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# RNA-Interference-Mediated Silencing of OCT4B1, Alters Expression Profile of Several TNF Ligand/ Receptor Transcripts in Human Tumor Cell Lines

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**ABSTRACT**

***Background:*** The OCT4B1 as a new discovered variant of OCT4 is expressed in both cancer cell and tissues. This variant with its anti-apoptotic properties aid cancer cells to scape from apoptosis. TNF ligands and receptors are amongst two categories of eleven gene families involved in the apoptosis pathway. Therefore, the aim of the present study was to investigate the effects of OCT4B1 suppression on several transcripts of both TNF ligands and receptors family in some tumor cell lines. ***Methods:*** The AGS (gastric adenocarcinoma), 5637 (bladder tumor) and U-87MG (brain tumor) tumor cell lines were transfected with specific OCT4B1 siRNA and , as well as a scrambled sequence and PBS, as controls, using Lipofectamine 2000 comerical kit. The expression of TNF ligand and receptor transcripts were evaluated in parallel with beta-actin (as housekeeping gene) using Real-Time PCR technique. ***Results:*** our results indicated that in TNF ligand transcripts family, the mRNA level of TNF transcripts was up-regulated and inversely TNFSF8, TNFSF7, TNFSF10, TNFSF1 and TNFSF6 was down-regulated. We observed also that in TNF receptor transcripts family, six transcripts including, TNFRSF 10A, TNFRSF10B, TNFRSF11B, TNFRSF1A, TNFRSF21 and TNFRSF25 were up-regulated, while TNFRSF9 and CD27 were down-regulated. ***Conclusions:*** According to these results, it may be concluded that OCT4B1 suppression can lead to apoptosis in tumor cell lines via up-regulation of several TNF ligand and receptor transcripts. Thus, OCT4B1 suppression effects on TNF and its receptors may be considered as promising target genes in future studies in cancr research and therapy.

## Introduction

OCT4 genes (octamer DNA binding transcription factor 4), belong to a family of transcription regulatory genes containing the PUO DNA binding domain, located on human chromosome 6 that potentially encodes at least three different variants (A, B and B1) via mRNA alternative splicing.[1] OCT4 is among the important and critical genes involved in both embryonic and adult stem cell proliferation and differentiation.[2] Expression of OCT4 induces stem cell pluripotency, while, it's silencing or down regulation is believed to be a sign of cell differentiation.[2] According to the most recent theories of cancer known as "cancer stem cell", cancers are originated from either adult stem cells (tissue stem cells) or reprogrammed tissue somatic cells.[3] There are gene expression pattern similarities between cancer stem cells and normal stem cells.[4] Recently it has been established that a new variant of OCT4, known as OCT4B1, is expressed in both normal stem cells (embryonic and adult) and cancer cell lines/cancer tissues[1]. Moreover, investigations showed that this variant has anti-apoptotic potency in cancer cell lines.[5]

Apoptosis, an important cellular pathway, occurs continually during development and aging to preserves cell populations in tissues.[6] There are at least twelve gene families related to apoptotic pathway, of which TNF ligand and TNF receptor genes are subclasses of these families.[7] TNF ligands and receptors not only play important roles in apoptosis but are also involved in normal developmental processes including regulation of immune cell functions and additional cell type-specific responses.[8]

TNF ligands are a set of genes that encode cytokines polypeptides with the ability to activate TNF receptor proteins located in the surface of cells.[9] This superfamily consists of 19 ligands recognized as TNFSF (tumor necrosis factor superfamily), most of which are type II of transmembrane proteins whose

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extracellular domains can be cleaved by specific metalloprotease to generate soluble cytokines[10,11]. TNF receptors are a set of 29 genes called TNFRSF (tumor necrosis factor receptor superfamily), encode oligomeric, and belong to type I or type III transmembrane proteins. Several TNF receptors contain death domains (DDs) that recruit caspase-interacting proteins following ligand binding to initiate the extrinsic pathway of caspases activation.[9] Additionally, due to the anti-apoptotic properties of OCT4B1, one hypothesis suggests that it can affect the expression of essential genes involved in cell survival and cell death, especially programmed cell death (apoptosis) to induce the self-renewal state of the cell.

