Pathology - Research and Practice 215 (2019) 506–511

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Contribution and prognostic value of TSGA10 gene expression in patients with acute myeloid leukemia (AML)

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ARTICLE INFO

Keywords:
TSGA10
AML
HIF
Angiogenic factor

ABSTRACT

Background: Different studies have investigated TSGA10 expression in various cancerous tissues but, so far no study has been conducted on newly diagnosed (ND) AML patients. The association of TSGA10 gene expression with hypoxia inducible factor (HIF) and angiogenic factors has remained to be fully elucidated and is still a controversial issue. The present study was designed to investigate this association in patients newly diagnosed with AML.

Methods: We evaluated TSGA10, HIF-1α and VEGF mRNA levels in ND AML patients and healthy subjects using real-time PCR technique. Data were analyzed via comparative Livak method.

Results: Based on the results of this study, TSGA10 gene expression was decreased in 28 out of 30 (93.3%) samples while VEGF and HIF-1α expression levels were increased in all ND AML patients compared to healthy controls. Diagnostic evaluation was performed by receiver operating characteristic (ROC) curve and area under the curve (AUC) calculation. Respectively, using cut-off relative quantification of 1.604, 0.0329, and 0.0042, the sensitivity values of TSGA10, VEGF, and HIF-1α gene expression were 86.7%, 90%, and 100%. Also, specificity values were 100%, 100% and 100%, respectively. TSGA10 expression was shown to be reduced in ND AML patients compared with healthy subjects and we found a negative correlation between TSGA10 and VEGF expression.

Conclusions: Since TSGA10 interacts with HIF-1 and affects its transcriptional activity, in ND AML patients with decreased TSGA10 expression, VEGF expression was high suggesting a TSGA10 mediated regulation of HIF-1 target genes. Altogether, the current study showed that TSGA10 could be considered as a tumor suppressor in AML patients.

1. Introduction

Acute myeloid leukemia (AML) is associated with rapid growth and accumulation of abnormal white blood cells in the bone marrow causing interference in the production of normal blood cells. AML is the most common leukemia which affects adults, and its incidence increases with age. Replacement of normal bone marrow with the abnormal cells mentioned earlier leads to a drop in red blood cells, platelets, and normal white blood cells [1–3]. AML, being known as a rapidly developing acute leukemia, is typically fatal within weeks or months if left untreated. In order to achieve appropriate treatment and complete remission, it is really crucial to make an early diagnosis. Genes and their products involved in leukemia might represent helpful diagnostic markers for the disease [4]. They could also be regarded as appropriate targets for achieving novel therapeutic perspectives.

The TSGA10 gene (NC_000002.12) expressed in testis during developmental stages of spermatogenesis and embryogenesis is located on the chromosome region 2q11.2 [5,6]. The length of TSGA10 mRNA is about 3 kb and contains up to 21 exons (ENST00000393483.7) in different transcripts. This gene was identified using differential mRNA display technique in 2000 [5]. The protein product weighs 82 kDa which would be broken down in cytoplasm of the cell into two fragments of 27 kDa and 55 kDa. These fragments exert inhibitory effects on hypoxia inducible factor-1α (HIF-1α) and inhibit its localization into

Abbreviations: AML, acute myeloid leukemia; AUC, area under the curve; CT, cancer-testis; CTA, cancer testis antigen; HIF, hypoxia inducible factor; ND, newly diagnosed; ROC, receiver operating characteristic; TSGA10, testis specific gene antigen 10; VEGF, vascular endothelial growth factor

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https://doi.org/10.1016/j.prp.2019.01.003
Received 31 August 2018; Received in revised form 15 December 2018; Accepted 5 January 2019

nucleus consequently preventing its transcriptional activity. In oxygenated cells, HIF-1α subunits are unstable since they are targeted for proteasomal degradation [7,8]. However, in hypoxic cells, HIF-1α degradation is suppressed allowing the transcriptional activation of this protein on target genes [9]. Hypoxia-inducible factors (HIFs) respond to the decrease of available oxygen in the cellular environment (hypoxia). Study on HeLa cells has confirmed that TSGA10, through binding to HIF-1α, prevents its nuclear localization leading to decreased HIF-1α transactivation activity [10]. Activated HIF, by transcriptional activation of over 100 downstream genes regulating vital biological processes involved in tumor survival and progression, plays a key role in adaptive responses of the tumor cells to changes in oxygen level. These genes are implicated in glucose metabolism, cell proliferation, migration and angiogenesis. Angiogenesis has been shown to play a role in hematological disorders, and low oxygen pressure might thereby affect leukemogenesis and chemo-sensitivity in human AML [11]. In comparison to most healthy tissues, bone marrow environment is characterized by a low oxygen tension which is a hallmark of the bone marrow stem cell niches. Hypoxia, by inducing the secretion of several growth factors and cytokines including CXCL-12, vascular endothelial growth factor (VEGF) and interleukin-6 (IL-6), is involved in the maintenance of hematopoietic stem cells (HSCs). Also, hypoxia may affect hematopoietic cells by the modulation of the stromal cells. It has been demonstrated that hypoxia affects survival, proliferation, differentiation as well as metabolism of mesenchymal stromal/stem cells (MSCs). Hypoxic microenvironment of the bone marrow has been shown to contribute to AML mainly via HIF-mediated signaling. Hypoxia is implicated in the pathogenesis of hematological malignancies (including AML) in different ways such as: angiogenesis induction, promotion of metastasis and spread of leukemic cells and induction of stemness and drug resistance. It has been reported that in AML, hypoxia, via CXCR4 upregulation, augments survival and migration of leukemic cells and hypoxic bone marrow niches increase resistance to treatments by protecting from various stresses including oxidative stress signals, DNA damage and cell death stimuli [12–14].

