMI-1640 medium supplemented with 20% FBS.

RNA extraction and cDNA synthesis

RNA extraction was performed using TRIzol reagent (Invitrogen) according to the instruction of manufacturer. Quantity and quality of isolated RNAs was evaluated by spectrometry and electrophoresis on the agarose gel. Total RNAs were digested with RNase-free DNaseI (Fermentase) to remove any unwanted DNA contamination. The first strand of cDNAs was synthesized using the Reverse Transcription System (Fermentase, Lithuania). GAPDH was used as internal control to assess quality of synthesized cDNAs.

RT-PCR

RT-PCR was performed with the red master mix (Amplicon) according to the instruction of manufacturer. Primer sets of B2S/R2b and GAPF/GAPR were used to amplify OCT4B3 and GAPDH, respectively. The sequences of used primers were as follow:

RT-PCR approach was performed using 0.5 µl of cDNA and No-RT samples and 4 pmol of mixed Forward and Reverse primers in total vol. of 10 µl. Polymerase chain reaction for OCT4B3 and GAPDH transcripts was performed under the following cycling conditions: initiation at 94℃ for 4 min, amplification for 35 cycles with denaturation at 94℃ for 30 sec, annealing and extension at 65℃ for 30 sec, with a final extension at 72℃ for 7 min. The thermal profile for GAPDH was performed for 28 cycles with annealing of 58℃ and extension of 15 sec.

The size of amplified PCR products was 511-bp and 116-bp for OCT4B3 and GAPDH, respectively. Consequently, PCR products were confirmed by DNA sequencing.

Results

Identification of a Novel OCT4 Spliced Variant, OCT4B3

During analysis the expression pattern of OCT4B variants, specifically OCT4B2, we detected another transcript about 207-bp smaller than OCT4B2 using B2S/R2b primer set. Performing PCR with nested primers did not eliminate this extra PCR product. Therefore, this extra product was cloned into T/A cloning vector (PTZ57R/T vector) and sequenced. The sequencing results was analyzed using Chromas software and Blast in NCBI database. Based on the obtained results we found that OCT4 generates another spliced variant, resembling to OCT4B2 which loses a nearly 207-bp fragment of Exon 1b (Fig1). This new OCT4 transcript was named OCT4B3.

OCT4B3 is expressed in human brain astrocytoma and bladder carcinoma cell lines

The expression pattern of OCT4B3 was evaluated in various human cell lines with RT-PCR. Among 17 human different cell types (AA172, Daoy, HEK293, HeLa, HepG2, MCF-7, NT2, 5637, 1321N1, NCCIT, Jurkat, Y79, HS5, PC3, Raji, OVCAR3 and A549, OCT4B3 expression was detected just in 1321N1 and 5637 cell lines (Fig1A).

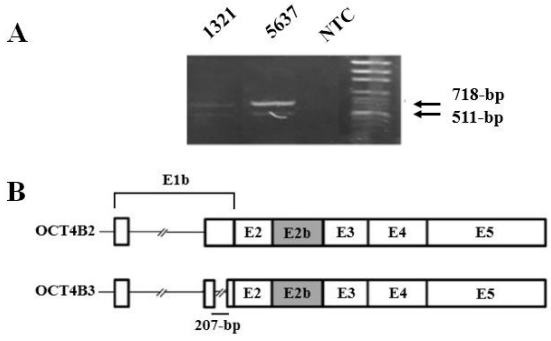

Figure1. (A) RT-PCR analysis of OCT4B3. Using primer set B2S/R2d we detected a 511-bp PCR product in the 5637 and 1321 cell lines which was corresponded to the

OCT4B3 transcript. The 718-bp band was related to the OCT4B2 variant. (B) Schematic structure of OCT4B3. This variant is composed of 6 exons (E1b, E2, E2b, E3, E4 and E5) which is very similar to the OCT4B2 transcript, except to missing a 207-bp from the E1b.

Discussion

Alternative splicing is an important mechanism which confer protein diversity in animals especially in mammals. This process permits a single gene produces several proteins with different functions in various tissues and conditions. Recent genome-wide analysis suggest that nearly 95% of human genes are alternatively spliced (8, 9). In many cases, alternative splicing patterns are tissue specific events (10). Therefore, regulatory elements in the pre-mRNA sequence (introns and exons) are associated with tissue specificity of these splice variants (11). OCT4 gene can generates various isoforms by alternative splicing that expressed in different tissues and have disparate functions (12). For example, OCT4A is an important transcription factor that confirms stemness properties into ES and EC cells (13, 14). OCT4B-190 and OCT4B-265 are two other OCT4 spliced variants that response to cell stress (15, 16). OCT4B1 is expressed in stem cells and undifferentiated cells and suggests to be another stemness marker. OCT4B2 is, also, expressed in different cancer cells and up-regulated under stress conditions (4, 17). Here, we introduced another OCT4 spliced variant, OCT4B3 that is very similar to the OCT4B2 transcript which lacks 207-bp of its Exon1b. This variant has a low expression as usual, and was detected in human bladder cancer (5637) and astrocytoma (1321N1) cell lines. We suggest OCT4B2 might lose a 207-bp fragment of its Exon1b by splicing and generates OCT4B3 under special conditions, however, we needs more experimental evidences to confirm this hypothesis definitely. Since cancer cell lines are a mixture of “cancer stem cells” and “cancer non-stem cells”, reflecting the complex mixture of these cancer stem cells, stromal cells and invasive immune cells in an in vivo tumor, the fact that internal special micro-environmental conditions affect a complex interaction of these cell types might induce the production of these various spliced variants.

Author Contributions

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Conflict of Interest

Authors declare no conflict of interst.

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