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Original Research Article

Illuminating the role of lncRNAs ROR and MALAT1 in cancer stemness state of anaplastic thyroid cancer: An exploratory study

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ABSTRACT

Background: Anaplastic thyroid cancer (ATC) is one of the most aggressive malignancies in humans that accounts for a considerable rate of cancer-associated mortality. Since conventional therapies are lacking sufficient efficacy, new treatment approaches are required. This goal could be achieved through a better understanding of the molecular pathogenesis of ATC. Thyroid tumorigenesis is initiated by a subpopulation of cells known as cancer stem cells (CSCs) with specific markers such as CD133 that confers to processes such as self-renewal and metastasis. Besides, some long non-coding RNAs (lncRNAs) promote tumorigenesis by mediating the aforementioned processes.

Methods: Here, we designed an exploratory study to investigate the role of lncRNAs ROR and MALAT1 and their related genes in CSC stemness. Using magnetic-activated cell sorting (MACS), the $CD133^-$ and $CD133^+$ subpopulations were separated in SW1736 and C643 ATC cell lines. Next, the expression profiles of the CD133 marker, MALAT1, and its associated genes (*CCND1*, *NESTIN*, *MYBL2*, *MCL1*, *IQGAP1*), as well as ROR and its related genes (POU5F1, SOX2, NANOG), were explored by qRT-PCR.

Results: We found significant up-regulation of *ROR*, *POU5F1*, *SOX2*, *NANOG*, *CD133*, *MALAT1*, *IQGAP1*, and *MCL1* in $CD133^+$ SW1736 cells compared to $CD133^-$ cells. As for $CD133^+$ C643 cells, *CCND1*, *IQGAP1*, *POU5F1*, *SOX2*, *NANOG*, and *NESTIN* were significantly up-regulated compared to $CD133^-$ cells.

Conclusions: This study suggests that these lncRNAs in CD133-positive SW1736 and C643 cells might regulate stemness behaviors in ATC.

1. Introduction

ATC is a rare and devastating type of thyroid cancer comprising about 2% of all cases of thyroid cancers [1]. Some of the well-known features of ATC include a high growth rate, aggression, invasiveness, poor prognosis, and response to therapy [2]. Various therapeutic approaches have been used for ATC, but the survival rate was not significantly improved in patients [3]. Therefore, the exploration of molecular mechanisms underlying the progression of thyroid cancer might pave the way toward the improvement of treatment strategies for aggressive thyroid cancers. One of the reasons behind the ineffective response of patients to various treatment modalities such as chemotherapy, surgery, and radiotherapy is the stemness of cancer cells [4]. There is evidence that the initiation of thyroid cancer tumorigenesis is marked by tumor-initiating cells, which are also known as CSCs. These clonogenic cells have the potential for self-renewal, drug resistance, tumor

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progression, and recurrence. One of the specific biomarkers used to identify thyroid CSCs is CD133 (PROMININ1) [5], a transmembrane protein recognized as a CSC marker in various other cancers, which is also involved in the epithelial-to-mesenchymal transition (EMT) [6].

The self-renewal potential and pluripotency of embryonic stem cells (ESCs) are regulated by epigenetic and transcriptional networks; at the level of transcriptional regulation, several transcription factors such as POU5F1 (OCT4), NANOG, and SOX2, known as the core transcription factors, maintain the ESC state and inhibit differentiation [7]. They are among the specific biomarkers that distinguish thyroid CSCs and have a higher expression rate in $CD133^+$ thyroid CSCs [8]. Similarly, NESTIN is a CSC marker that regulates stemness, invasion, migration, and proliferation of lung adenocarcinoma [9].

ROR is a well-known regulatory long non-coding RNA (lncRNA) for stem cell self-renewal [10] that contains the same miRNA response elements located within core transcription factors (POU5F1, SOX2, NANOG) and acts as a sponge for repressive miRNAs. Therefore, ROR might prevent the miRNA-mediated suppression of core transcription factors [7]. Up-regulation of core transcription factors as well as CD133 has been observed in gastric CSCs, which indicated an association between ROR and stemness factors, and the pluripotency of stem cells [11].

Another important lncRNA associated with EMT in cancer is metastasis-associated lung adenocarcinoma transcript 1 (MALAT1); this lncRNA forms a ribonucleoprotein complex with the RNA-binding protein HuR (MALAT1/HuR), binds to the *PROM1* gene (*CD133*) gene, and regulates its expression. It is shown that the down-regulation of MALAT1/HuR complex components leads to the up-regulation of *CD133* and presentation of more basal-like cellular features [6].

Several studies have separately investigated potential links between MALAT1 and gene profiles including *CCND1*, *MCL1*, *MYBL2*, *IQGAP1*, and *NESTIN*. *CCND1*-encoded protein, formerly known as cyclin D1, is one of the regulators of the G1 phase whose continuous production during cellular reprogramming might inhibit the generation of induced pluripotent stem cells (iPSCs) [12].

Myeloid cell leukemia-1 (MCL1) is an anti-apoptotic protein and a member of the BCL2 family engaged in embryonic development and cell survival [13]. This protein is also involved in the regulation of self-renewal in human stem cells. More specifically, the self-renewal capability of human stem cell hierarchy is functionally dependent on MCL1 [14]. MYBL2, formerly known as B-MYB, is a transcription factor and a member of the MYB family. It is a prominent regulator of cellular processes such as proliferation, survival, and differentiation. There is evidence that the dysregulation of *MYBL2* is associated with the initiation and progression of cancer. Besides, over-expression of *MYBL2* is linked with poor prognosis in different cancers [15]. IQGAP1 is a scaffold protein that interacts with cell adhesion proteins and signaling molecules and modulates cellular processes such as adhesion and migration [16].