Vascular endothelial growth factor (VEGF) gene expression is regulated by hypoxia, in a HIF-dependent manner. VEGF signaling increases autocrine AML blast cell proliferation and survival as well as renders them resistant to chemotherapy. In addition, VEGF signaling, in a paracrine manner, mediates vascular endothelial cell-controlled angiogenesis contributing to AML progression [15]. Expression of VEGF, as a key angiogenic factor first identified as an essential growth factor for vascular endothelial cells [16], shows a positive correlation with therapeutic response failure and can be evaluated for prognosis prediction of AML patients [17]. There are controversial studies about the exact role of TSGA10 in cancer. While several studies point to increased TSGA10 expression levels in cancer cells compared to normal ones and introduce it as a cancer-testis antigen (CTA) [6,18–20], recent studies have reported that TSGA10 expression in cancer cells is decreased as compared to normal cells identifying it as a tumor suppressor [21–23]. According to the previous reports, TSGA10 is expressed in skin (66%), brain (83%), breast (66%) and gastrointestinal tumors (58%) [19]. According to another report, TSGA10 is expressed in 70% of breast cancer patients [20]. Different studies have investigated TSGA10 expression in various cancerous tissues, but so far no study has been conducted on newly diagnosed (ND) AML patients. On the other hand, the association of TSGA10 gene expression with HIF-1 and angiogenic factors in such patients has not been determined. Therefore, the present study was conducted to explore these issues.

2. Material and methods

Peripheral blood was obtained from 30 ND AML patients and 10 healthy volunteers (age 18–68). Based on FAB classification which categorizes AML subtypes according to type of the cell from which the leukemia develops and its degree of maturity, AML was divided into subtypes: M0 through M7 by morphological evaluation and immunophenotyping. The research protocol was approved by Ethics Committee of Kermanshah University of Medical Sciences in accordance with international agreements (ID number: 2264) (Table 1).

Table 1
The clinical characteristics of acute myeloid leukemia patients.

Cod	FAB class	Age	WBC × 10^3	Hb	Blast (%)	PLT × 10^3	AML
1	AML-M5	33	14	7.5	23	38	New
2	AML-M2	18	2.2	8.9	30	2	New
3	AML.M4	58	65	6.7	19	46	New
4	AML-M2	45	97.7	10.5	24	72	New
5	AML-M0	28	14.9	9.7	24	20	New
6	AML.M2	25	3.9	9.5	27	25	New
7	AML- M2	30	6.1	11.9	16	270	New
8	AML.M5	45	80.5	8	30	8	New
9	AML.M2	25	25	10.1	34	21	New
10	AML.M2	59	14.1	10	36	9	New
11	AML- M0	34	9.9	8.5	22	63	New
12	AML.M2	47	4.6	7.7	42	12	New
13	AML.M2	36	9	7.7	25	17	New
14	AML.M2	37	5.2	12	17	128	New
15	AML.M4	55	10.4	7.1	25	39	New
16	AML.M4	50	7.6	6.2	30	166	New
17	AML.M2	45	174	8	28	51	New
18	AML.M2	32	27.5	12.2	26	25	New
19	AML.M1	43	1.4	13	29	135	New
20	AML-M1	44	40.8	8.6	30	30	New
21	AML-M2	26	37	9.2	38	4	New
22	AML-M7	58	1.4	5.2	24	23	New
23	AML-M2	28	187.7	8.6	29	45	New
24	AML-M2	59	55.5	5.5	31	50	New
25	AML-M2	38	11.2	7.2	29	40	New
26	AML-M2	27	12.9	9.3	28	63	New
27	AML M4	75	60	7.5	27	42	New
28	AML M4	51	74.4	10.3	24	41	New
29	AML-M2	63	1.0	9.3	21	112	New
30	AML-M2	21	2.0	10.6	46	67	New

Total RNA was extracted from the peripheral blood of patients and healthy controls using Trizol reagent (Invitrogen), as recommended by the supplier. Sense and antisense primers of target genes were designed by robust oligo primer design software (synthesized by the Bioneer Company). The primers used for each gene are as follows: TSGA10 forward 5‘-ATCAACAACGGCACATGCTAT-3′, reverse 5′-AGACTATCTC GTTCTGTGGTCAT- 3′, VEGF forward 5‘-GGCTGGCAACATAACAGA GAA-3′, reverse 5‘ – CCCCACATCTATACACACCTCC-3′, HIF-1α forward 5′-CTG AGG TTG GTT ACT GTT GGT ATC-3′, reverse 5′-AGT GTA CCC TAA CTA GCC GAG GAA-3′, BETA forward 5′-TGGCACCCAGCACAAT GAA-3′, reverse 5′-CTAAGTCATAGTCCGCCTAGAAGCA -3′ (Table 2).

