Iranian Journal of Basic Medical Sciences

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Down-regulation of HSP40 gene family following *OCT4B1* suppression in human tumor cell lines

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ARTICLE INFO

Article type:
Original article

Article history:
Received: Jun 16, 2015
Accepted: Nov 5, 2015

Keywords:
HSP40 gene family
OCT4B1
siRNA
Tumor cell lines

ABSTRACT

Objective(s): The *OCT4B1*, as one of *OCT4* variants, is expressed in cancer cell lines and tissues more than other variants and plays an important role in apoptosis and stress (heat shock protein) pathways. The present study was designed to determine the effects of *OCT4B1* silencing on expressional profile of HSP40 gene family expression in three different human tumor cell lines.

Materials and Methods: The *OCT4B1* expression was suppressed by specific siRNA transfection in AGS (gastric adenocarcinoma), 5637 (bladder tumor) and U-87MG (brain tumor) cell lines employing Lipofectamine reagent. Real-time PCR array technique was employed for RNA qualification. The fold changes were calculated using RT^2 Profiler PCR array data analysis software version 3.5.

Results: Our results indicated that fifteen genes (from 36 studied genes) were down-regulated and two genes (*DNAJC11* and *DNAJC5B*) were up-regulated in all three studied tumor cell lines by approximately more than two folds. The result of other studied genes (19 genes) showed different expressional pattern (up or down-expression) based on tumor cell lines.

Conclusion: According to the findings of the present study, we may suggest that there is a direct correlation between *OCT4B1* expression in tumor cell lines (and tissues) and HSP40 family gene expressions to escape from apoptosis and cancer expansion.

► *Please cite this article as:*
Mirzaei MR, Asadi MH, Mowla SJ, Hassanshahi GhH, Ahmadi Z. Down-regulation of HSP40 gene family following *OCT4B1* suppression in human tumor cell lines. Iran J Basic Med Sci 2016; 19:187-193.

Introduction

The heat shock proteins (HSPs) are considered as the most conserved proteins which exist in both prokaryotic and eukaryotic cells and their level of expression increases during stresses (1, 2). A wide spectrum of physiological and environmental factors including heat shock stress could alter the expression of HSPs. This family of proteins plays essential roles in correct assembling of nascent and stress-accumulated misfolded proteins, hence preventing their aggregation (3, 4). Based on the structural localization, HSPs (either intracellular or extracellular) functions as protective and/or inductive the intracellular HSPs aid cells to survive under stress conditions while extracellular or membrane receptor like HSPs interact with some of apoptosis family member proteins to regulate a programmed cell death (apoptosis) pathway (5). As mentioned above, HSPs are expressed by normal cells; however, under stress conditions (i.e. heat-shock) their expression is up-regulated.

The mammalian HSPs are divided according to their molecular size and function, into two main groups: high and small molecular weight. HSP60, HSP70 and HSP90, are members of the high molecular weight group while other HSPs are categorized as small molecular weight group. A variety of HSPs members are constitutively expressed, but, some of them are stress-induced (6). Within stressful circumstances, the HSPs could induce proteasomal-mediated degradation of some selected trigger proteins. Altogether, these properties make HSPs as mediators that are enable to regulate the processes of cell death pathways (7).

The HSPs are also up-regulated in a considerable range of human cancers and several cellular functions (varying from proliferation, differentiation, to invasion and metastasis) associated with tumor genes (7). Especially HSP70, as an anti-apoptotic molecule, is reported to be up-regulated in malignant human tumors. Interestingly, HSP70 enhances the tumorigenic potency of rodent cells (8).

Investigations showed that HSP90 is an ATP-dependent chaperone molecule which is crucial for eukaryotic cells. It is essential for both activation and continues stabilization of several proteins involved

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in various cellular pathways like apoptosis. Therefore, it appears thatHSP90is considered as an remarkable target for cancer treatment(9).

Studies evidenced an important relation between HSPs expression and cancer (10). Accordingly, in all cancer tissues and cancer cell lines, different subtypes of HSPs are expressed. Several different hypotheses were purposed to explain the initiation of cancer. The "cancer stem cell" hypothesis is almost widely accepted by the scientists. With regard to this theory, adult stem cells (which are present in nearly all human tissues) or reprogrammed tissue somatic cells are the primary sources for triggering tumor tissue expansion (11, 12). According to this hypothesis, specific biological marker of embryonic stem cells (such as, *OCT4* series) are re-expressed in tumor tissues and cancer cell lines, which are currently employed as either tumor markers for diagnosis or treatments of cancers.