Apoptosis is a specialized mechanism for removal of the aggressive cells including cancer cells; however, within the cancer tissues and cancer cell lines, apoptosis would be suppressed. Delineation of both cellular and molecular mechanisms that contribute to these suppression processes could be considered as promising targets in cancer therapy.

Based on the aforementioned introductory facts, because OCT4B1 can inhibit apoptosis, the aim of the present investigation was to explore the relation between the OCT4B1 suppression and expression profile of several TNF ligand and TNF receptor family genes in three human tumor cell lines including AGS (gastric adenocarcinoma), 5637 (bladder tumour) and U-87MG (brain tumour).

## Materials and Methods

### *Cell culture and transfection*

AGS (gastric adenocarcinoma), 5637 (bladder tumour) and U-87MG (brain tumour) were maintained in RPMI-1640 (Gibco) culture medium containing 10% fetal bovine serum (Gibco) and antibiotics (100 mg/mL streptomycin and 100 U/mL penicillin, Gibco company) at 37 °C in a humidified incubator containing 5% CO2. The cells were subcultured three times (to assurance of cell viability) and then cultured in tow test and control groups ($10^5$ cells/ml). To suppress OCT4B1 variant, two specific siRNA were utilized based on the specific OCT4B1 sequences. One irrelevant or scrambled siRNA (with no complementary target sequence in the human genome) and a vehicle in PBS mock were transfected under the same conditions as controls. The siRNA were designed by a selection program (Whitehead Institute for Biomedical Research, htt://jura.wi.mit.edu/) and the process of synthesis was performed by MWG Company (Germany) (Table 1).

**Table 1.** sequences and criteria of designed siRNAs.

| siRNA name | **Target** | **Sequences** |
|---|---|---|
| Version I | Target | AAGGAGTATCCCTGAACCTAG |
| | Sense | (GGAGUAUCCCUGAACCUAG)dTdT |
| | Anti-sense | (CUAGGUUCAGGGAUACUCC)dTdT |
| Version II | Target | AAGAGGTGGTAAGCTTGGATC |
| | Sense | (CAGUGGUAAGCUUGGAUC)dTdT |
| | Anti-sense | (AAUCCAAGCUUACCACCUC)dTdT |
| Scramble | Sense | GCGGAGAGGCUUAGGUGUAdTdT |
| | Anti-sense | UACACCUAAGCCUCUCCGCdTdT |

For siRNA transfection, lipofection method was used; briefly, a suspension of freshly cultured cells was prepared ($10^5$ cells/ml) in the RPMI1640 culture media without antibiotics, seeded in six well plates in two test and control groups. During logarithmic growth phase (confluences 30-50%), cells were transfected with 50 nmol/ml synthetic siRNA (in the control group, scrambled sequence siRNA), using lipofectamin 2000 (Invitrogen, USA) and Opti-MEM media, according to the manufacturers' instructions. In brief, 5µl of siRNA (25µM) and 4.5 µl RNAi-MAX reagents were diluted in 250 µl Opti-MEM and incubated for 10 min at room temperature. The mixture was then added to the cells in a final volume of 2.5ml, and then the cells were incubated at 37ºC in a humid atmosphere of 5% $CO_2$ for 72 hours.

### *The OCT4 variants expression*

OCT4 variant expression was detected in the studied cell lines, total RNA was extracted from cultured cells ($10^6$ cells/ml) using TRIzol reagent (Invitrogen, UK). The contaminated DNA in the RNA samples was removed by TURBO DNase I (Fermentase, Lithuania). The RNA fidelity purity and integrity were measured by spectrophotometer (260/280 NM ratio) and gel electrophoresis (1% agarose gel). The first strand cDNA was synthesized at 42ºC for 60 min using 100 pmol oligo-dT primer, 200 units of MMuLV reverse transcriptase and 1µg of total RNA according to the manufacturer's instructions (Fermentase, Lithuania). Quantitative Real Time PCR (polymerase chain reaction) was performed to detect OCT4 variants gene expression in studied cells as described later. Specific primers were designed for OCT4 variants (A, B and B1), interested TNF ligands and receptors transcripts and ß-actin, using Gene Runner (version 3.02) and Allele ID (version 4.0) software (Table 2).