Real-time PCR was performed on RNA samples using Takara Kit by Rotor Gene 6000 system (Corbett Research, Australia). Cycling conditions were 95 °C for 30 s. In addition, 40 cycles of 95 °C for 5 s and 60 °C for 1 min at the optimized conditions were also run (for PCR step). Finally, melting was carried out at 60–95 °C (with 0.5 °C increments) for 5 s for each step. Relative quantification analysis was carried out using Livak method [24]. Finally, by plotting receiver operating characteristic (ROC) curves and defining area under the curve (AUC), the clinical performance of TSGA10, VEGF, and HIF-1α relative quantification was specified. The optimal cut off point values for relative quantification that separates case and control groups was determined. The analysis used the samples’ crossing point, the efficiency of the reaction, and the number of cycles completed and other values to compare the samples.

3. Statistical analysis

The obtained data are presented as means ± SD and depicted by GraphPad Prism (version 6.0, Inc, La Jolla, CA, USA) [25]. The correlation calculation between TSGA10 and VEGF gene expression was performed using Bivariate Correlation analysis and Pearson coefficient

Table 2
The sequence of primers used in this study.

GENE	Forward	Reverse	Product length
TSGA10	5- ATCAACAACGGCACATGCTAT -3	5- AGACTATCTCGTTCTGTGGTCAT -3	96 bp
VEGF	5- GGCTGGCAACATAACAGAGAA -3	5- CCCCACATCTATACACACCTCC -3	159 bp
HIF-1α	5- CTGAGGTTGGTTACTGTTGGTATC-3	5- AGTGTACCCTAACTAGCCGAGGAA-3	113 bp
BETA	5-AGCTGCCGTTATACTGTTCTG-3	5-ACTGCCTCCTGTGTCTTCAATCTT-3	186 bp

(Two-tailed) determination.

4. Results

The data obtained from 30 ND AML samples and 10 healthy controls are illustrated in Table 1. After obtaining the original data, the expression data were analyzed using Livak method.

Gene expression profile of AML cases and normal controls were analyzed using real-time PCR arrays which showed a significant difference between AML patients and normal controls.

However, no significant association was found between TSGA10 expression level with neither age nor the type of AML. The results of our study showed that 28 (93.3%) patients exhibited lower levels of TSGA10 expression while all of them had higher levels of VEGF and HIF-1α expression in comparison to normal controls (Figs. 1–3). Our results show that there is a significant association between expression of TSGA10 and VEGF genes in AML patients (****$P <$ 0.0001, r = -0.8925). (Fig. 4).

Diagnostic test evaluation was performed based on sensitivity and specificity by ROC curve and AUC calculation. The optimal cut off for relative quantification that separates AML patients from controls was also determined. According to expression level of TSGA10 in patients and controls, the AUC was 0.889 (95% CI, 0.780–0.998; $p < 0.001$) (Fig. 5a). Also, the optimal cut off value was defined as 1.604 and the sensitivity and specificity values of 86.7% and 100% were achieved, respectively. In diagnostic performance evaluation of VEGF gene expression, cut off point was 0.0329 and AUC was 0.962 (95% CI, 0.910–1.015; $p < 0.001$) (Fig. 5b). For this test, the sensitivity and specificity values were 90% and 100%, respectively. Finally, HIF-1α cut off point was 0.0042, and the ROC curve showed an AUC of 1 (95% CI, 1-1; $p < 0.001$) (Fig. 5c). The sensitivity and specificity values were 100% and 100%, respectively (Table 3).

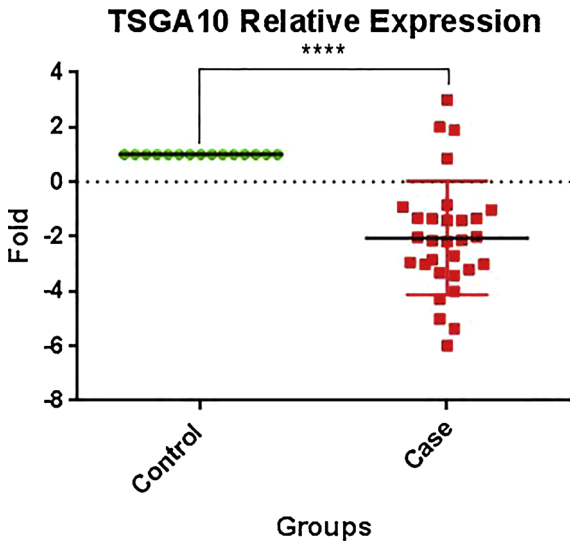

Fig. 1. TSGA10 gene expression in samples of AML patients were analyzed using real-time PCR. Relative gene expression was determined using Livak method compared to the control (****$P <$ 0.0001, -2.05167 ± 2.0743E0).