OCT4 (octamer-binding transcription factor 4) is considered as the central factor of regulation of the self-renewal potency of proteins belonging to a family of transcription regulatory proteins and contains the POU DNA binding domain (13). The high level of *OCT4* expression has been primarily restricted to embryonic stem cells (ESC), and in response to its suppression, the cell differentiation processes are enhanced (14). Recent investigations showed that, *OCT4* is also involved in modulating survival of cancer cells (15-17). The *OCT4* gene potentially encodes different variants by various capacity via alternative splicing among which three variants (A, B and B1) of *OCT4* are more famous (18-20). The *OCT4A* is expressed in embryonic and some adult stem cells (located in nucleus) and control the pluripotency properties of cells. While the OCT4B is widely expressed in both stem and tumor cells (more located in the cytoplasm) by not effects on stemness pathways. The OCT4B1, a new variant of *OCT4* that is expressed by both pluripotent normal and cancerous stem cell lines and tissues (21-24). Complementary studies showed up-regulation of *OCT4B1* in gastric (8), colorectal (9), bladder (10), and germ cell tumors (11), where it functions as an anti-apoptotic factor (23, 24, 25).

Our previous investigations showed possible involvement of the OCT4B1 variant in stress pathway (10). In the present study, we aimed to suppress *OCT4B1* in three human tumor cell lines and check the profile of the stress-related genes which may either over-expressed or suppressed following *OCT4B1* suppression.

Materials and Methods

Cell culture

Three human tumor cell lines, namely, AGS, 5637, and U87MG were purchased from the Iranian national cell bank (Pasteur institute of Iran, Tehran) and were further cultured in RPMI-1640 (Gibco) supplemented with 10% (v/v) of heat-inactivated FBS (Fetal bovine serum), 2 mM L-glutamine (Sigma, St. Louis, USA) and penicillin-streptomycin antibiotics (Gibco) at 37 ℃ and 5% CO_2 atmosphere.

OCT4 variants expression profile

Real-time RT-PCR was carried out to evaluate the expression status of the *OCT4* variants in the studied tumor cell lines. Total RNA was extracted from cultured cells (10^6 cells/ml) using Trizol reagent (Invitrogen). According to the manufacture's guidelines, the isolated RNA was then treated with TURBO DNase to avoid possible DNA contaminations. The RNA purity and fidelity were examined by evaluation of optical density (calculation of 260/280 nm ratio) and by observation of the samples on agarose gel following their electrophoresis (1% agarose gel), respectively. The first strand cDNA was synthesized at 42 ℃ for 60 min using 100 pmol oligo(dT) primers, 1µg of the extracted RNA and a cDNA synthesis kit (Pars Tous, Iran) according to the manufacturer's comments. Specific primers were designed for *OCT4A*, *OCT4B*, *OCT4B1*, and ß-actin (as a housekeeping gene) using Gene Runner (version 3.02) and Allele ID (version 4.0) softwares (Table 1).

Quantitative real-time PCR was performed by addition of SYBR green master mix (Parstous, Iran), 200 ng of the generated cDNA, and 2 pg/µl of each the appropriate primer. The following program was set on a BIO-RAD CFX96 system (Bio-Rad Company, USA): one cycle of 95 ℃ for 15 min, 40 cycles of 95 ℃ for 30 sec, 60 ℃ for 30 sec (for *OCT4B1* 61 ℃ for 20 sec) and 72 ℃ for 30 sec. Real-time PCR was carried out in triplicate and ß-actin was employed as a housekeeping gene for normalizing of the amplified signals of the target gene. The relative measures of the PCR product were determined using the $2^{-\Delta\Delta Ct}$ formula. 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 of the PCR products were visualized following electrophoresis on agarose to check the size and quality of the PCR product.

