Indian Journal of Biochemistry & Biophysics
Vol. 61, October 2024, pp. 620-626
DOI: 10.56042/ijbb.v61i10.9564

Identification of a new MYBL2 isoform with distinct subcellular distribution

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Received 13 March 2024; *revised 19 June 2024*

Different splicing variants of genes may have different functions, and the identification of a new Splicing variant may broaden the role of the gene in many respects. MYBL2 plays a crucial role in the regulation of cell growth and differentiation, cell survival and cell cycle progression. In human embryonic stem cells (hESCs), there are usually two splicing forms of MYBL2 reported, MYBL2a and MYBL2b. Here, we successfully cloned the novel splicing variant of the MYBL2, which is named MYBL2c *in vitro*. MYBL2c lacks exon 3 and exon 4, as well as most of the regions encoding the DNA-binding domain. More importantly, MYBL2cis located in both the nucleus and cytoplasm, while MYBL2a is dominantly located in the nucleus. In addition, this splicing variant is not only expressed in undifferentiated human embryonic stem cells but also expressed in the neural stem cell, with a similar expression pattern to that of SOX2. Therefore, our study implies that the alternative splicing of MYBL2 is closely related to the maintenance of stem cell pluripotency.

Keywords: Human embryonic stem cells, Isoform, MYBL2, Neural stem cell

The MYB family consists of three members, namely MYB (c-Myb) , MYBL1 (A-Myb) , and MYBL2 (B-Myb). MYB was initially identified as the mammalian homolog of the retroviral v-Myb oncogene, which causes acute leukemia. MYBL1 and MYBL2 have subsequently been cloned due to their similarity to MYB[1,2]. In mammals, expression of MYB is primarily found in hematopoietic cells, colonic crypts, and the brain[3,4], whereas MYBL1 is expressed in various regions of the developing central nervous system, germinal B-lymphocytes, and the reproductive systems of both genders[5]. In contrast, MYBL2 is expressed in nearly all cells undergoing cell division, which may explain why mice lacking MYBL2 experience early embryonic death due to the impairment of inner cell mass formation[6]. In conclusion, of all three MYB family members, MYBL2 seems to play a more important role in regulating cell growth and differentiation, cell survival and cell cycle progression[7].

MYBL2 is expressed in stem cells in many tissues and plays a crucial role in the activation of genes that regulate the transition from G2 to the M phase[8,9]. The MuvB core acts in concert with MYBL2 to facilitate the recruitment of FOXM1 to specific gene promoters, including Cyclin B, survivin, and the CDC25 phosphatases, which are responsible for the control of the G2-M checkpoint[10,11]. In addition, a recent study identified a signaling pathway involving ATR-CDK1-FOXM1, which collaborates with MYBL2 to ensure a smooth transition of cells from the S phase to the G2 phase[12]. Moreover, several evidence indicate that MYBL2 contributes to the maintenance of the undifferentiated state of stem cells. Mechanistically, it has been suggested that MYBL2 participates in regulating the transcriptional network that governs cell cycle progression and cell fate, and this regulation is crucial for sustaining self-renewal and pluripotency of embryonic stem cells (ESCs) [13]. Specifically, MYBL2 directly controls the expression of key mediators of differentiation and pluripotency in ESC, such as POU5F1, SOX2, and NANOG[14,15]. Similarly, MYBL2 has been shown to govern the self-renewal and differentiation of hematopoietic stem cells[16]. This regulation was achieved by down-regulating ID1 and CEBPα, which are known to promote cellular differentiation, while simultaneously up-regulating GATA2, a transcription factor that stimulates proliferation at the expense of differentiation[16,17].

Here, we report a novel splice variant of MYBL2 that lacks the exon encoding part of the DNA binding

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domain, and since it shares the same ATG and termination codon as the other two splice variants, it is likely to be a protein-encoding RNA. In our current study, different from the MYBL2 protein located in the nucleus, the new isoform of MYBL2 coding by a new splicing variant distributed both in the nucleus and cytoplasm, indicating distinct roles in gene regulation. In addition, overexpression of the new splicing variant in human embryonic stem cells (hESCs) promotes the cell-cycle-dependent genes and core factors, which are identical to MYBL2. Moreover, during the neural differentiation of hESCs, both MYBL2 and its new splicing variant had the similar expression pattern to SOX2, further demonstrating its crucial role in stem cell maintenance.