Bearing in mind the significance of lncRNAs in the regulation of stemness in various cancers, the goal of this study is to investigate the presumptive role of ROR and MALAT1 in the maintenance of CSCs stemness state in ATC. Our approach was to use MACS to isolate the CSC subpopulation in SW1736 and C643 ATC cell lines based on the CD133 marker expressed on the cell surface and to evaluate the expression profiles of genes including *MALAT1*, *ROR*, *CCND1*, *NESTIN*, *MYBL2, MCL1, IQGAP1,* and *CD133* as well as stemness transcription factor-encoding genes such as *POU5F1*, *SOX2,* and *NANOG* in ATC cell lines.

2. Material and methods

2.1. Cancer cell lines and cell culture

The SW1736 and C643ATC cell lines were purchased from the CLS Cell Lines Service GmbH, Germany.

Cells were cultured in RPMI 1640 medium (Gibco, Carlsbad, CA) containing 10% fetal bovine serum (Invitrogen, Carlsbad, CA) and incubated at 37 °C with 5% CO_2. The Institutional Review Board of Endocrinology and Metabolism Research Institute of Tehran University of Medical Sciences approved this study.

2.2. MACS and flow cytometry

The MACS indirect CD133 MicroBead, Human Kit (MiltenyiBiotec, Bergisch Gladbach, Germany) was used to separate $CD133^+$ cells in SW1736 and C643 cell lines according to the manufacturer's instructions.

The purity of $CD133^+$ cell lines was evaluated by flow cytometry. Cells were incubated with phycoerythrin (PE)-conjugated anti-CD133 antibodies (Miltenyi Biotec, Bergisch Gladbach, Germany) for 45 min at 4 °C, and then were added to 1×10^6 cell/100 μL in PBS and incubated for 20 min on ice in darkness. Anti-mouse IgG1 antibody (Miltenyi Biotec, Bergisch Gladbach, Germany) was used as isotype control. Flow cytometry was used to analyze and separate the labeled cells, and data were analyzed by CELLQuest software (Becton–Dickinson Immunocytometry Systems, San Jose, CA, USA). Gating was based on negative control staining profiles.

2.3. RNA extraction and cDNA synthesis

Total RNA obtained from thyroid carcinoma cell lines (SW1736 and C643) was extracted by TRIzol Reagent. Complementary DNA (cDNA) was synthesized using the PrimeScriptTM RT Kit (Takara, Dalian, China) according to the manufacturer's protocol. We made use of a NanoDrop-2000 spectrophotometer (Thermo Scientific, Waltham, MA) to determine the yield and purity of total RNA.

2.4. Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)

Expression of genes including *MALAT1*, *IQGAP1, CCND1, CD133, POU5F1, SOX2, NANOG*, *ROR, NESTIN, MCL1, MYBL2* was measured by qRT-PCR using the ABI7500 system (Applied Biosystems, CA, USA) and SYBR green premix (Takara, Dalian, China). The expression of the *ACTB* gene (*β-actin*) was also evaluated as the endogenous control, and all the samples were normalized to human *β-actin* according to the $2^{-\Delta\Delta Ct}$ method. Primer sequences and annealing temperatures are listed in

Table 1
List of designed primer sequences.

Gene	Sequence
ROR	F: ATGAGTTATAGTTCTTCCAGGTC
	R: GGAGTCATTGAAGGTTCTAAGC
MALAT1	F: GTAACGATGGTGTCGAGGTC
	R: CAGCATTACAGTTCTTGAACATG
POU5F1	F: CGCCGTATGAGTTCTGTG
	R: GGTGATCCTCTTCTGCTTC
NESTIN	F: GAAGGTGAAGGGCAAATCTG
	R: CCTCTTCTTCCCATATTTCCTG
SOX2	F: GGACTGAGAGAAAGAAGAGGAG
	R: GAAAATCAGGCGAAGAATAAT
NANOG	F: CTCCTTCCATGGATCTGCTTATTC
	R: AGGTCTTCACCTGTTTGTAGCTGAG
CD133	F: GGTCCAACAGGGCTATCAATC
	R: TGAATAGGAAGACGCTGAGTTAC
CCND1	F: GATGCCAACCTCCTCAACG
	R: GAAGCGGTCCAGGTAGTTC
MYBL2	F: TGTGGATGAGGATGTGAAGC
	R: GCAGGTGTCGTGAAGTGG
IQGAP1	F: CAGTCACAGTGGAGAGGATAC
	R: CAGCATTGATGAGAGTCTTGTAG
MCL1	F: AACAAAGAGGCTGGGATG
	R: ATTGCACTTACAGTAAGGCTATC
β-actin	F: CTTCCTTCCTGGGCATG
	R: GTCTTTGCGGATGTCCAC

Table 1. After primary heating at 95 °C for 10 min, the thermal cycling condition included 40 cycles of 95 °C for 15s, 60 °C for the 30s, and 72 °C for 30s.