**Table2.** Demonstrates designed primers for OCT4 variants, TNF ligand and receptor genes families and ß-actin.

| Target genes (Gene Symbol) | Gene bank number | Designed Oligo | Relative Sequence | Fragment length |
|---|---|---|---|---|
| OCT4A | NM-002701 | F | CGCAAGCCCTCATTTCAC | **111** |
| | | R | CATCACCTCCACCACCTG | |
| OCT4B | NM-203289 | F | CAGGGAATGGGTGAATGAC | **177** |
| | | R | AGGCAGAAGACTTGTAAGAAC | |
| OCT4B1 | EU518650 | F | GGTTCTATTTGGTGGGTTCC | **128** |
| | | R | TTCTCCCTCTCCCTACTCCTC | |
| CD40LG | NM-000074 | F | AACCCTGGAAAATGGGAAAC | **222** |
| | | R | CCTCCCAAGTGAATGGATTG | |
| FASLG | NM-000639 | F | TGGCCTTGTGATCAATGAAA | **155** |
| | | R | TCATCATCTTCCCCTCCATC | |
| LTA | NM-000595 | F | CCCACCAGTGGCATCTACTT | **174** |
| | | R | CAGCCCTGGATACACCATCT | |
| TNF | NM-000594 | F | CAGAGGGCCTGTACCTCATC | **219** |
| | | R | GGAAGACCCCTCCCAGATAG | |
| TNFSF10 | NM-003810 | F | TTCACAGTGCTCCTGCAGTC | **192** |
| | | R | ACGGAGTTGCCACTTGACTT | |
| CD70 | NM-001252 | F | TGGTACACATCCAGGTGACG | **212** |
| | | R | AAGTGTCCCAGTGAGGTTGG | |
| TNFSF8 | NM-001244 | F | ACTCTGGCTCTGTGCCTTGT | **152** |
| | | R | AATGGAGCCCTTTTCAGGAT | |
| CD40 | NM-001250 | F | GCAGGCACAAACAAGACTGA | **191** |
| | | R | TCGGGAAAATTGATCTCCTG | |
| FAS | NM-000043 | F | CAAGGGATTGGAATTGAGGA | **203** |
| | | R | TGGAAGAAAAATGGGCTTTG | |
| LTBR | NM-002342 | F | ACCAGGTGTGAGAACCAAGG | **153** |
| | | R | GAGCAGAAAGAAGGCCAGTG | |
| TNFRSF10A | NM-003844 | F | AGAGAGAAGTCCCTGCACCA | **154** |
| | | R | GTCACTCCAGGGCGTACAAT | |
| TNFRSF10B | NM-003842 | F | CACCAGGTGTGATTCAGGTG | **221** |
| | | R | CCCCACTGTGCTTTGTACCT | |
| TNFRSF11B | NM-002546 | F | GGCAACACAGCTCACAAGAA | **241** |
| | | R | CTGGGTTTGCATGCCTTTAT | |
| TNFRSF1A | NM-001065 | F | GTGCCTACCCCAGATTGAGA | **175** |
| | | R | TGTCGATTTCCCACAAACAA | |
| TNFRSF21 | NM-014452 | F | TGAACAAGACCCTCCCAAAC | **215** |
| | | R | CAGCAGGAAAAGCACAATCA | |
| TNFRSF25 | NM-003790 | F | GTGTGTCCCCAAGACACCTT | **206** |
| | | R | GGTTGGCAGTAGAAGGGTGA | |
| CD27 | NM-001242 | F | CAGCCCACCCACTTACCTTA | **226** |
| | | R | TCCTTCGTTGATGGAGGAAC | |
| TNFRSF9 | NM-001561 | F | CACTCTGTTGCTGGTCCTCA | **158** |
| | | R | CACAGGTCCTTTGTCCACCT | |
| ß-actin | NM-001101 | F | AGGCACCAGGGCGTGAT | **184** |
| | | R | GCCCACATAGGAATCCTTCTGAC | |