The siRNA transfectionprocedure

In order to suppress *OCT4B1*, two specific siRNAs complementary to the unique region of *OCT4B1* sequence (exon2b), and one irrelevant (scramble) siRNA (with no complementary target sequence in the human genome) were designed, using the siRNA selection program (Whitehead institute for biomedical research, htt://jura.wi.mit.edu/) and were further ordered and synthesized (MWG Germany, Table 2). Tumor cell lines (1×10^5 cells/ml) were seeded onto the six-well plates in RPMI1640 medium lacked antibiotics in two groups (test and control). Once cultured cells achieved a confluency of 30-50%, cells were transfected with 50 nmol/ml of OCT4B1-siRNA

Table1. Sequences of designed primers of OCT4A, OCT4B, OCT4B1 and ß-actin. F=forward R=reverse

Target genes	Designed Oligo	Relative Sequence	Fragmentlength
OCT4A	F	CGCAAGCCCTCATTTCAC	111
	R	CATCACCTCCACCACCTG	
OCT4B	F	CAGGGAATGGGTGAATGAC	177
	R	AGGCAGAAGACTTGTAAGAAC	
OCT4B1	F	GGTTCTATTTGGTGGGTTCC	128
	R	TTCTCCCTCTCCCTACTCCTC	
ß-actin	F	CACACCTTCTACAATGAGC	160
	R	ATAGCACAGCCTGGATAG	

Table 2. 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 control group, scramble siRNA), using Lipofectamin 2000 (Invitrogen, USA) and opti-MEM media, according to the manufacture's instruction. In brief, 5µl of siRNA (25 µM) 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 at a final volume of 2.5ml, and incubated at 37 ˚C in a humidified atmosphere and 5% CO_2 for further 72 hr.

To determine the efficiency of gene suppression, OCT4B1 expression was quantified in OCT4B1-siRNA (test group) and scramble-siRNA transfected (control group) cells, 24, 48 and 72 hr after transfection processes.

HSP40 gene family profiling

Following 48 hr of transfection, cells were heat-shocked at 45 ˚C for 1 hr. As previously described, the total RNA was extracted from cells of either control or test groups and cDNA was synthesized immediately (26). Quantitative gene expression analysis was performed using a real-time PCR array approach (SABiosciences Company, USA).

Statistical analysis

Gene expression analysis and chart drawing were carried out using CFX96 manager software (Bio-Rad, USA), and RT² Profiler PCR Array Data Analysis version 3.5, respectively.

Results

OCT4 variants were expressed and OCT4B1 was down-regulated after siRNA transfection

Present findings indicated that all of the three *OCT4* variants (A, B and B1) were expressed in the studied cell lines (Figure 1). We found the highest expression level of *OCT4B1* in U87-MG cell line. As expected, in response to siRNA transfection, *OCT4B1* expression was dramatically decreased by 24, 48 and 72 hr. As it is clearly demonstrated in Figure 2, the highest level of *OCT4B1* suppression was observed 48 hr post-transfection.

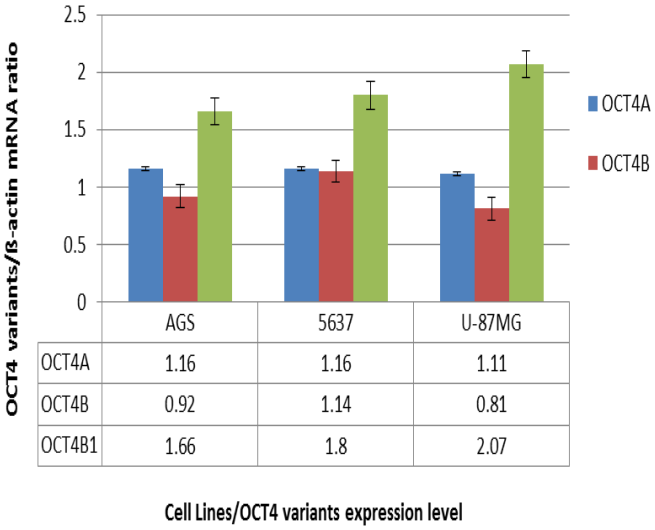

Figure 1. Expression status of OCT4 variants in tumor cell lines. The real-time PCR data showed that all three OCT4 variants (A, B and B1) were expressed in the studied cell lines. The Y axis shows the OCT4B1 variant mRNA expression level compared to ß-actin (as housekeeping control gene) and the X axis indicates three studied tumor cell lines (AGS, 5637 and U87MG)