2.5. Statistical analysis

We performed all experiments in duplicates for qRT-PCR and triplicates for flow cytometry. Differential gene expression analysis for $CD133^+$ and $CD133^-$ cells was performed using $2^{-\Delta\Delta Ct}$ Method. Statistical significance is expressed as $p < .05$ (*); $p < .01$ (**); $p < .001$ (***).

3. Results

3.1. Isolation of CD133-positive/-negative cells by MACS

SW1736 and C643 cells were sorted based on the CD133 marker. This transmembrane protein is known as a CSC marker for thyroid [5] and several other cancers [6]. It is assumed that the aggressive trait of ATC could be related to high levels of CD133 [17].

Our results demonstrated the existence of both $CD133^+$ and $CD133^-$ subpopulations in SW1736 and C643 cell lines (see Fig. 1).

3.2. Expression of CD133 marker, ROR, and stemness factors NANOG, SOX2, POU5F1

We observed a significantly higher expression of *ROR* and *CD133* marker in the $CD133^+$ cell subset of the SW1736 cell line compared to $CD133^-$ cells (Fig. 2) (*ROR*: $p < .001$ (***), *CD133*: $p < .01$ (**). Up-regulation of both *ROR* and *CD133* in the $CD133^+$ cell subset of C643 cell line was also detected. However, as shown in Fig. 3, the increase was not significant ($p > 0.05$). It should be noted that all the expression data were normalized to one, so that over-expression has values higher than one and vice versa.

As for *POU5F1*, *SOX2*, and *NANOG*, a significantly higher expression was recorded in $CD133^+$ cells compared to the $CD133^-$ subset in both SW1736 (*POU5F1*: $p < .01$(**), *SOX2*: $p < .001$ (***), and *NANOG*: $p <$

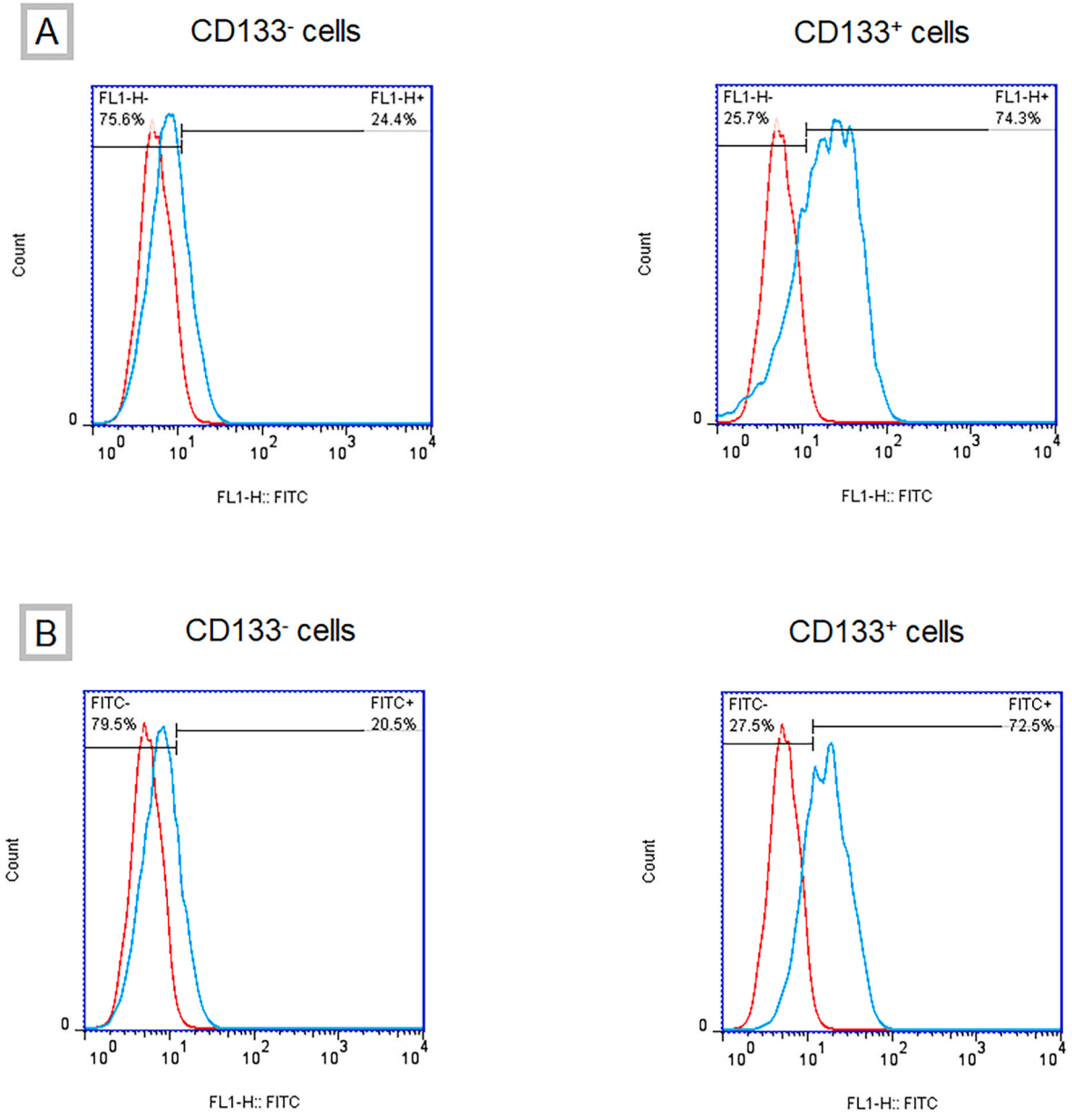

Fig. 1. Validation of MACS results by flow cytometry. Flow cytometry analysis of CD133 in A) C643 and B) SW1736 cell lines. Blue lines exhibit positive staining for CD133 and red lines show negative control with matched isotype antibody. Data were the averages of at least three independent runs.