### *MTT Assay*

The cell viability was determined using 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay on the basis of the ability of viable cells to metabolize MTT. MTT is a water-soluble tetrazolium salt which can be metabolized into a water-insoluble formazan product by mitochondrial succinate dehydrogenase in viable cells.[12] Briefly, $10^4$ cells/ml were seeded onto a 96-well plate at a final volume of 200 µL. Subsequent to cell transfection, the resultant supernatant was replaced by 200 µL of pre-warmed RPMI 1640 (without phenol red) and continued by addition of 10 µL of 5 mg/ml MTT to each well. The plate was incubated at 37 ˚C for 3.5 h in the dark until a

purple precipitate was visible under light microscopy. Finally, 200 µL of DMSO (dimethyl sulphoxidase) was added to each well and 15 min after the absorbance (at 570 nm) was read after with a reference filter of 620 nm.

### *Apoptosis analysis*

The ratio of apoptotic cell in OCT4B1 siRNA transfected cells (test group) against the scrambled sequence siRNA transfected cell (control group) was detected 48 hours after transfection. Single cell suspension was carried out, and the Annexin V-FLOUS kit (Roch, Germany) was used for detection of the exposed phosphatidylserin on cell surfaces, as described elsewhere.[13] Then, 48 hours after transfection, cells ($1\times10^6$) were centrifuged and resuspended in 200µl of binding buffer. Annexin V-FLOUS (1µl) and propidium iodide (1µl) were added to tubes after 5 minutes and were further incubated for 5 minutes at room temperature (in the dark) and then analyzed by Beckman-Coulter Elite flow cytometer. Annexin V-FLOUS binding was detected using a FITC signal detector (FL1) and propidium iodide staining by the phycoerythrin emission signal detector (FL3). The ratio of FITC/PI positive cells (apoptosis) was calculated from flow histograms.[14]

### *TNF ligand and receptor transcripts profiling*

Followed suppression of OCT4B1 variant, mRNA synthesis of seven transcripts in TNF ligand and eleven transcripts in TNF receptor gene families were detected. Total RNA from test and control group were extracted and cDNA was synthesized as described. Quantitative Real Time PCR was performed by the addition of a SYBER green master mix (Qiagene, USA), 200ng of the generated cDNA and 2 pgr/µl of appropriate primers (Table 2). The following cycling program was set on a BIO-RAD CFX96 system (Bio-Rad Company, USA): one cycle at 95ºC for 15 min, 40 cycles at 95ºC for 30 S, 58-61ºC for 30 S and 72ºC for 30 S. Real-time PCR was carried out in triplicate and ß-actin was assessed as a housekeeping gene for the normalization of amplification signals of the target genes. The proportional amounts of PCR products were determined using the $2^{-\Delta\Delta Ct}$ formula. Relative expression is determined by comparison of interested gene expression in test and control group using data from the real-time cycler and the ΔΔCT method. The dissociation stages, melting curves and quantitative analyses of the data were performed using CFX manager software version 1.1.308.111 (Bio-Rad, USA). All PCR products were visualized by electrophoresis on a 1% gel containing 0.5 mg/ml ethidium bromide to check the size of PCR products.

## Results

### *siRNA transfection caused down-regulation of OCT4B1*

The expression of OCT4B1 variant was assessed at 24, 48 and 72 hour time points, following siRNA transfection in test (OCT4B1 siRNA transfected cells) and control (scrambled sequence siRNA transfected cells) groups. In the test group version I and II of designing siRNA was carried out in triplicate and the results showed more suppression in the version I siRNA transfection. Our data showed that optimal knockdown of the gene occurred after 48 hours of treatment (Figure 1).