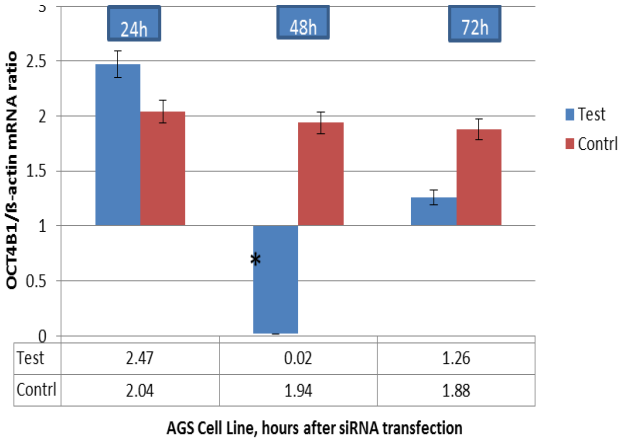

Figure 2. Expression status of OCT4B1 following 24, 48 and 72 hours in response to siRNA transfection.The Y axis shows OCT4B1 variance mRNA expression compared to ß-actin (housekeeping as control gene) and the X axis indicates AGS tumor cell lines, Test; transfected cells with OCT4B1 siRNA and Control; transfected cells with scramble siRNA.
*shows there is a significant difference between control and test groups after 48 hr (P<0.05)

Status of changes in expression of HSP40 gene family in response to OCT4B1 suppression

Our panel PCR data indicated that the expression profile of 36 studied genes in HSP40 gene family in studied tumor cell lines after *OCT4B1* suppression showed approximately unique pattern of expression. Interestingly, 15 genes were down-regulated while two genes (*DNAJC11* and *DNAJC5B*) were up-regulated in all three studied tumor cell line (Figure 2 and Table 3). Other studied genes (19 genes) were variously regulated, so that in some cases we observed up-regulation and in some cases down-regulation was seen in tumor cell lines.

Discussion

Previous reports (23, 24, 27), emphasized on the potential roles played by *OCT4B1* in apoptosis and stress-related response pathways. Farashahi and colleagues reported that under stress condition (heat shock), the expression level of *OCT4B1* was significantly elevated in tumor cell lines (24).

.We have already reported a significant relation between *OCT4B1* and expressional profile of apoptosis regulated genes (25). Here, to decipher the molecular targets in stress pathway (heat shock) which are controlled by *OCT4B1*, we examined the status of the expression of 36 genes from HSP40 gene family following suppression of *OCT4B1* in three different tumor cell lines, by employing RNAi strategy.

According to the results obtained in this investigation, the profile of gene expression of HSP40 gene family was almost similar in all of the studied cell lines. We observed that 34 of 36 studied genes were down-regulated at least in one of three studied cell lines, while 15 genes were down-regulated in all three cell lines. We also observed that 9 genes were down-regulated more than two folds in all of investigated cell lines. Interestingly, two genes (*DNAJC11* and *DNAJC5B*) were up-regulated in all of studied tumor cell lines. Some genes revealed different framework of regulatory, DNAJA1 was down- regulated by -52.03 folds in 5637 and -8.16 folds in U87MG but -1.7 folds in AGS tumor cell lines, respectively. *DNAJB2* was down-regulated -8.13 and -16.43 folds in AGS and 5637 tumor cell lines, respectively while it was also down-regulated by -1.5 in U87Mg tumor cell line (Table 3).

The co-chaperones heat shock protein 40kDa (HSP40) constitute the largest and most diverse sub-group of the heat shock protein (HSP) family. HSP40 genes members are involved in regulation of HSP70 gene family function, as well as HSP90 chaperone machine (28).

In the present study, it was revealed that following *OCT4B1* suppression, the expression of fifteen genes in HSP40 family was decreased (in all three studied tumor cell lines) and other genes were down-regulated in some cell lines. Interestingly, just two genes (*DNAJC11* and *DNAJC5B*) showed up-regulation in all three cell lines suggesting that up-regulation of *OCT4B1* in cancer cells and tissues induce the expression of HSP40 genes family. Consistent with our results, Hi.H.L *et al* showed over-expression of some HSP40 gene members such as *DNAJC12* in rectal cancer (29) and Morita *et al* reported that *DNAJB8* (HSP40) was over-expressed in colon cancer (30).