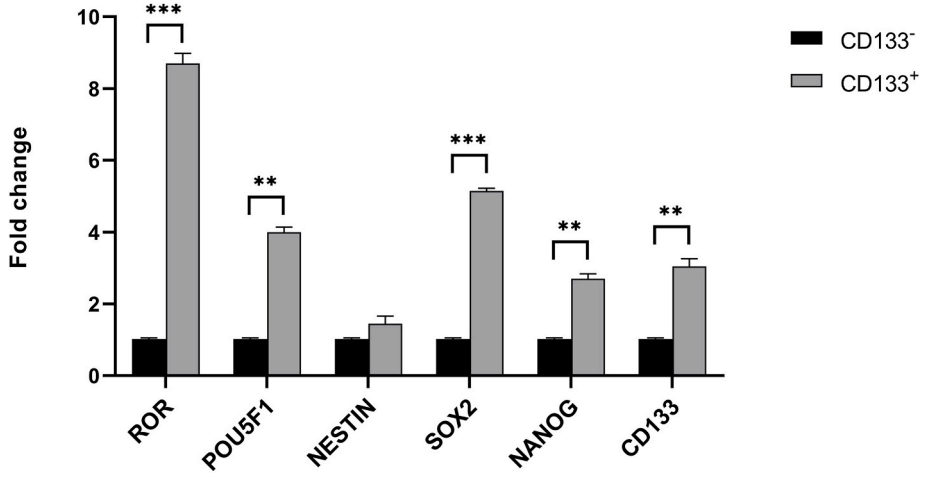

Fig. 2. Expression levels of *CD133* marker, *ROR*, and stemness factors *NANOG*, *SOX2*, *POU5F1* in SW1736 cell line. Data were the averages of at least two independent runs; bars, SD ($p < .01$ (**); $p < .001$ (***)).

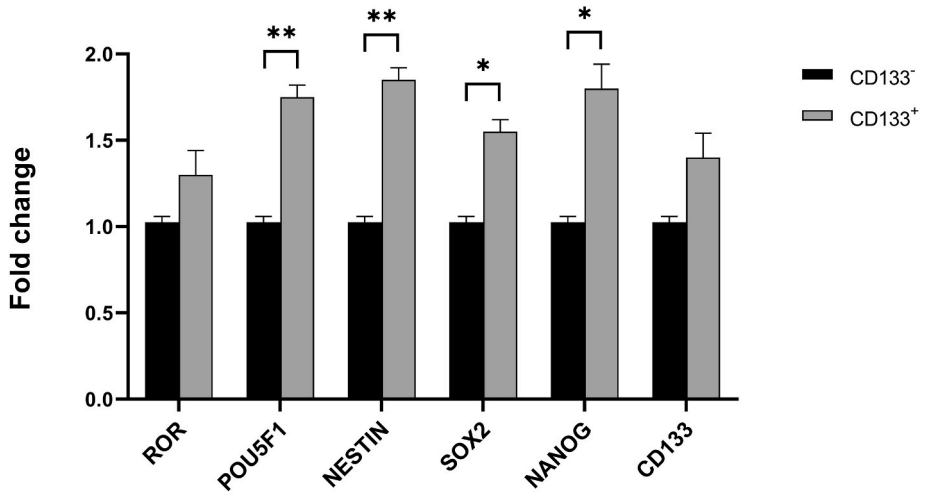

Fig. 3. Expression levels of *CD133* marker, *ROR*, and stemness factors *NANOG*, *SOX2*, *POU5F1* in C643 cell line. Data were the averages of at least two independent runs; bars, SD ($p < .05$ (*); $p < .01$ (**)).

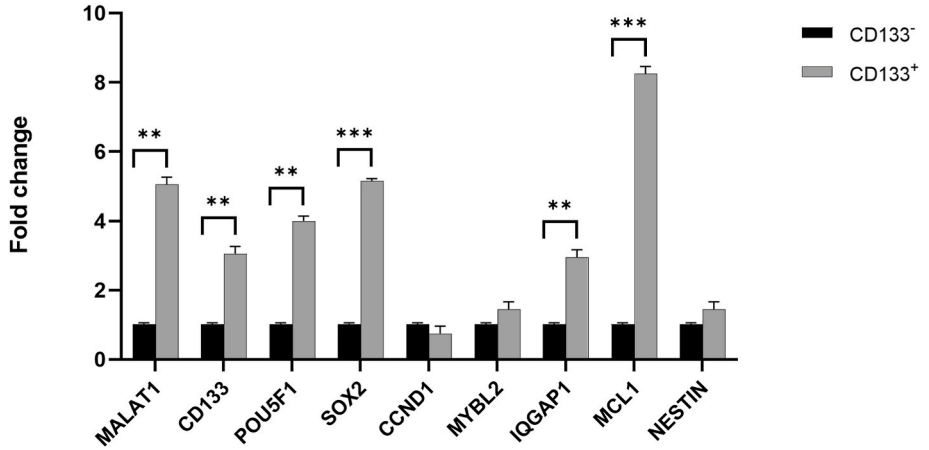

Fig. 4. Expression levels of *MALAT1*, *IQGAP1*, *MYBL2*, *MCL1*, *NESTIN*, and *CCND1* in SW1736 cell line. Data were the averages of at least two independent runs; bars, SD ($p < .01$ (**); $p < .001$ (***)).