Materials and methods

Cell culture and neural differentiation

The HEK293FT cells (Invitrogen) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). The hESCs (H9, WA09) were maintained on irradiated mouse embryonic fibroblasts (MEFs)[18]. The hESC culture medium (hESCM) consisted of DMEM/F12, 20% Knockout serum replacer, 1×minimal essential medium (MEM) non-essential amino acids solution, 1×L-glutamine solution, 0.1 mM β-mercaptoethanol, and 4 ng/mL fibroblast growth factor 2[19]. The hESCs were passaged every 5 days using dispase digestion (Gibco, 17105) , and the medium was changed daily. Neural differentiation of hESCs followed a published protocol[20,21]. Briefly, the hESCs were dissociated from the MEF layers, broken into suitable pieces, and suspended in hESCM to form embryoid bodies for the first 4 days. Then, the medium was switched to a neural induction medium (NIM) to induce neuroectoderm fate. Six days after differentiation, cell aggregates were plated on a laminin-coated surface. At day 17, neural progenitor cells were detached and maintained in suspension to form neurospheres. The NIM recipe consisted of DMEM/F12, 1×N2 supplement, 1×MEM non-essential amino acids solution, and 2 mg/mL heparin.

Transfection and virus package

The calcium phosphate precipitation method was used for transient transfection[22]. Briefly, 2 μg plasmids (pLVX-Tight-HA-MYBL2a and pLVX-Tight-HA-MYBL2c) were used for transfection together with 2 μg ef1α-rtTA. Doxycycline was added to induce gene expression to a final concentration of 2 μg/ml. To produce lentivirus, HEK293FT cells (Invitrogen) cultured in a 10 cm dish were co-transfected with 10 μg of lentiviral transfer vector, 7.5 μg of Δ8.9, and 5 μg of VSVG using the calcium phosphate precipitation method. After 16 h, the medium was replaced with a fresh-cell culture medium. Two days later, the medium containing viral particles was collected and filtered through a 0.45 μm filter (Millipore). The viral particles were then concentrated by ultracentrifugation (SW28 rotor, Beckman) at 50,000 × g for 2.5 h. Finally, the pellet was resuspended in hESCM.

Western blotting

Following a transient transfection lasting 48 h, whole-cell protein extracts were prepared from HEK293 FT cells by utilizing 1 mL of 1× sample loading buffer. After boiling for 5 min, 30 μL of whole-cell extracts were subjected to electrophoresis on a 10% SDS polyacrylamide gel. After being transferred on a nitrocellulose membrane, blots were immunostained with rabbit anti-GFP antibody (Invitrogen) followed by horseradish peroxidase (HRP) -labeled goat anti-rabbit secondary antibody (Jackson Immuno Research Laboratories). Blots were then striped and mouse anti-β-actin antibody (Sigma-Aldrich) was probed as an internal control.

Immunochemistry

HEK293 cell lines stably infected with HA-MYBL2a or HA-MYBL2c genes were grown on coverslips in 24-well plates. For studying protein distribution, cells were treated with doxycycline for 24 h to induce protein expression and were then immediately fixed for 10 min at room temperature in 4% paraformaldehyde (PFA). Nuclei were stained with Hoechst for 5 min after membrane permeabilization with 0.2% Triton X-100/PBS.

Real-time PCR

The total RNA was purified with Trizol and 1 μg of total RNA was used for the reverse transcription with random primers (Invitrogen) and SuperScript III (Invitrogen). Quantitative PCR was performed on a CFX Connect Real-Time PCR System (Bio-Rad) with SYBR green detection PCR Mastermix (Bio-Rad). For Otx2c, the annealing temperature was set to 62°C to secure the specificity of the primers[23,24]. For all other primers, the annealing temperature was 60°C. The primers were used as follows (Table 1).

Statistical analysis

Data were analyzed using Student's t-test for comparison of independent means with pooled estimates of common variances.