The expression of *OCT4B1* in cancer cells and tissue (22), in accordance with anti-apoptotic effects of the variant (23, 26), as well as its association with stress signaling pathway (24), may propose that *OCT4B1* up-regulation in tumor cells in turn leads to over-expression of HSP40 family members. Accordingly, it may be considered as a probable mechanism of anti-apoptotic activity of *OCT4B1* in tumor cells. By other means, down-regulation of *OCT4B1* could be assumed as an alternative strategy for HSP40-mediating the molecular-based cancer therapies.

Furthermore, little is known concerning the molecular mechanisms of survival functions of HSPs. Overall, regarding results presented here, in response to *OCT4B1* suppression in tumor cells, expression of 34 (out of 36) members of HSP40 family was decreased at least in one of three studied tumor cell lines, while, two genes showed up-regulation in all three studied tumor cell lines.Therefore, our data may probably demonstrate a general direct association between *OCT4B1* suppression and HSP40 genes down- expression.

Table 3. Gene expression of HSP40 family member following *OCT4B1* suppression in tumor cell lines

Symbol	Description of Genes	AGS	5637	U87MG
DNAJA1	DNAJ (HSP40) homolog, subfamily A, member 1	-1.70	-52.03	-8.16
DNAJA2	DNAJ (HSP 40) homolog, subfamily A, member 2	-5.29	-7.25	-2.54
DNAJA3	DNAJ (HSP 40) homolog, subfamily A, member 3	-1.85	-2.53	1.13
DNAJA4	DNAJ (HSP 40) homolog, subfamily A, member 4	1.60	-4.18	-1.47
DNAJC21	DNAJ (HSP 40) homolog, subfamily C, member 21	-2.51	-4.35	-15.03
DNAJB1	DNAJ (HSP 40) homolog, subfamily B, member 1	-2.06	-2.82	1.01
DNAJB11	DNAJ (HSP 40) homolog, subfamily B, member 11	-3.72	-5.09	-46.40
DNAJB12	DNAJ (HSP 40) homolog, subfamily B, member 12	-6.98	-15.54	-2.39
DNAJB13	DNAJ (HSP 40) homolog, subfamily B, member 13	1.13	-1.21	5.79
DNAJB14	DNAJ (HSP 40) homolog, subfamily B, member 14	-1.78	1.96	5.59
DNAJB2	DNAJ (HSP 40) homolog, subfamily B, member 2	-8.13	-16.43	-1.50
DNAJB5	DNAJ (HSP 40) homolog, subfamily B, member 5	-2.54	-3.48	-1.22
DNAJB6	DNAJ (HSP 40) homolog, subfamily B, member 6	-1.57	-7.88	1.32
DNAJB7	DNAJ (HSP 40) homolog, subfamily B, member 7	-31.94	-43.75	2.45
DNAJB8	DNAJ (HSP 40) homolog, subfamily B, member 8	-2.49	-3.41	-1.19
DNAJB9	DNAJ (HSP 40) homolog, subfamily B, member 9	-22.69	-11.62	-3.47
DNAJC1	DNAJ (HSP 40) homolog, subfamily C, member 1	-2.68	-3.68	-1.29
DNAJC10	DNAJ (HSP 40) homolog, subfamily C, member 10	1.20	-1.14	2.50
DNAJC11	DNAJ (HSP 40) homolog, subfamily C, member 11	12.26	7.42	6.75
DNAJC12	DNAJ (HSP 40) homolog, subfamily C, member 12	-1.58	-2.17	1.31
DNAJC13	DNAJ (HSP 40) homolog, subfamily C, member 13	-4.10	-5.77	-2.05
DNAJC14	DNAJ (HSP 40) homolog, subfamily C, member 14	-1.23	-1.68	1.70
DNAJC15	DNAJ (HSP 40) homolog, subfamily C, member 15	67.46	20.28	-1.24
DNAJC16	DNAJ (HSP 40) homolog, subfamily C, member 16	-1.31	-1.80	1.58
DNAJC17	DNAJ (HSP 40) homolog, subfamily C, member 17	-9.81	-13.44	-2.36
DNAJC18	DNAJ (HSP 40) homolog, subfamily C, member 18	-1.33	-1.83	1.56
DNAJC19	DNAJ (HSP 40) homolog, subfamily C, member 19	-	-	-
DNAJC3	DNAJ (HSP 40) homolog, subfamily C, member 3	-1.33	1.35	3.85
DNAJC4	DNAJ (HSP 40) homolog, subfamily C, member 4	-1.17	-1.60	1.78
DNAJC5	DNAJ (HSP 40) homolog, subfamily C, member 5	24.75	-2.20	3.03
DNAJC5B	DNAJ (HSP 40) homolog, subfamily C, member 5 beta	2.37	1.73	4.94
DNAJC5G	DNAJ (HSP 40) homolog, subfamily C, member 5 gamma	-1.35	-1.40	-2.38
DNAJC6	DNAJ (HSP 40) homolog, subfamily C, member 6	-1.44	-1.66	-2.70
DNAJC7	DNAJ (HSP 40) homolog, subfamily C, member 7	1.08	-1.27	2.24
DNAJC8	DNAJ (HSP 40) homolog, subfamily C, member 8	-12.50	-19.27	-2.65
DNAJC9	DNAJ (HSP 40) homolog, subfamily C, member 9	-8.84	-4.49	-2.84
SERPINH1	Serpin peptidase inhibitor, clade H 1	-4.01	-8.27	3.82