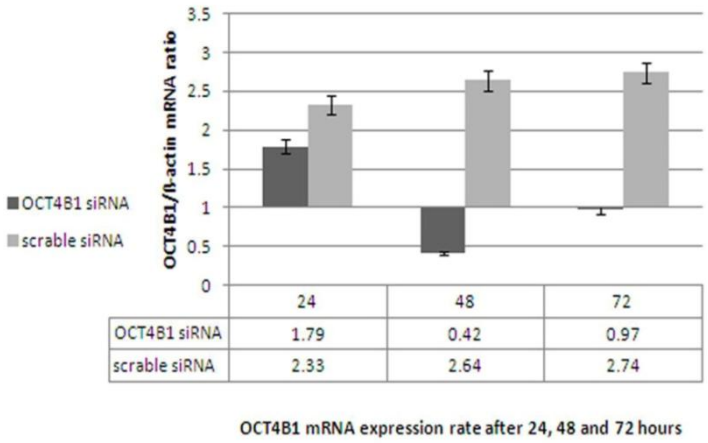


**Figure1.** Demonstrates OCT4B1 expression after 24, 48 and 72 hours of siRNA transfection in AGS cell line. Y axis showed rate of OCT4B1 variance expression compared to ß-actin (housekeeping gene as control) and X axis showed AGS cell line transfected with OCT4B1 siRNA (test) and scramble siRNA transfected cell (control) after 24, 48 and 72 hours of transfection.

### *Elevated apoptosis and decreased viability of studied tumor cell lines treated with specific OCT4B1 siRNA*

Flow cytometry analysis of the cells stained with Annexin V and PI demonstrated that two days after siRNA transfection up to 34% of the test group cells (transfected by specific OCT4B1 siRNA) underwent apoptosis, in comparision to control cells (transfected by scrambled sequence siRNA)(Figure not shown).

MTT assay also indicated reduced cell activity after OCT4B1 siRNA transfection compared to cells

transfected by scrambled sequence siRNA and mock transfected control cells. (Figure 2).

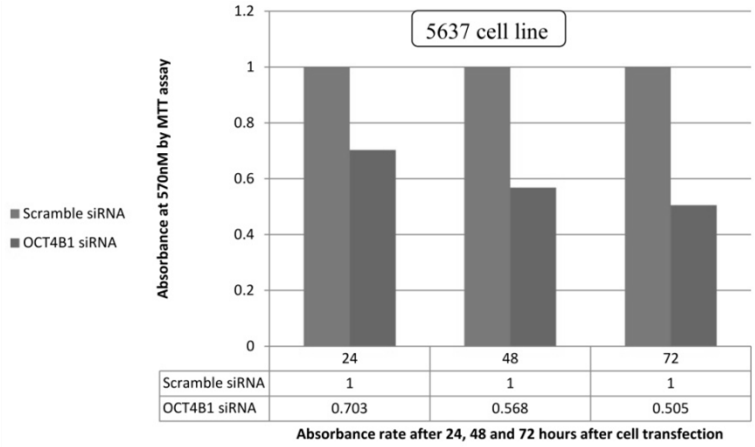


**Figure 2**. The figure shows OCT4B1 siRNA transfected cells had lower viability in compare to control groups (scramble siRNA transfected cells).