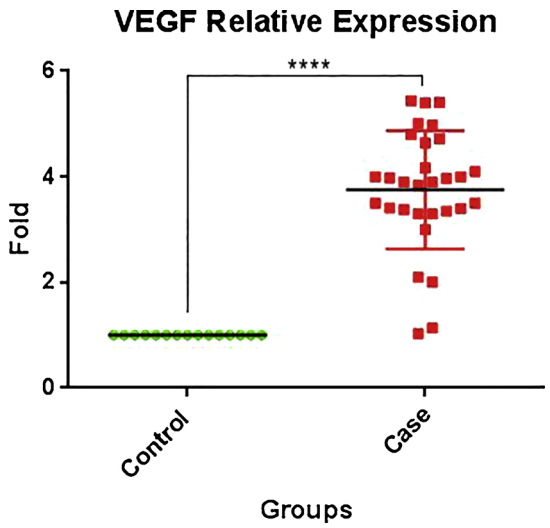

Fig. 2. VEGF gene expression in samples of AML patients were analyzed using real-time PCR. Relative gene expression was determined using Livak method compared to the control (****$P <$ 0.0001, 3.7520E0 ± 1.1208E0).

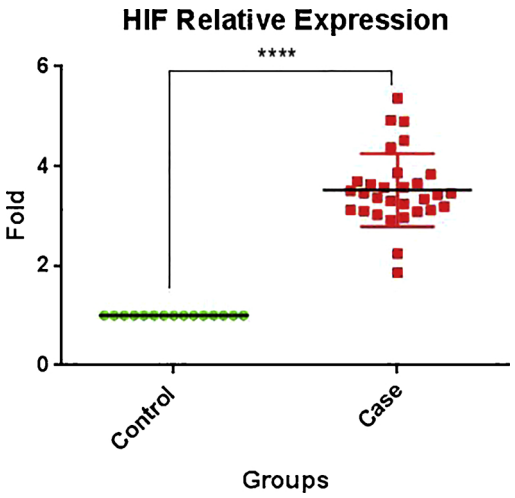

Fig. 3. HIF-1α gene expression in samples of AML patients were analyzed using real-time PCR. Relative gene expression was determined using Livak method compared to the control (****$P <$ 0.0001, 3.51967 ± 0.732791).

5. Discussion

Prevention and early diagnosis of different cancers, such as AML, are important issues in human health which can be achieved through continuous researches in this regard. According to previous studies, various genes are involved in AML and their expression significantly affects AML incidence. At present, there is no evidence on the exact role of TSGA10 in AML. Therefore, the current study was conducted to evaluate TSGA10 gene expression in ND AML patients and delve into its association with HIF-1α and the angiogenic factor VEGF.

Previous studies have shown higher expression levels of VEGF and

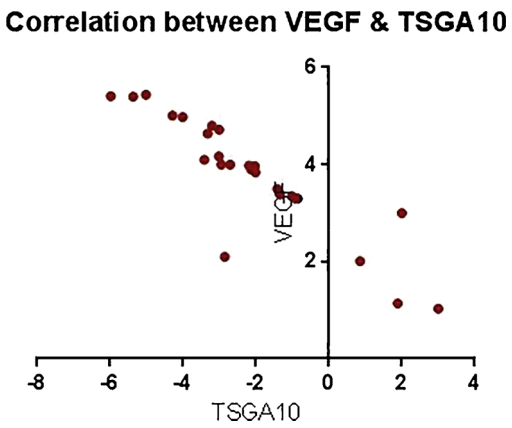

Fig. 4. The association between TSGA10 and VEGF gene expression (****$P < 0.0001$, r = -0.8925).

HIF genes in AML patients compared to normal controls [26,27]. Chen.p et al. showed that HIF-1α over expression is closely related to poor prognosis of AML [28]. Augmented VEGF plasma levels are associated with lower rates of complete remission and a reduced survival of AML patients. In AML bone marrow biopsies, elevated angiogenesis is often observed and micro-vessel density is found to be higher in bone marrow biopsies of these patients [15]. HIF-1, a key regulator of angiogenesis expressed in cancer cells under hypoxic conditions, plays a crucial role in tumor growth and stability. Up regulated in tumor cells during hypoxia, HIF-1 is able to induce angiogenesis initiating factors such as VEGF, MMP2, MMP9, IGF, bFGF, CXCR4, CXCL12 [8,29]. HIF-1 expression in cancer cells leads to resistance to chemotherapy and radiotherapy, hence its inhibition can improve cancer prevention and treatment.