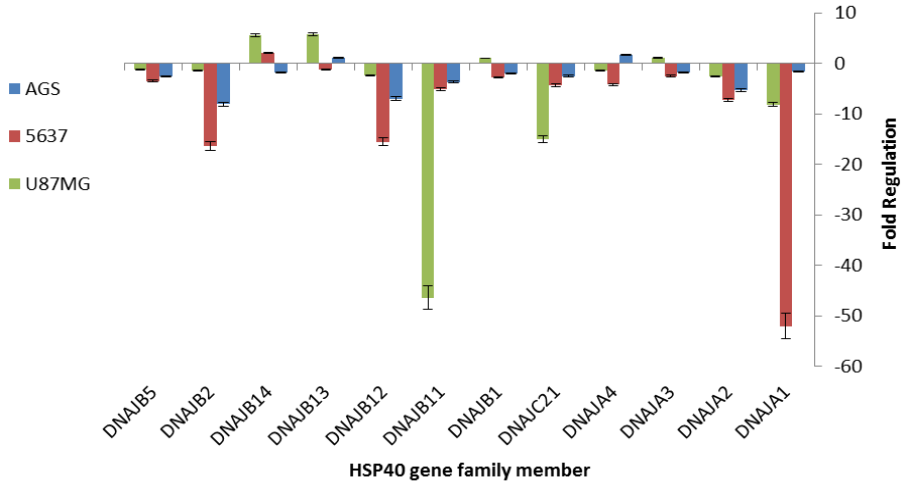

Figure 3-1. HSP40 gene expression, 48 hr after siRNA transfection in studied tumor cell lines. Y axis showed fold gene regulation and X axis showed 36 genes of HSP40 gene family

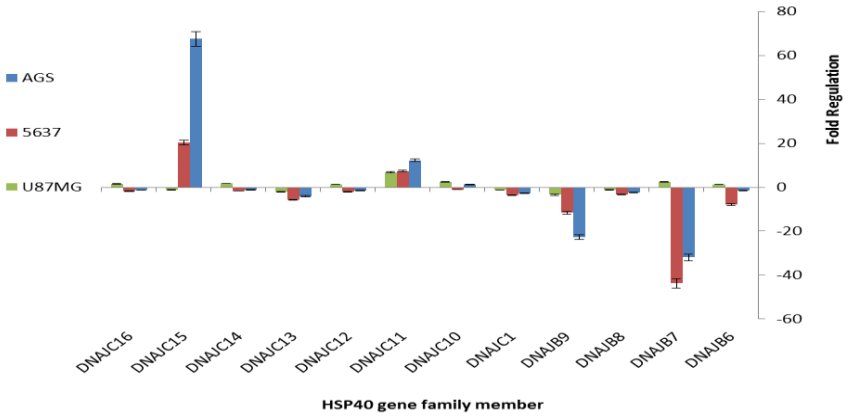

Figure 3-2. HSP40 gene expression, 48 hr after siRNA transfection in studied tumor cell lines. Y axis showed fold gene regulation and X axis showed 36 genes of HSP40 gene family