.01 (**), respectively) (Fig. 2) and C643 cell lines (*POU5F1*: $p < .01$ (**), *SOX2*: $p < .05$ (*), and *NANOG*: $p < .05$ (*), respectively) (Fig. 3).

3.3. Expression of *MALAT1, IQGAP1, MYBL2, MCL1, NESTIN, and CCND1*

In SW1736 cells, a significant up-regulation of *MALAT1* ($p < .01$ (**)), *IQGAP1* ($p < .01$ (**)), and *MCL1* ($p < .001$ (***)) was demonstrated in $CD133^+$ cells compared to $CD133^-$ cells, Nevertheless, the up-regulation of *NESTIN* and *MYBL2* was not significant ($p > .05$). As for *CCND1*, our results demonstrated a lower, but insignificant level of expression in the $CD133^+$ subset compared to the $CD133^-$ subset ($p > .05$) (Fig. 4).

In C643 cells, a significant up-regulation of *IQGAP1* ($p < .05$ (*)), *CCND1* ($p < .05$ (*)), and *NESTIN* ($p < .01$ (**)) was observed in $CD133^+$ cells compared to $CD133^-$ cells. In contrast, the up-regulation of *MALAT1*, *MYBL2*, and *MCL1* was not significant ($p > .05$) (Fig. 5).

Overall, in SW1736 cells, we observed significant co-expression of '*ROR*, *POU5F1*, *SOX2*, *NANOG*, *CD133*' and '*MALAT1*, *POU5F1*, *SOX2*, *IQGAP1*, *MCL1*, *CD133*'.

In C643 cells, we found significant co-expression of '*POU5F1*, *SOX2*, *CCND1*, *IQGAP1*, *NESTIN*' and '*POU5F1*, *SOX2*, *NANOG*, *NESTIN*'.

4. Discussion

Over years of research, a great body of evidence has accumulated, suggesting that thyroid tumorigenesis is driven by a subpopulation of heterogenous cells known as cancer stem cells. These stem-like cells are of major importance in many aspects, namely contributing to drug resistance, recurrence, tumor growth, and malignant progression [5]. Self-renewal and stemness of cells are regulated by various key players such as transcription factors [7] and lncRNAs [18].

In an attempt to explore the molecular mechanism underlying the pathogenesis of ATC, we investigated the expression of stemness-associated genes in two ATC cell lines, (i.e., SW1736 and C643). Because of their availability and ease of handling, cell lines are useful *in vitro* models of CSCs for preliminary hypothesis testing. We selected these cell lines since they both comprise subpopulations that resemble CSCs. Moreover, $CD133^+$ cells derived from these cell lines show gene expression patterns similar to those of CSCs [19].

We evaluated the expression levels of genes encoding the CD133 marker, *ROR*, and stemness factors including *NANOG*, *SOX2*, and *POU5F1* in SW1736 cells. Our results indicated significant up-regulation of *CD133* ($p < .01$), *ROR* ($p < .001$), *SOX2* ($p < .001$), *POU5F1* ($p < .01$) and *NANOG* ($p < .01$) genes in $CD133^+$ cells compared to $CD133^-$ cells. We also observed the up-regulation of *NESTIN* in $CD133^+$ cells compared to $CD133^-$ cells. However, it was not significant ($p > .05$).

Expression of *SOX2*, *POU5F1*, and *NANOG* genes in $CD133^+$ thyroid CSCs has been indicated in various studies [8]. According to Tseng et al. [20], these genes were expressed in the $CD133^+$ cells isolated from ATC tumors as well as ATC cell lines.

On the other hand, Wang et al. [11] observed that lncRNA ROR was highly expressed in gastric CSCs and that it up-regulated genes including *CD133*, *SOX2*, *POU5F1*, and *NANOG* in these cells. Their findings suggested an association between ROR and stemness factors and the pluripotency of gastric CSCs.

In our study, a possible explanation for significant co-expression of *ROR* and stemness genes including *POU5F1*, *SOX2*, and *NANOG* in CD133-positive SW1736 cells might be that all these factors contain a response element that ROR binds to and acts as a sponge for repressive miRNAs that inhibit the expression of these stemness transcripts. ROR sponges the activity of miR-145, which in turn leads to an increase in the expression levels of miR-145 targets, such as *OCT4*, *SOX2*, and *NANOG* [7]. In a similar manner, we think that the co-expression pattern in the current study might have occurred via ROR by preventing miR-145.

