Mosul Journal of Nursing

www.mjn.mosuljournals.com

Gene Expression Profile Of Lncrna Meg3 In Prostate Cancer And Normal Cell Lines

Article information

Article history:
Received January 7, 2021
Accepted January 22, 2021
Available online April 22, 2021

DOI: 10.33899/mjn.2021.168003

https://mjn.mosuljournals.com/article_168003.html

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Abstract

Background and Aim: Cancer is one of the major of human problems and prostate cancer is a popular healthy problem in cancers. In spite of the developing treatment methods, prostate cancer cannot be treated effectively.Therefore, the identification of novel genes that will be role in the treatment and diagnosis of prostate cancer is of greatest interestmeg3 (meg3 family tumer suprosser gene are key regulators of programed death cell .

Objective Although studies have saw that this meg 3 has processes, its usefulness in the treatment and diagnosis of prostate cancer has not been fully elucidated.At the same time, previous studies have shown that the meg 3gene is associated with important protein apoptosis triggering proteins such as HAGLR and TSIX. In this study, by using gene expression level, we aimed to investigate the relationship between the levels of meg3,and haglrgene which the prognosis and apoptosis processes of prostate cell line.

Materials and Methods: Collected of the sample from normal and cancer tissue type in the cell line, RNA isolated from the cell, RNA quantitation, cDNA synthesised, cDNA quantitation ,and Real -time PCR was performed to see the level between two types of genes.

Results: meg3 tumer supresur level was significantly lessened in regulator molecule tissues of a cell line with prostate cancer . In contrast to HAGLRprotein, no significant difference was detected in TSIX expression level. However, Our study also showed that low meg3 expression level is associated with important clinic characteristics of the patient such as tumor grade, stage and breast cancer type.

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Conclusion: Findings of our study show that meg3 has roles in prostate cancer formation and might be a novel biomarker for the diagnosis and treatment of cancer.

Key words: Apoptosis, MEG3, prostate Cancer, gene expression , MEG3, HAGL

INTRODUCTION

Prostate cancer: is one of the most common types of cancer in men. Usually prostate cancer is a major disease that effect mens health .worldwide .it is a second most common form of cancer in men [1].where it may not cause serious harm. However, while some types of prostate cancer grow slowly and may need minimal or even no treatment, other types are aggressive and can spread quickly. [15]

Aims: A substantial fraction of cancer's LncRNAs exert diverse biological functions by regulating gene expressions and functions[20].. Recent studies show that several cancer risk loci are transcribed into lncRNAwhich plays key roles in tumorigenesis [3].The dysregulation and alteration of several lncRNAs has been reported in various cancer types. Based on the expression lncRNAs,expression profiling of human tumors as identified marks associated with diagnosis, staging, progression, prognosis, and response to treatment [2]. MEG3 is suggested to function as a tumor suppressor lncRNA [5]. In this study, determination of expression profile of MEG3 gene in various cancer and normal cell lines was aimed in order to lead more information about its function in cancer.

In many types of cancer, meg3 inhibiting the expression of sense gene , leading to disruption of the linc RNA mechanism[7]. Meg3 one of the key molecules that lead to multidrug resistance to the cell,. (MEG3) (lncRNA) located on chromosome 14q32.3. [8]. The excess level of meg3 inhibits cell death whereas low levels of meg3 increase the susceptibility of the cell to various toxins. [9].

Meg3 tumer suppruser gene are classified in three subfamilies. The meg3 and HAGLR, are of high interest for cancer treatment, with major key questions yet to be elucidated[10].HAGLR is aLong non-coding RNAs , have limited or no protein- coding , regulation network of cancer and play an important role in tumourigenesis and progression [11] The HOXD-AS1 (also known as HAGLR gene is located between the HOXD1 and HOXD3 genes in the HOXD cluster[12] are new players in gene regulation but their mechanisms of action are mainly undocumented[13] .In this study that regulate cancer cell MEG3 and HAGLR may have important roles for several tumorigenic processes. To explore their roles, ATCC normal and cancer (PC,) cell lines were subjected to examine through RNA isolation, cDNA conversion, quantitative RT-PCRfor expression analyses. (MEG3 and HAGLR) genes have differential expression pattern in both normal and cancer cell lines. The obtained results indicate their importance for biological processes in cancer. Tsix is instead required to silence Xist on the active-X[14] TSIX siRNA reduced the mRNA expression of type I collagen [15] Genetic variation at 8q24 is a major contributor to prostate cancer (PCa) [17]. The therapeutic landscape of prostate cancer has been transformed over the last decade by new therapeutics, [18]. Precision medicine for advanced prostate cancer may identify new treatment strategies and change clinical

practice [19]. prostate cancer that facilitates tumor formation, disease progression and therapeutic resistance[21]. FOXAl regulatory plexus harboring somatic singlenucleotide variants in primary prostate tumors [22]. Genome sequencing and gene expression analyses of prostate tumours[23].