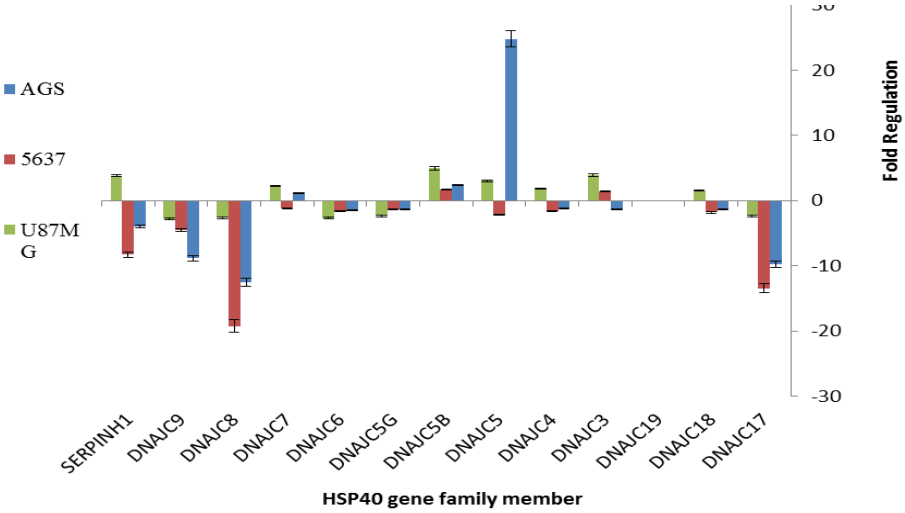

Figure 3-3. HSP40 gene expression, 48 hr after siRNA transfection in studied tumor cell lines. Y axis showed fold gene regulation and X axis showed 36 genes of HSP40 gene family

Conclusion

The data presented here may support the theory of possible direct association between the expression of *OCT4B1* and HSP40 gene family. Accordingly, OCT4B1 suppression in different tumor cell lines lead to a significant down-regulation of the expression of the members of HSP40 family.

Acknowledgment

This project was financially supported by the Rafsanjan University of Medical Sciences, Rafsanjan, Iran and the results described in this paper were part of a student thesis.

Conflict of interest

None of authors of the present article declared conflict of interest.