In C643 cells, we observed a significant up-regulation of *NESTIN* ($p < .01$), *SOX2* ($p < .05$), *POU5F1* ($p < .01$), and *NANOG* ($p < .05$) in the $CD133^+$ subpopulation compared to the $CD133^-$ subset while the up-regulation of *ROR* and *CD133* genes was not significant ($p > .05$). Although CD133 is a common thyroid CSC marker, we have not detected a significant up-regulation of the *CD133* gene in $CD133^+$ cells compared to CD133 cells. In a study reported by Carina et al. [21], CD133 was not expressed in the SW1736 cell line. Moreover, CD133-positive cells have shown variable frequency among thyroid cancers of the same type [5]. It appears that, although routinely expressed and used for CSC isolation, CD133 is not always solely sufficient to characterize of CSCs. Therefore, we suggest using other CSC-specific features for the precise detection of these cells.

Analysis of NESTIN protein expression in 23 ATC patients showed that only 26.1% of samples were NESTIN-positive. Meanwhile, patients with positive expression of *NESTIN* had a worse outcome compared to NESTIN-negative individuals, suggesting a potential prognostic value for this marker in patients with ATC [22].

A recent study reported that *MALAT1* contributes to the stemness of glioblastoma cells by regulating *SOX2* and *NESTIN* [23]. Down-regulation of *MALAT1* in the SHG139S glioma stem cell line resulted in the down-regulation of SOX2 and NESTIN stemness markers and promoted cell proliferation [24]. Hence, lncRNA MALAT1 seems to

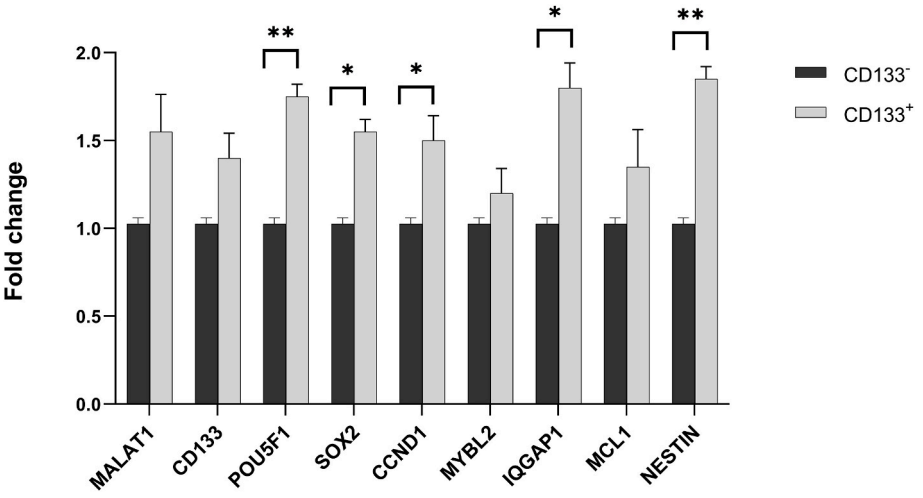

Fig. 5. Expression levels of *MALAT1*, *IQGAP1*, *MYBL2*, *MCL1*, *NESTIN*, and *CCND1* in C643 cell line. Data were the averages of at least two independent runs; bars, SD ($p < .05$ (*); $p < .01$ (**)).

perform a key role in the pathogenesis of these cancers.

Interestingly, bioinformatics analysis predicted that MALAT1 has a complementary sequence to that of miR-145, which inhibits the expression of *SOX2*. As a result, it was assumed that MALAT1 might be an endogenous sponging RNA that targets miR-145 and negates the suppressive impact of miRNA on *SOX2*, leading to an increase in *SOX2* expression [25].

Here, we observed that *MALAT1* was significantly up-regulated ($p < .01$) in $CD133^+$ cells compared to $CD133^-$ SW1736 cells. Similarly, we observed significant up-regulation of *IQGAP1* ($p < .01$) in $CD133^+$ cells compared to the $CD133^-$ subset. Although *MALAT1* and *IQGAP1* were up-regulated in $CD133^+$ C643 cells compared to the $CD133^-$ subset, the increase of *MALAT1* was not significant ($p > .05$).

IQGAP1 is an outstanding regulator of invasion in thyroid cancer [26]. In thyroid cancer cells, *MALAT1* has been shown to contribute to the invasion and proliferation of thyroid cancer by up-regulating IQGAP1 scaffold protein [27]. Our data also demonstrated simultaneous up-regulation (positive co-expression) of *MALAT1* and *IQGAP1*, which might be due to the regulatory impact of MALAT1 on the expression of *IQGAP1* mentioned above. IQGAP1 might contribute to tumorigenesis via a MALAT1-mediated mechanism. However, further studies and functional assays are required to validate this assumption.

Our results demonstrated a remarkable increase in the expression of *MCL1* ($p < .001$) in the $CD133^+$ subset compared to the $CD133^-$ subset of the SW1736 cell line. In contrast, *MCL1* up-regulation was not significant in the C643 cell line ($p > .05$). Given that each of these cell lines has a unique genetic profile [4,28], different expression patterns of the *MCL1* gene in C643 and SW1736 cell lines may be a result of the difference between the genetic content of these cells. MCL1 is an anti-apoptotic protein involved in the regulation of self-renewal in hematopoietic stem cells [29] and cancer stem-like side population cells in non-small cell lung carcinoma [30].