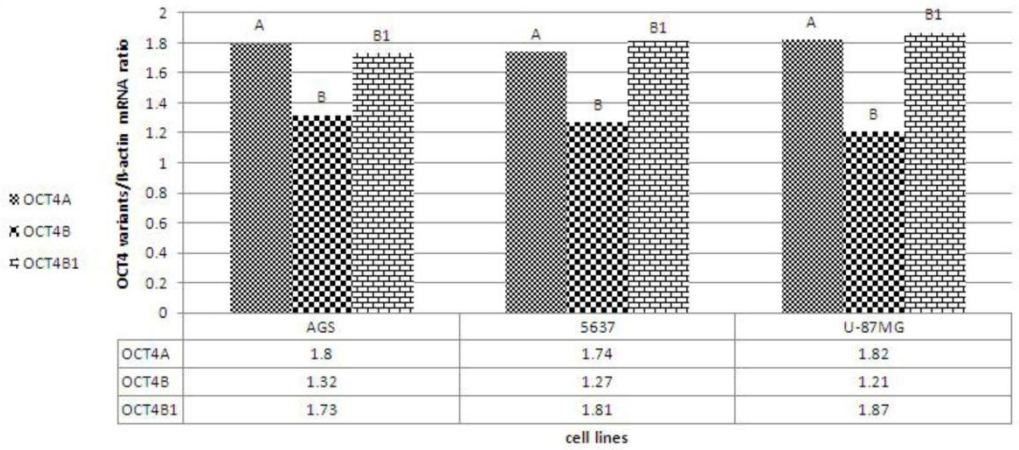


**Figure 3.** Demonstrates level of OCT4 variants expression in studied tumor cell lines. Y axis shows OCT4B1 variance mRNA expression level compared to ß-actin (as housekeeping control gens) and X axis indicates three studied tumor cell lines (AGS, 5637 and U87MG).

**Table 3.** TNF ligand genes family up/down regulated after OCT4B1 suppression in 5637, AGS and U87MG tumor cell lines.

| Gene bank number | Gene symbol | Protein/gene function | Cell lines/ Fold changes | | |
|---|---|---|---|---|---|
| | | | 5637 | AGS | U87MG |
| **NM-000074** | **TNFSF5** | Tumor necrosis factor superfamily, 5 | **1.67** | **1.51** | **1.46** |
| **NM-000639** | **TNFSF6** | Tumor necrosis factor superfamily, 6 | **-3.51** | **2.29** | **-1.49** |
| **NM-000595** | **TNFSF1** | Tumor necrosis factor superfamily, 1 | **-3.53** | **-6.31** | **-1.7** |
| **NM-000594** | **TNF** | Tumor necrosis factor | **13.95** | **5.32** | **2.3** |
| **NM-003810** | **TNFSF10** | Tumor necrosis factor superfamily, 10 | **-12.28** | **-4.26** | **-10.54** |
| **NM-001252** | **TNFSF7** | Tumor necrosis factor superfamily, 7 | **-10.18** | **-6.68** | **-6.18** |
| **NM-001244** | **TNFSF8** | Tumor necrosis factor superfamily, 8 | **-8.21** | **-12.64** | **-7** |

### *Quantitative Real-time PCR results*

Our data revealed that all three variants were expressed in the studied cell lines (Figure 3). Expressional profile of the studied transcripts in three cell lines is almost similar (Table 3 and 4). In TNF ligand transcript family, TNF transcript was up-regulated (>8 fold), while TNFSF8, TNFSF7, TNFSF10, TNFSF1 and TNFSF6 were down-regulated between 2-10 fold (Table 3 and Figure 4). In the TNF receptor transcript family, six transcripts including, TNFRSF10A, TNFRSF10B, TNFRSF11B, TNFRSF1A, TNFRSF21 and TNFRSF25 were up-regulated by more than 5 fold, while TNFRSF9 and CD27 were down-regulated by more than 3 fold (Table 4 and Figure 5).

**Table 4.** TNF receptor genes family up/down regulated after OCT4B1 suppression in 5637, AGS and U87MGtumor cell lines.

| Gene bank number | Gene Symbol | Protein/gene function | Cell Lines/Fold changes 5637 | AGS | U87MG |
|---|---|---|---|---|---|
| **NM-001250** | **TNFRSF5** | Tumor necrosis factor receptor superfamily, 5 | **1.8** | **-3.59** | **-1.35** |
| **NM-000043** | **TNFRSF6** | Tumor necrosis factor receptor superfamily, 6 | **-2.83** | **-2.09** | **-1.24** |
| **NM-002342** | **TNFRSF3** | Tumor necrosis factor receptor superfamily, 3 | **1.63** | **-2.97** | **3.16** |
| **NM-003844** | **TNFRSF10A** | Tumor necrosis factor receptor superfamily, member 10a | **6.74** | **9.72** | **7.53** |
| **NM-003842** | **TNFRSF10B** | Tumor necrosis factor receptor superfamily, member 10b | **6.42** | **9.65** | **12.48** |
| **NM-002546** | **TNFRSF11B** | Tumor necrosis factor receptor superfamily, member 11b | **12.4** | **9.26** | **8.89** |
| **NM-001065** | **TNFRSF1A** | Tumor necrosis factor receptor superfamily, member 1a | **17.29** | **-** | **9.14** |
| **NM-014452** | **TNFRSF21** | Tumor necrosis factor receptor superfamily, member 21 | **12.4** | **12.91** | **11.1** |
| **NM-003790** | **TNFRSF25** | Tumor necrosis factor receptor superfamily, member 25 | **5.83** | **8.94** | **8.01** |
| **NM-001242** | **CD27** | CD27 molecule | **-3.8** | **-9.91** | **-4.85** |
| **NM-001561** | **TNFRSF9** | Tumor necrosis factor receptor superfamily, member 9 | **-8.35** | **-12.04** | **-7.68** |