Table 3
Diagnostic value analysis for determining statistically significant difference between AML patients and control group.

Parameter	AUC	Cut-off	Sensitivity (%)	Specificity (%)	PPV	NPV
HIF-1α	1	0.262	100	100	1	1
VEGF	0.962	0.0329	90	100	1	0.909
TSGA10	0.889	1.604	86.7	100	1	0.882

In the present study, by evaluating TSGA10 gene expression in ND AML patients, we showed an up to 6-fold reduction of TSGA10 expression in some AML patients. TSGA10 expression was found to be decreased in AML patients compared to normal controls. Since TSGA10 interacts with HIF-1α and inhibits its activity, VEGF expression was found to be higher in these patients compared to controls, indicating a negative correlation between TSGA10 and VEGF expression.

The interaction of TSGA10 with HIF1α results in a decreased VEGF secretion. Mansouri et al. showed that TSGA10 binding to HIF-1α C-terminal, by preventing P300 recruitment, leads to HIF-1α inactivation and prevents its transcriptional activity thereby interfering with tumor growth, angiogenesis and metastasis. Increased TSGA10 levels could reduce expression of the genes involved in angiogenesis as well as

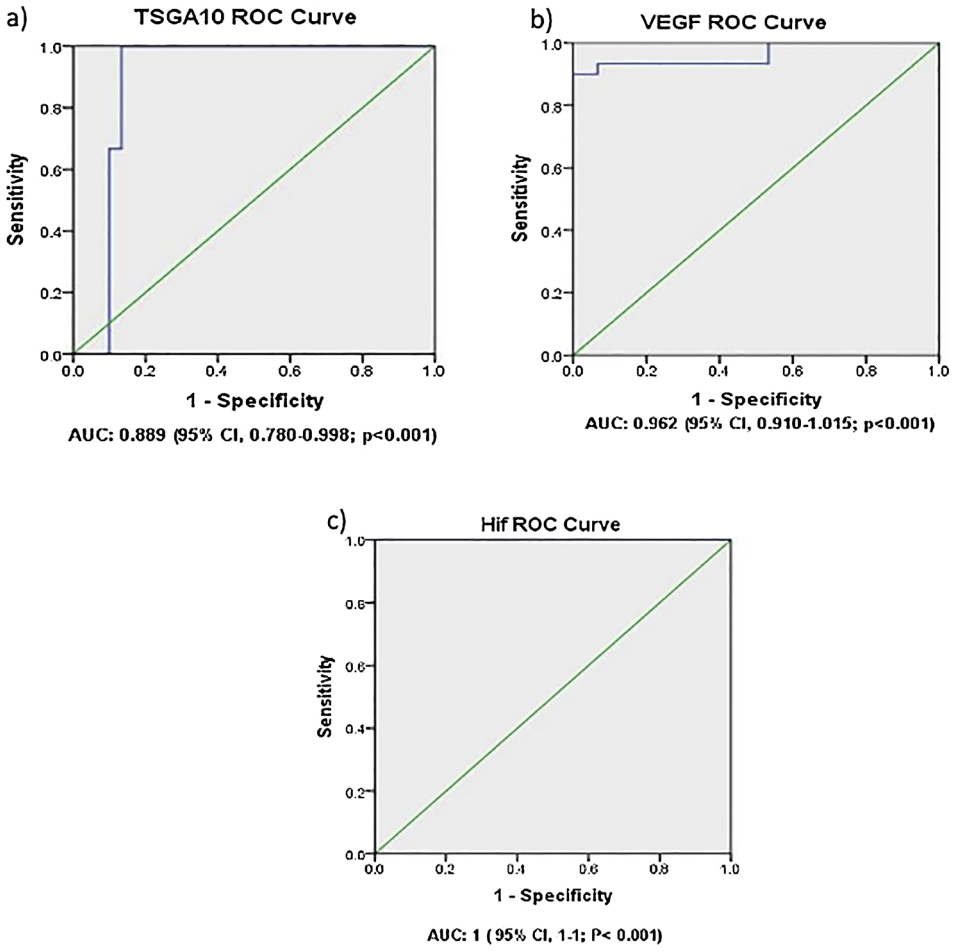

Fig. 5. Area under the curve (AUC) of receiver operating characteristic (ROC) for TSGA10 (a), VEGF (b) and HIF-1α (c) have been shown, respectively. HIF-1α gene expression has the highest sensitivity and specificity and the second suitable test is VEGF. AUC, cut-off points, 95% confidence interval (CI), sensitivity, specificity and the P-values are also shown.