MATERIALS AND METHODS

Participants

Collection of the sample from normal and cancer tissue type in cell line RNA isolation from the cell, RNA quantitation,cDNA synthesis,cDNA quantitation ,and Real Time- PCR analysis. We can do real -time PCR to see the level between of genes.

RNA isolation from cell line

The RNA isolation protocol is as follows; The cells removed with Trypsin, and DMEM containing FCS is added to stop the effect of trypsin Cells are centrifuged at 3500 rpm for 5 min. The supernatant is removed without touching the pellet. The remaining pellet is resuspended in 200 Oil PBS. Add 400 pl of Lysis Buffer to this mixture and vortex for 15 seconds. The whole mixture is transferred to filter tubes, centrifuged for 30 seconds at 9200 rpm. The lower part is discarded. Add 100 p1 (10 JilDNAse and 90 JilDNAse incubation buffer) to the filtered tubes and wait at room temperature for 45 minutes. Add 500 ql Wash Buffer I and centrifuge at 9200 rpm for 30 seconds. The lower part is discarded. Add 500 Oil Wash Buffer II and centrifuge at 9200 rpm for 30 seconds. The bottom tube is replaced with the new one. Add 200 ql of Wash Buffer II and centrifuge for 2 minutes at 11800 rpm. The lowertube is discarded and a new tube is inserted. Add 50 p1 of Elution Buffer and wait for1 minute at room temperature. Centrifuge at 9200 rpm for 1 minute. The filtered tube is discarded. Measurements are made on the NanoDrop 1000 to determine the amount of RNA. RNAs are stored at -80 °C until the working period.

RNA Quantitation

Determination of quantity and quality of obtained RNA samples were done by detecting A260/A280 ratio using Nanodrop spectrophotometer. For PCR reactions, RNA was diluted according to their density.

Real-Time PCR (qPCR) Analyzes

This experiment, a Rotor- Gene Q (QIAGEN,) Real-Time PCRinstrument was used. qPCR experiments were performed in the direction of the manufacturer's firm using Maxima SYBR Green / ROX qPCR Master Mix (# K0251). For this study, appropriate synthetic primers were designed for exonregions of MEG3 and HAGLRgenes by using NCBI/Primer Blast database. Table 1 in a detailed manner.

	sequence (5'->3')	Primer lenght	Tempertur e (°C)	GC%	PCR product(bp)
TSIX	GTTGCATCAGCTGTCCTCCT	17	57.75	64.71	221
	AAAAAGGGGTTGGGGTAGG	20	61.90	60.00	
HAGLR	ACCAGACCTACTCTTCCGCT	20	59.88	55.00	246
	GGGAAGAGCCAAGTCAGAC	20	60.03	55.00	
MEG3	ATCTGGTGAGCCAGGTAGGA	20	59.53	55.00	207
	GGGAAGAGCCAAGTCAGAC	21	59.44	52.38	

RESULTS

As figure 1 shows the expression levels of meg3 was found to be significantly diminished in the tumoral tissues of prostate cancer patients as compared to normal tissues (p=0.0078).

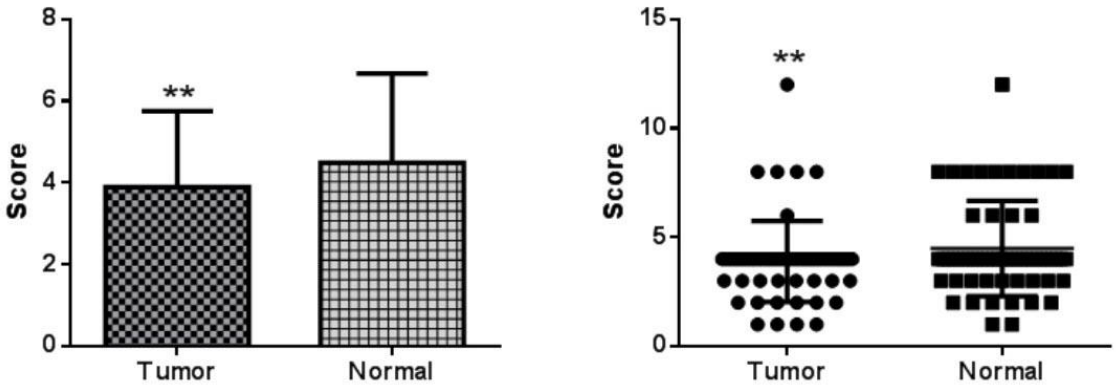


Figure 1.Meg3gene expression levels according to prostate cancer diagnosis,* p=0.0078.