References

1. Sanchez Y, Parsell DA, Taulien J, Vogel JL, Craig EA, Lindquist S. Genetic evidence for a functional relationship between Hsp104 and Hsp70. J Bacteriol 1993; 175:6484-6491.
2. Parcellier A, Gurbuxani S, Schmitt E, Solary E, Garrido C. Heat shock proteins, cellular chaperones that modulate mitochondrial cell death pathways. Biochem Biophys Res Commun 2003; 304:505-512.
3. Gupta SC, Sharma A, Mishra M, Mishra RK, Chowdhuri DK. Heat shock proteins in toxicology: how close and how far? Life *S*ci 2010; 86:377-384.
4. Alexiou GA, Vartholomatos G, Stefanaki K, Patereli A, Dova L, Karamoutsios A, *et al*. Expression of heat shock proteins in medulloblastoma. J Neurosurg Pediatr 2013; 12:452-457.
5. Schmitt E, Gehrmann M, Brunet M, Multhoff G, Garrido C. Intracellular and extracellular functions of heat shock proteins: repercussions in cancer therapy. J Leukoc Biol 2007; 81:15-27.
6. Lanneau D, Brunet M, Frisan E, Solary E, Fontenay M, Garrido C. Heat shock proteins: essential proteins for apoptosis regulation. J Cell Mol Med 2008; 12:743-761.
7. Ciocca DR, Calderwood SK. Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications. Cell Stress Chaperones 2005; 10:86-103.
8. Reed JC. Mechanisms of apoptosis avoidance in cancer. Curr Opin *O*ncol 1999; 11:68-75.
9. Li J, Buchner J. Structure, function and regulation of the hsp90 machinery. Biomed J 2013; 36:106-117.
10. Ischia J, So AI. The role of heat shock proteins in bladder cancer. Nat Rev Urol 2013 ; 10:386-395.
11. Clarke MF, Fuller M. Stem cells and cancer: two faces of eve. Cell 2006; 124:1111-1115.
12. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature 2001; 414:105-111.
13. Scholer HR, Ruppert S, Suzuki N, Chowdhury K, Gruss P. New type of POU domain in germ line-specific protein Oct-4. Nature 1990; 344:435-439.
14. Lee J, Kim HK, Rho JY, Han YM, Kim J. The human OCT-4 isoforms differ in their ability to confer self-renewal. J Biol Chemi 2006; 281:33554-3365.
15. Prud'homme GJ. Cancer stem cells and novel targets for antitumor strategies. Curr Pharm Des 2012; 18:2838-2849.
16. Yasuda H, Tanaka K, Okita Y, Araki T, Saigusa S, Toiyama Y, *et al*. CD133, OCT4, and NANOG in ulcerative colitis-associated colorectal cancer. Oncol Lett 2011; 2:1065-1071.
17. Ricci MS, Zong WX. Chemotherapeutic approaches for targeting cell death pathways. Oncologist 2006; 11:342-357.
18. Gao Y, Wang X, Han J, Xiao Z, Chen B, Su G, *et al*. The novel OCT4 spliced variant OCT4B1 can generate three protein isoforms by alternative splicing into OCT4B. J Genet Genomics 2010; 37:461-465.
19. Cheng L, Sung MT, Cossu-Rocca P, Jones TD, MacLennan GT, De Jong J, *et al*. OCT4: biological functions and clinical applications as a marker of germ cell neoplasia. J Pathol 2007; 211:1-9.
20. Rijlaarsdam MA, van Herk HA, Gillis AJ, Stoop H, Jenster G, Martens J, *et al*. Specific detection of OCT3/4 isoform A/B/B1 expression in solid (germ cell) tumours and cell lines: confirmation of OCT3/4 specificity for germ cell tumours. Br J Cancer 2011; 105:854-863.
21. Atlasi Y, Mowla SJ, Ziaee SA, Gokhale PJ, Andrews PW. OCT4 spliced variants are differentially expressed in human pluripotent and nonpluripotent cells. Stem Cells 2008; 26:3068-3074.
22. Asadi MH, Mowla SJ, Fathi F, Aleyasin A, Asadzadeh J, Atlasi Y. OCT4B1, a novel spliced variant of OCT4, is highly expressed in gastric cancer and acts as an antiapoptotic factor. Int J C*a*ncer 2011; 128:2645-2652.
23. Farashahi Yazd E, Rafiee MR, Soleimani M, Tavallaei M, Salmani MK, Mowla SJ. OCT4B1, a novel spliced variant of OCT4, generates a stable truncated protein with a potential role in stress response. Cancer Lett 2011; 309:170-175.
24. Mirzaei MR, Najafi A, Arababadi MK, Asadi MH, Mowla SJ. Altered expression of apoptotic genes in response to OCT4B1 suppression in human tumor cell lines. Tumour Biol 2014 ; 35:9999-10009.
25. Asadzadeh J, Asadi MH, Shakhssalim N, Rafiee MR, Kalhor HR, Tavallaei M, *et al*. A plausible anti-apoptotic role of up-regulated OCT4B1 in bladder tumors. Urol J 2012; 9:574-580.
26. Momeni M, Reza Mirzaei M, Zainodini N, Hassanshahi G, Arababadi MK. MiR-143 *i*nduces *e*xpression of AIM2 and ASC in jurkat *c*ell line. Iran J Immunol 2013; 10:103-109.
27. Li D, Yang ZK, Bu JY, Xu CY, Sun H, Tang JB, *et al*. OCT4B modulates OCT4A expression as ceRNA in tumor cells. Oncol Rep 2015; 33:2622-2630.
28. Sterrenberg JN, Blatch GL, Edkins AL. Human DNAJ in cancer and stem cells. Cancer Lett 2011; 312:129-142.
29. He HL, Lee YE, Chen HP, Hsing CH, Chang IW, Shiue YL, *et al*. Overexpression of DNAJC12 predicts poor response to neoadjuvant concurrent chemoradiotherapy in patients with rectal cancer. Exp Mol Pathol 2015; 98:338-345.
30. Morita R, Nishizawa S, Torigoe T, Takahashi A, Tamura Y, Tsukahara T, *et al*. Heat shock protein DNAJB8 is a novel target for immunotherapy of colon cancer-initiating cells. Cancer *S*ci 2014; 105:389-395.