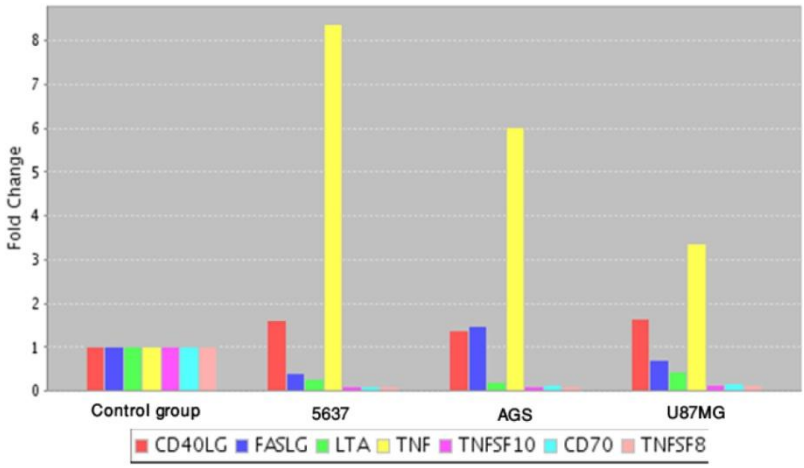


**Figure 4.** Demonstrates TNF ligand family gene expression 48 hours after siRNA transfection in studid tumor cell lines. Y axis showed fold gene regulation and X axis showed 25 genes of anti-apoptotic gene family in test and control groups. group1; AGS, group2; 5637 and grou3; U87MG tumor cell lines.

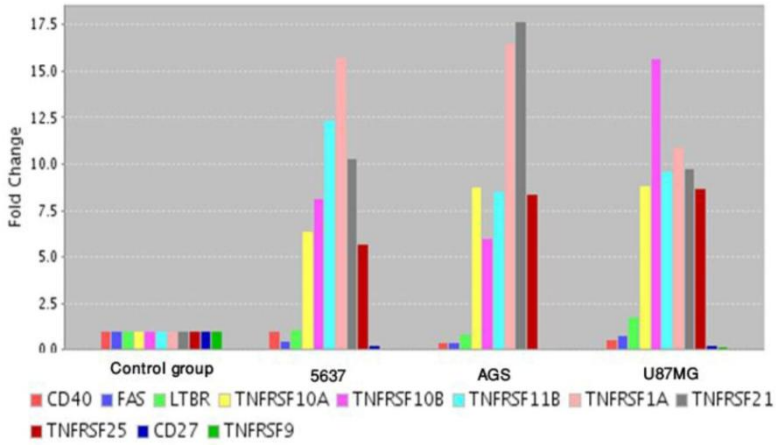


**Figure 5.** Demonstrates TNF receptor family gene expression 48 hours after siRNA transfection in studid tumor cell lines. Y axis showed fold gene regulation and X axis showed 25 genes of anti-apoptotic gene family in test and control groups. group1; AGS, group2; 5637 and grou3; U87MG tumor cell lines.