MMP2, MMP9 and VEGF secretion from cancer cells consequently hindering angiogenesis [8]. In line with our findings, previous results have demonstrated that higher TSGA10 expression levels, are associated with lower levels of HIF expression [30]. Rad et al. investigated the association of HIF-α subunits with TSGA10 transcripts in HeLa, MCF-7 and MDA-MB-231 cell lines and found a negative relationship between expression of TSGA10 and HIF-α subunits. As demonstrated by their study, cell lines with lower expression of TSGA10 showed higher levels of HIF-α isoforms [30]. Furthermore, according to a study conducted by Hegels et al., TSGA10 C-terminal interaction with HIF-1α in the spermatozoa midpiece inhibits its nuclear accumulation thereby decreasing HIF-1 transcriptional activity [31]. Also, TSGA10 was stated as a tumor suppressor gene by Yuan et al in 2013. In their study, TSGA10 downregulation was shown to be associated with increased malignancy and clinical features of esophageal squamouse cell carcinoma (ESCC). They found significantly lower TSGA10 expression levels (which correlated with clinicopathologic features) in ESCC samples compared to adjacent normal tissues and TSGA10 expression levels negatively correlated with proliferative capacity of ESCC cell lines. Also, TSGA10 knockdown promoted *in vivo* tumor formation in nude mice whereas its increased expression impeded tumor formation. They found two binding sites for miR-577 in the 3′ UTR of TSGA10 gene and further investigations revealed a negative correlation between miR-577 and TSGA10 expression levels in tumor samples and ESCC cell lines [21]. Also consistent with our results, Bao et al. in 2018 provided another evidence concerning antitumor activities of TSGA10 gene further identifying this interesting molecule as a tumor suppressor. Overexpressed in human nasopharyngeal carcinoma (NPC) specimens, miR-23a was shown to be associated with higher angiogenic activity leading to increased metastatic progression in such patients. With a relative lower expression in NPC samples compared to non-cancerous nasopharyngeal samples, TSGA10 was shown to be a direct target of proangiogenic miR-23a as illustrated by different *in vitro* and *in vivo* experiments. It was shown that miR-23a contained in the exosomes secreted by NPC cells can be transferred to endothelial cells thereby promoting angiogenesis in the adjacent tumor endothelium. They showed for the first time that miR-23a, by binding to the specific site in the 3′ UTR of human/zebrafish TSGA10, directly represses its expression resulting in elevated migration of endothelial cells and angiogenesis. Collectively, miR-23a was suggested to modulate angiogenesis through directly targeting TSGA10 [23].

In contrast to the abovementioned studies showing decreased expression of TSGA10 in cancers and identifying it as tumor suppressor, there are several studies regarding its increased expression in cancers considering it as a CTA while it is expressed in normal tissues as well. Mobasheri et al in 2006 showed RNA expression of TSGA10 in 84.6% (44/52 samples) of bone marrow samples and all peripheral blood samples from patients with acute lymphoblastic leukemia (ALL) but not peripheral blood samples from healthy controls. However, regarding bone marrow specimens 52% (27 samples) showed high expression of TSGA10 as detected in the first PCR and 30% (16 samples) had low levels of TSGA10 expression as detected in the second amplification or semi-nested PCR. Also, among peripheral blood samples from ALL patients 57.1% (8 samples) were positive in the first PCR reaction (high expression level) and the remaining 42.9% (6 samples) were positive in the semi-nested PCR reaction indicating a low expression of TSGA10 [18]. In the next year, Mobasheri et al. evaluated TSGA10 expression in different cancers (156 tumor samples) including: brain, skin, gastrointestinal, soft tissue, breast, urogenital, bone and other tumors. They detected TSGA10 expression in 60.9% (96/156) of tumor samples among which 17.3% showed expression in the first round of PCR and 82.7% in nested-PCR [19]. Also, in another study Dianatpour et al showed that TSGA10 was expressed in 70% (35/50 samples) of breast cancer samples among which 10% (5 samples) showed expression in the first RT-PCR reaction (high expression level) and 60% (30 samples) in the reamplification reaction or semi-nested PCR (low expression level). Next, they analyzed TSGA10 expression in the breast cancer cell lines MCF-7 and MDA-231 and observed a high expression of the gene in the first round of RT-PCR. In their study TSGA10 expression was not detected in adjacent noncancerous tissue (ANCT) specimens as normal breast tissue [20]. In comparison with these studies, Tanaka et al. reported that TSGA10 is also expressed in normal tissues (other than testis). With a predominant expression in testis and overexpression in some tumors, TSGA10 was suggested to be a cancer testis-like (CT-like) antigen. TSGA10 mRNA copy number per 10^5 GAPDH mRNA copies was shown to be 16,000 in testis but 1000 in pancreas, lung, endothelium and breast and less than 200 in other 15 normal tissues. Also, TSGA10 mRNA overexpression which exceeded 10-fold of the average expression in normal tissues was observed in bladder cancer (8/21, 38%), ovarian cancer (7/21, 35%), hepatocellular carcinoma (4/20, 20%), prostate cancer (3/20, 15%), colon cancer (1/20, 5%) and malignant melanoma (1/21, 4.8%) while TSGA10 overexpression was not detected in lung cancer (0/18), endometrial cancer (0/31) or breast cancer (0/20) [6].