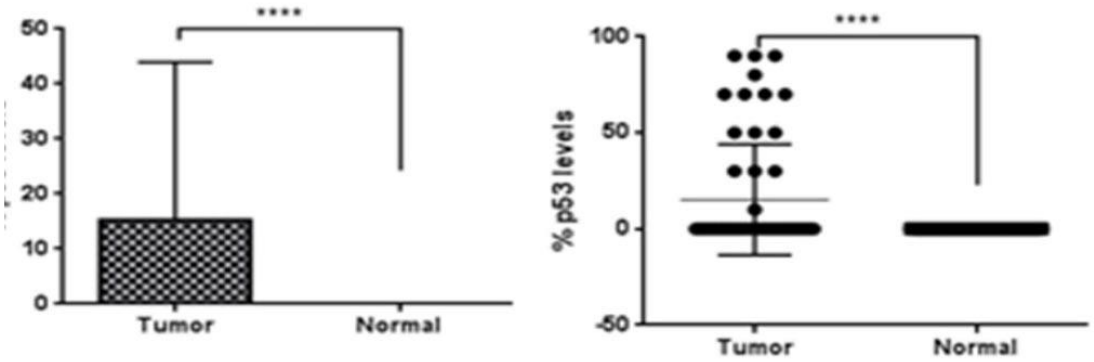


Figure 2.Expression levels of HAGLRgene in invasive prostate cancer subtype. *** p=0.0001.

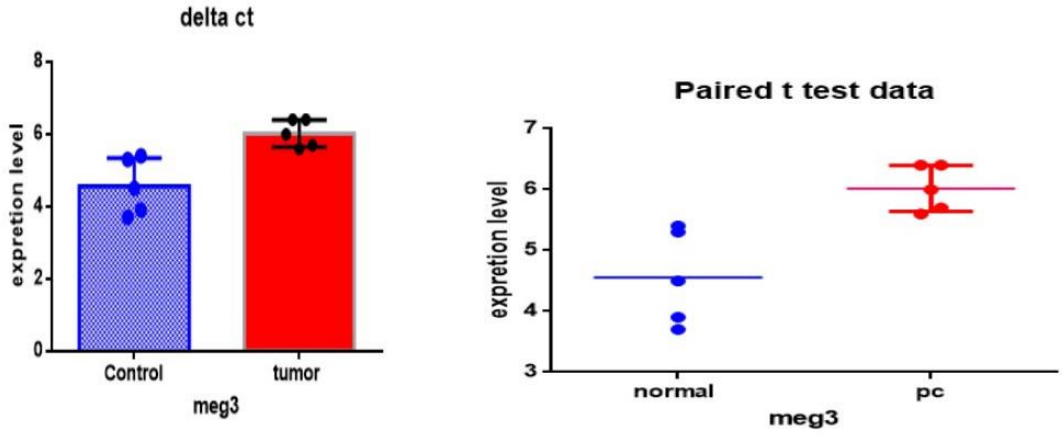


Figure 3.Meg3gene expression levels according to prostate cancer* p=0.0271.

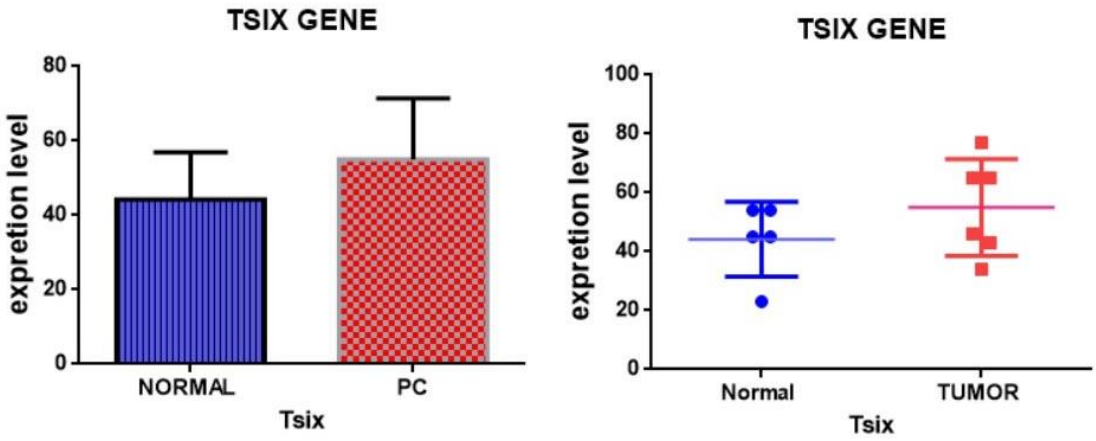


Figure 4.Tsix gene expression levels according to prostate cancer* p=0.0581.