Another important function of this protein is the regulation of cellular self-renewal in human stem cells. The human stem cell hierarchy has a functional dependence on MCL1 for self-renewal capability [14]. Over-expression of *MCL1* in breast cancer cells increased stemness features and resistance to chemotherapy while its inhibition had the opposite effect [31]. MALAT1 indirectly affects the expression of its downstream related genes through its interplay with mediators such as miRNAs [32]. The studies by Wang et al. [33] as well as Samimi et al. [34] indicated that down-regulation of *MALAT1* leads to decreased expression of *MCL1*. They suggested that MALAT1 sponged the *MCL1* inhibitor miR-363-3p, which resulted in the up-regulation of *MCL1*.

We observed the up-regulation of *MYBL2* in the $CD133^+$ subset compared to $CD133^-$ cells in both SW1736 and C4643 cell lines. However, the difference was not significant ($p > .05$).

MYBL2 is a crucial transcription factor associated with the maintenance of stem cell-like and undifferentiated features of cells [15]. It is a putative pluripotency gene [35] and a chromatin-bound partner for core transcription factors (POU5F1, SOX2, and NANOG) and forms a pluripotency network in ESCs [36]. Over-expression of *MYBL2* was associated with poor prognosis in different cancers [15]. It was shown that in MALAT1-negative cells, the cellular level of MYBL2 and its mitotic targets were reduced. On the other hand, the exogenous introduction of MYBL2 led to the expression of several mitosis-associated genes in these cells. It was suggested that MYBL2 might regulate the expression of *MALAT1* through binding to its promoter region and that they are involved in a positive regulatory loop [37].

On the other hand, MYBL2 directly binds to the promoter of the *POU5F1* gene and regulates its activity *in vitro*. Furthermore, the knockdown of *MYBL2* in murine embryonic cells decreased the levels of *SOX2* and *POU5F1* RNAs and proteins while *MYBL2* over-expression slightly increased the expression of the *POU5F1* and *SOX2* genes [38]. Interestingly, it was shown that POU5F1 binds to the enhancer of the *MALAT1* gene, regulates its expression, and contributes to the regulation of cell proliferation [39]. It seems that the MYBL2/POU5F1/MALAT1 triple are connected via a regulatory loop where POU5F1 regulates the expression of *MALAT1* and *MYBL2* acts as the upstream regulator of both POU5F1 and MALAT1.

CCND1 is the regulator of the G1 phase. Pluripotent stem cells in the inner cell mass have a short G1 phase to prevent the signal-induced differentiation of ESCs into endoderm or neuroectoderm. Continuous production of this protein during cellular reprogramming might inhibit the generation of iPSCs [12]. In one study, the role of MALAT1 in the regulation of *CCND1* expression was investigated. In cells with down-regulated MALAT1, CCND1 was up-regulated and colony formation and cell proliferation were exacerbated [40].

Here, we observed insignificant down-regulation of *CCND1* in the $CD133^+$ subset of SW1736 cells compared to the $CD133^-$ population ($p > .05$). In contrast, the expression of *CCND1* in $CD133^+$ C643 cells was significantly higher compared to $CD133^-$ cells ($p < .05$). This observation might be, to some extent, due to different genetic profiles of these cell lines. In conclusion, *MALAT1* and *CCND1* do not exhibit a significant co-expression pattern in our study.

Overall, in $CD133^+$ SW1736 cells, we observed significant co-expression of mRNAs in the following groups: '*ROR, POU5F1, SOX2, NANOG, CD133*' and '*MALAT1, POU5F1, SOX2, IQGAP1, MCL1, CD133*'.

In $CD133^+$ C643 cells, we found significant co-expression of '*POU5F1, SOX2, CCND1, IQGAP1, NESTIN*' and '*POU5F1, SOX2, NANOG, NESTIN*'. These co-expression patterns might be due to potential interactions between genes in each of these groups (Fig. 6).

Altogether, in this exploratory study, some gene expression patterns in $CD133^+$ cells derived from SW1736 cells were different from those in $CD133^+$ C643 cells. This observation might be related to the variable nature of $CD133^+$ CSCs as they might carry different mutation profiles in each cell line, thus producing different behavior patterns in terms of molecular/genetic complexity in cancer.

Our findings need further validation to explain whether ROR and MALAT1 play a role in stemness regulation via these genes. As thyroid cancer cell lines are valuable models to study this disease [41], and similar to other studies [42], the findings of our study could be further evaluated and confirmed using more diverse ATC cell lines [43]. As the next step, functional experiments are required to evaluate the impact of induced over-expression and silencing of each lncRNA and the subsequent effects on their associated genes. We also suggest that a more comprehensive network-based analysis across lncRNA/miRNA/mRNA axes and subsequent experimental validation could help identify regulatory miRNAs of genes evaluated in our study and find more pieces to complete this puzzle.

Despite limitations, this study highlighted the potential role of key lncRNAs in the regulation of CSC stemness by focusing on the molecular aspect of ATC. Expanding our knowledge of the molecular mechanisms behind anaplastic thyroid tumorigenesis is a helpful approach to detect candidate biomarkers that provide an efficient diagnosis of ATC at an early stage, hence eliminating the rate of metastasis-related mortality.