## Discussion

In general, development of tumor is characterized by increasing of tumor cell mass. This could be due to increased proliferation and/or decreased apoptosis, or both. A coordination and balance between cell proliferation and apoptosis is crucial for normal development. [15]

TNF ligand and receptors are two gene families with a critical role in apoptosis pathway, they alter TNF ligands and receptors expression, and dramatically change apoptosis rates.[16]

There are contradictory reports for expression of OCT4 in cancer cells and cancer tissues, some are impressed to express this gene in cancer tissues, while other reports indicate OCT4 does not express in cancer cell line and tissues.[17,18] However, it has been documented that OCT4 expression induces cell stemness and down-regulation or silencing caused differentiation.[19] Atlasi et al. defined and introduced a novel variant of OCT4, designated as OCT4B1, which is highly expressed in tumor cell lines and down-regulated upon differentiation.[1] Consistant with our results, Asadi and collaborators revealed that OCT4B1 has anti-apoptotic potency.[5] Farashahi et al. also determined a direct relation between stress (heat shock) and up-regulation of OCT4B1.[20] Over-expression of OCT4B1 in tumor cell lines and cancer tissues in compared to normal cells and tissues and so, anti-apoptotic potency of this variant, suggests OCT4B1 play an important role in cancer origination and progression in tumor cell lines.

In the current research all three OCT4 variants were expressed in studied cell lines and following the suppression of the OCT4B1, the mRNA rate of this variant was significantly decreased compared to cells that transfected by scrambled siRNA. OCT4A expression maybe related to OCT4 pseudogenes that have the most similarity to OCT4A variant.[21] OCT4B is expressed in a variety of cells (somatic and tumor cell lines and tissues) and studies have revealed this variant does not have a role in stemness state. Results of Annexin V-FLOS test showed that approximately 29% of OCT4B1 siRNA transfected cells underwent apoptosis in compared to scrambled siRNA transfected cells. Based on these results it can be concluded that following suppression of OCT4B1, the apoptosis pathway is activated and triggers further processes of apoptosis. After OCT4B1 suppression (48 hours), mRNA synthesis rate of the five studied TNF ligand genes including: TNFSF8, TNFSF7, TNFSF10, TNFSF1 and TNFSF6 were decreased (down-regulated) by 2-10 fold and expression of TNF gene was elevated (up-regulated) more than 8 fold (Table 2). From 11 TNF receptor genes analyzed, 7 genes including: TNFRSF10A, TNFRSF10B, TNFRSF11B, TNFRSF1A, TNFRSF21, TNFRSF25 and TNFRSF9 were up-regulated by (5-15 fold), and CD27 and FAS genes were down-regulated 2-3 fold, and TNFRSF3 (LTBR) gene expression shows no change (Tables 3). Most TNF ligands and receptors are expressed in the immune system although several studies have emphasized expression of these ligands and receptors in cancer cell lines with different patterns.[22,23]

Due to the fact that TNF Superfamily molecules induce apoptosis via extrinsic pathway, based on our results it may be concluded that OCT4B1 suppression leads to apoptosis via extrinsic pathway.

Previous studies showed, siRNA transfections may caused different behavior in transfected cells especially in different cell lines,[24] but our results revealed that expression pattern of studied gene families in three studied tumor cell lines was the same, hence it may be concluded that OCT4B1 plays an alike role in tumor cell lines and inhibits apoptosis, maybe, via down-regulation of TNF superfamily molecules.

To the best of our knowledge this is the first investigation regarding the relation between OCT4B1 suppression and gene expression profile of TNF ligands and receptors in tumor cell lines. As the results have shown, there is a tight collaboration between suppression of this variant and up/down regulation of the studied TNF ligands and receptors genes. Therefore, the data demonstrated that OCT4B1 potentially targets TNF ligands and receptors members, as changes the self-renewal genes.[25]

In conclusion, the present study indicates that OCT4B1suppression triggered the cells toward apoptosis and reduced cell viability, so, suppression of this variant has significant effects on expressional pattern of TNF ligand and receptors genes family. Based this findings, OCT4B1, perhaps plays a critical role in Commutation of normal cell to cancer situation so, it may be considered in later investigation around cancer.

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