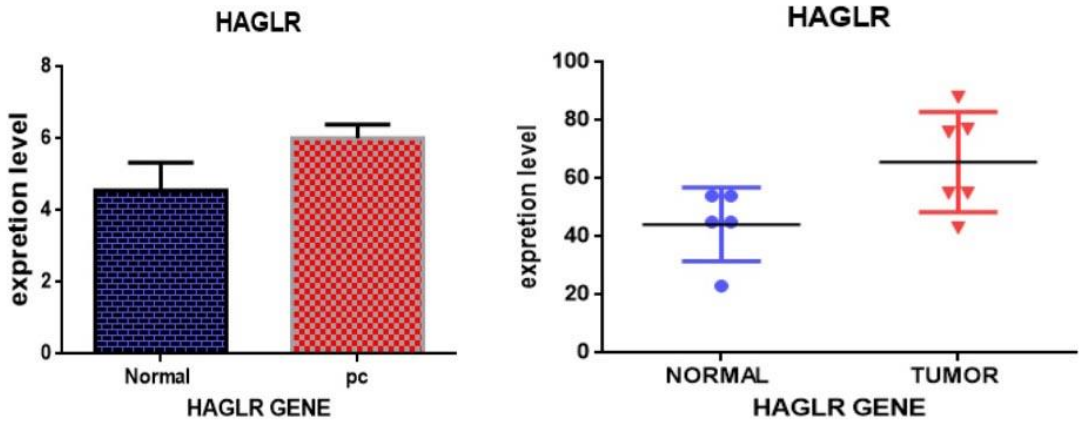
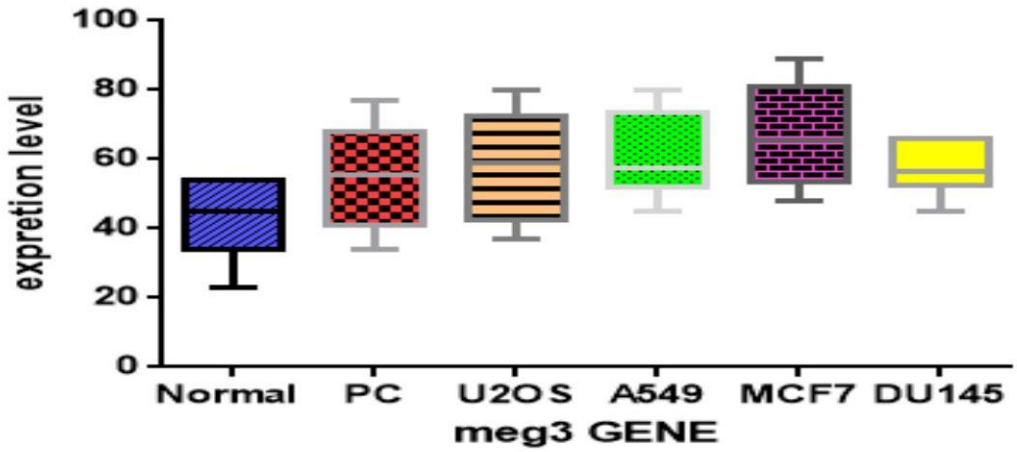


Figure 5.HAGLR gene expression levels according to prostate cancer* p=0.0001.



Figur :6 expression level from different cell line in meg3 gene .

DISCUSSION

prostate cancer is one of the most common solid tumors in men, with an year considered of 1.3 million cases and approximately 500,000 deaths.[2,] therapeutic and prognostic reasons, prostatecancer is treated on the expression level Another more differentiated methodology used in the characterization of prostate cancers is tumor grading

In this study, the range of expression of meg3gene was determined in a total number of cell line were diagnosed with prostate cancer. figer (1).The expression level of HAGLR can be increased of of tumer we compare with the normal tissue depend ona grade of the tumor . the expression level of tsix was no changed between normal and tumor it means there is significant according to figure 3.

The number new cases of prostate cancer in men over 60 years of age in China was about 56 600 in 2015[24]. in this study, we aimed to reveal the roles of meg3, HAGLR ,TSIX , which play roles in prostate cancer progress. Healthy tissues of patients were used as controls. Particularly, meg3 gene can expressed levels were significantly increase in tumor tissues of prostate cancer patients ($p = 0.0078$). In addition, there was significant difference in the expression level of HAGLR gene between normal and tumor tissues patients ($P= 0.0001$)in prostate cancer figer (2). In addition, High-risk disease accounts for approximately 15% of prostate cancer diagnoses[25], the Tsix gene showed that the expression level was find to be significantly higher in tumor tissues of breast cancer patients ($p <0.0001$). However, while approximately 24% of the patients included in the study were P53 positive, 75% of the breast cancer patients were P53 negative.

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