5. Conclusions and future perspectives

Due to the overly aggressive nature of ATC and the poor prognosis in patients, early diagnosis is particularly demanding. Therefore, gaining a better understanding of the molecular mechanisms underlying ATC pathogenesis could facilitate achieving this goal. Concomitantly, given the crucial role of stemness and self-renewal in CSCs, the potential initiators of thyroid tumorigenesis, it is especially important to focus on the mechanisms behind these key processes to discover novel and competent diagnostic biomarkers. These biomarkers might also serve as therapeutic targets, or better yet help us develop molecular signatures that are unique to ATC and improve the outcome in patients. Here, we compared the expression of lncRNAs *ROR* and *MALAT1* in $CD133^+$ and $CD133^-$ populations derived from the ATC cell lines including SW1736 and C643. Regarding the co-expression of these two lncRNAs and some

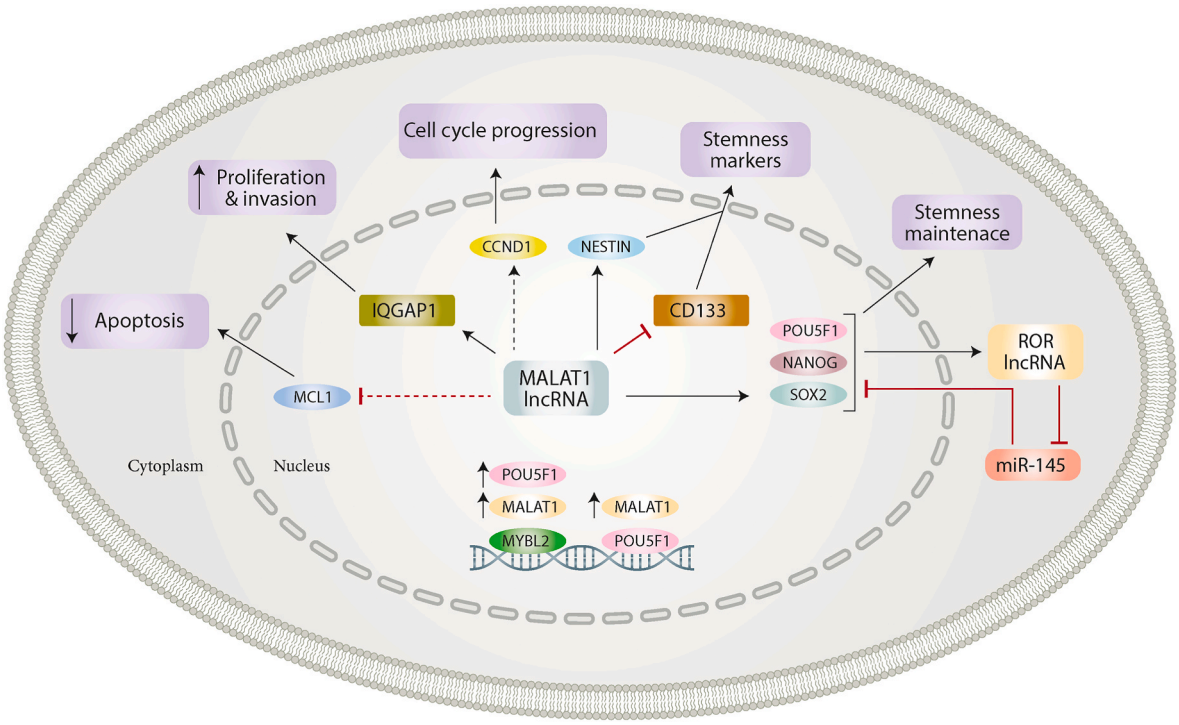

Fig. 6. Schematic representation of MALAT1 and ROR roles in stemness. ROR lncRNA acts as a sponge and prevents stemness factors including SOX2, POU5F1 and NANOG from miRNA-mediated degradation. On the other hand, SOX2, POU5F1 and NANOG modulate *ROR* transcription. MYBL2 and POU5F1 regulate *MALAT1* expression via binding regulatory elements in the *MALAT1* gene. In addition, MALAT1 down-regulates *CD133*, but up-regulates *IQGAP1* through binding the promoter of these genes. MALAT1 down-regulates *MCL1* mRNA by sponging miR-363-3p, and also promotes the expression of *CCND1* gene by modulating the Wnt/β-catenin signaling pathway. Solid and dashed lines indicate direct and indirect effects, respectively.

of their target genes, which regulate cellular processes such as stemness and self-renewal, it might be possible that ROR and MALAT1 affect stemness by regulating these targets. More studies are required to evaluate the role of these lncRNAs and to reveal the key players in stemness that might serve as the potential diagnostic, prognostic, and one-person trial therapeutic tools [44].

Ethics approval statement

This article does not contain any studies with human participants or animals performed by any of the authors. The Institutional Review Board of Endocrinology and Metabolism Research Institute of Tehran University of Medical Sciences approved this study.

Data availability statement

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

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CRediT authorship contribution statement

Mojdeh Mahdiannasser: Conceptualization, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Samaneh Khazaei:** Formal analysis, Methodology, Validation. **Mahshid Akhavan Rahnama:** Formal analysis, Methodology, Validation. **Mina Soufi-Zomorrod:** Formal analysis, Validation. **Fereshteh Soutodeh:** Formal analysis, Validation. **Somayeh Parichehreh-Dizaji:** Formal analysis, Validation. **Hassan Rakhsh-Khorshid:** Formal analysis, Methodology, Validation. **Hilda Samimi:** Conceptualization, Methodology, Visualization, Writing – review & editing. **Vahid Haghpanah:** Conceptualization, Supervision, Resources, Writing – review & editing.

Declaration of competing interest

The authors have no conflicts of interest to declare.

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