Taken together, in Mobasheri's, Dianatpour's and Tanaka's studies TSGA10 RNA level was shown to be increased in cancer cells compared to normal cells and therefore TSGA10 was claimed to likely act as an indicator for cancer prognosis. This is while the studies conducted by Yuan, Jakhesara, Mansouri and Bao have indicated decreased expression of TSGA10 in tumor cells and described it as a tumor suppressor gene. Our findings calls for further detailed investigations in this field to clarify the exact role of this interesting testis-specific gene in the context of hematological malignancies.

6. Conclusions

Regarding the defined role of TSGA10 in inhibiting HIF-1 activity, increased HIF-1α and VEGF expression levels observed in AML patients are suggested to be due to the significant decrease of TSGA10 expression in such patients compared to healthy individuals. Therefore, based on the findings of this study, decreased TSGA10 expression in AML patients could be considered as one of the underlying causes predisposing to AML progression. Collectively, according to the results of the current study, TSGA10 could be considered as a tumor suppressor gene, however further studies are warranted to precisely determine its function in tumor biology.

Funding

This research was supported by Medical Biology Research Center, Kermanshah University of Medical Sciences (3003623).

Availability of data and materials

All data generated or analyzed during this study are included in the published article.

Authors' contributions

Conception and design: K.M, Z.H, Development of methodology: K.M, Z.H, H.M and F.O Acquisition of data: K.M, A.B.R, A.M, S.A and M.R.P, Aanalysis and interpretation of data: K.M, Z.H, Writing, review and/or revision of the manuscript: K.M, Z.H and M.R.P.

Disclosure of conflict of interest

None.

Acknowledgments

This work was supported by Grants-in-Aid for Scientific Research (No: 3003623) from Kermanshah University Of Medical Sciences. We

would like to specially thank to Dr. Davood Rezazadeh and Mrs. Mozhgan Jahani for discussions and help with manuscript preparation.

References

[1] A. Jemal, A. Thomas, T. Murray, M. Thun, Cancer statistics, 2002, CA Cancer J. Clin. 52 (1) (2002) 23–47.

[2] B. Lowenberg, J.R. Downing, A. Burnett, Acute myeloid leukemia, N. Engl. J. Med. 341 (14) (1999) 1051–1062.

[3] S. Molica, A. Vacca, D. Levato, F. Merchionne, D. Ribatti, Angiogenesis in acute and chronic lymphocytic leukemia, Leuk. Res. 28 (4) (2004) 321–324.

[4] H. Döhner, D.J. Weisdorf, C.D. Bloomfield, Acute myeloid leukemia, N. Engl. J. Med. 373 (12) (2015) 1136–1152.

[5] M.H. Modarressi, J. Cameron, K.E. Taylor, J. Wolfe, Identification and characterisation of a novel gene, TSGA10, expressed in testis, Gene 262 (1) (2001) 249–255.

[6] R. Tanaka, T. Ono, S. Sato, T. Nakada, F. Koizumi, K. Hasegawa, et al., Over-expression of the testis-specific gene TSGA10 in cancers and its immunogenicity, Microbiol. Immunol. 48 (4) (2004) 339–345.

[7] M.H. Modarressi, B. Behnam, M. Cheng, K.E. Taylor, J. Wolfe, F.A. van der Hoorn, Tsga 10 encodes a 65-kilodalton protein that is processed to the 27-Kilodalton fibrous sheath protein, Biol. Reprod. 70 (3) (2004) 608–615.

[8] K. Mansouri, A. Mostafie, D. Rezazadeh, M. Shahlaei, M.H. Modarressi, New function of TSGA10 gene in angiogenesis and tumor metastasis: a response to a challengeable paradox, Hum. Mol. Genet. 25 (2) (2015) 233–244.

[9] P. Jaakkola, D.R. Mole, Y.-M. Tian, M.I. Wilson, J. Gielbert, S.J. Gaskell, et al., Targeting of HIF-α to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation, Science 292 (5516) (2001) 468–472.

[10] S. Hägele, B. Behnam, E. Borter, J. Wolfe, U. Paasch, D. Lukashev, et al., TSGA10 prevents nuclear localization of the hypoxia-inducible factor (HIF)-1α, FEBS Lett. 580 (15) (2006) 3731–3738.

[11] K. Rouault-Pierre, A. Hamilton, D. Bonnet, Effect of hypoxia-inducible factors in normal and leukemic stem cell regulation and their potential therapeutic impact, Expert Opin. Biol. Ther. 16 (4) (2016) 463–476.

[12] B. Muz, P. de la Puente, F. Azab, M. Luderer, A.K. Azab, The role of hypoxia and exploitation of the hypoxic environment in hematologic malignancies, Mol. Cancer Res. 12 (10) (2014) 1347–1354.

[13] M. Irigoyen, J.C. García-Ruiz, E. Berra, The hypoxia signalling pathway in haematological malignancies, Oncotarget 8 (22) (2017) 36832.

[14] M. Deynoux, N. Sunter, O. Hérault, F. Mazurier, Hypoxia and hypoxia-inducible factors in leukemias, Front. Oncol. 6 (2016) 41.

[15] K.R. Kampen, A. Ter Elst, E.S. de Bont, Vascular endothelial growth factor signaling in acute myeloid leukemia, Cell. Mol. Life Sci. 70 (8) (2013) 1307–1317.

[16] J.-W. Gu, K.L. Makey, K.B. Tucker, E. Chinchar, X. Mao, I. Pei, et al., EGCG, a major green tea catechin suppresses breast tumor angiogenesis and growth via inhibiting the activation of HIF-1α and NFκB, and VEGF expression, Vasc. Cell 5 (1) (2013) 9.

[17] T. Padro, R. Bieker, S. Ruiz, M. Steins, S. Retzlaff, H. Bürger, et al., Overexpression of vascular endothelial growth factor (VEGF) and its cellular receptor KDR (VEGFR-2) in the bone marrow of patients with acute myeloid leukemia, Leukemia 16 (7) (2002) 1302.

[18] M.B. Mobasheri, M.H. Modarressi, M. Shabani, H. Asgarian, R.-A. Sharifian, P. Vossough, et al., Expression of the testis-specific gene, TSGA10, in Iranian patients with acute lymphoblastic leukemia (ALL), Leuk. Res. 30 (7) (2006) 883–889.

[19] M.B. Mobasheri, I. Jahanzad, M.A. Mohagheghi, M. Aarabi, S. Farzan, M.H. Modarressi, Expression of two testis-specific genes, TSGA10 and SYCP3, in different cancers regarding to their pathological features, Cancer Detect. Prev. 31 (4) (2007) 296–302.

[20] M. Dianatpour, P. Mehdipour, K. Nayernia, M.-B. Mobasheri, S. Ghafouri-Fard, S. Savad, et al., Expression of testis specific genes TSGA10, TEX101 and ODF3 in breast cancer, Iran. Red Crescent Med. J. 14 (11) (2012) 722.

[21] X. Yuan, J. He, F. Sun, J. Gu, Effects and interactions of MiR-577 and TSGA10 in regulating esophageal squamous cell carcinoma, Int. J. Clin. Exp. Pathol. 6 (12) (2013) 2651.

[22] S.J. Jakhesara, P.G. Koringa, C.G. Joshi, Identification of novel exons and transcripts by comprehensive RNA-Seq of horn cancer transcriptome in Bos indicus, J. Biotechnol. 165 (1) (2013) 37–44.

[23] L. Bao, B. You, S. Shi, Y. Shan, Q. Zhang, H. Yue, et al., Metastasis-associated miR-23a from nasopharyngeal carcinoma-derived exosomes mediates angiogenesis by repressing a novel target gene TSGA10, Oncogene (2018) 1.

[24] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method, Methods 25 (4) (2001) 402–408.

[25] H. Motulsky, Analyzing Data with GraphPad Prism: GraphPad Software Incorporated, (1999).

[26] K.J. Hatfield, S.L. Bedringsaas, A. Ryningen, B.T. Gjertsen, Ø. Bruserud, Hypoxia increases HIF-1α expression and constitutive cytokine release by primary human acute myeloid leukaemia cells, Eur. Cytokine Netw. 21 (3) (2010) 154–164.

[27] S. Iranparast, M.-A. Assarehzadegan, Y. Heike, M. Hossienzadeh, A. Khodadadi, Wilms' tumor gene (WT1) expression correlates with vascular epithelial growth factor (VEGF) in newly acute leukemia patients undergoing chemotherapy, Asian Pac. J. Cancer Prev. 15 (2014) 9217–9223.

[28] P. Chen, X. Jiang, H.-F. Huang, Q. Yuan, J.-Y. Wu, Y.-F. Guo, et al., Expression of HIF-1α in primary acute myeloid leukemia cells and its relationship with prognosis, Zhongguo shi yan xue ye xue za zhi 23 (1) (2015) 19–23.

[29] G.L. Semenza, Targeting HIF-1 for cancer therapy, Nat. Rev. Cancer 3 (10) (2003) 721.

[30] P.R. Rad, M.B. Mobasheri, M. Hashemzadeh-Chaleshtori, M.H. Modarressi, Association of Hypoxia-inducible factor α subunits with TSGA10 transcripts in HeLa, MCF7 and MDA-MB231-cell lines, Basic Clin. Cancer Res. 9 (3) (2017).

[31] S. Hägele, B. Behnam, E. Borter, J. Wolfe, U. Paasch, D. Lukashev, et al., TSGA10 prevents nuclear localization of the hypoxia-inducible factor (HIF)-1α, FEBS Lett. 580 (15) (2006) 3731–3738.