Table1 — Real-time PCR primers sequence

Gene	Forward primer	Reverse primer
GAPDH	atgacatcaagaaggtggtg	cataccaggaaatgagcttg
NANOG	attcttccaccagtcccaaa	atctgctggaggctgaggta
SOX2	gccctgcagtacaactccat	tggagtgggaggaagaggta
POU5F1	acatcaaagctctgcagaaagaact	ctgaataccttcccaaatagaaccc
SOX1	gtttttttgtagttgttaccgc	gcatttacaagaaataatac
PAX6	tctttgcttgggaaatccg	ctgcccgttcaacatccttag
MYBL2 total	cttgagcgagtccaaagactg	agttggtcagaagacttccct
MYBL2a	gtgaggcagtttggacagca	gattcaaaactctcagccacctg
MYBL2c	cccatgaggaggtcatcgag	ctggcaacatcttggcgat
CCNA2	cgctggcggtactgaagtc	gaggaacggtgacatgctcat
CCND2	accttccgcagtgctccta	cccagccaagaaacggtcc
CDK1	aaactacaggtcaagtggtagcc	tcctgcataagcacatcctga

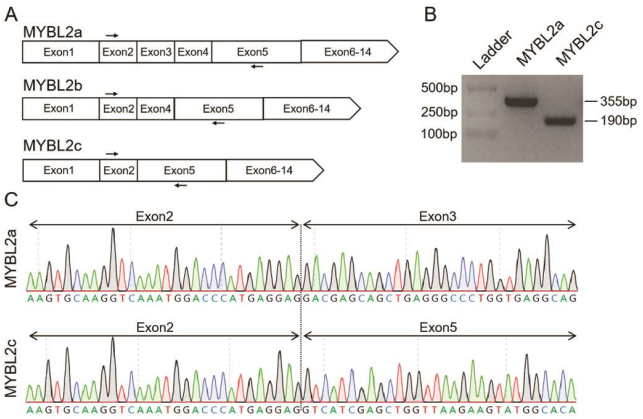

Fig. 1 — MYBL2c is an alternative splicing variant of the MYBL2 gene. (A) Schematic structure of MYBL2a, MYBL2b and MYBL2c; (B) PCR amplification of hESC cDNA with MYBL2 specific primers yields two clear bands; and (C) Sanger-sequencing data of MYBL2a and MYBL2c

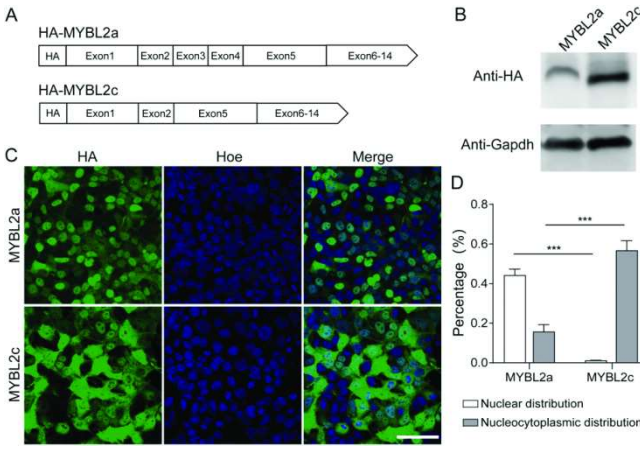

Fig. 2 — MYBL2c displays a different cellular distribution than MYBL2a. (A) Schematic structure of HA-MYBL2a and HA-MYBL2c; (B) Western blotting shows fusion protein expression of HA-MYBL2aand HA-MYBL2c in HEK 293 FT cells; and (C) HA-MYBL2a and HA-MYBL2c show different cellular distributions when transiently transfected into HEK293 FT cells; and (D) Quantification analyses of HA-MYBL2a and HA-MYBL2c in cellular distribution when transiently transfected into HEK293 FT cells (***P< 0.001 show statistical significance)

Results

MYBL2b is 24 amino acids shorter than MYBL2a based on different 5′splicing sites on exon 3 (72bp). However, when cloning MYBL2afrom hESCs, we identified a new variant that is much smaller than the MYBL2a (165bp) , which may be a new isoform of MYBL2.We termed this splicing variant as MYBL2c in comparison with MYBL2a and MYBL2b

(Fig. 1A). We ligated MYBL2a and MYBL2c into an inducible pLVX-Tight-Puro lentiviral vector (Clontech). Sequencing data showed that MYBL2a covers all 14 exons, while MYBL2c represented another splicing form of MYBL2 lacking both exon 3 and exon 4 different from MYBL2b only lacking exon3. A pair of primers were designed to distinguish different variants of MYBL2, with MYBL2a showing a band around 355 bp and MYBL2c showing a band around 190 bp (Fig. 1B). Sanger-Sequencing of the bands verified the lack of exon 3 and exon 4 in MYBL2c (Fig. 1C). Both PCR and Sanger-Sequencing results identified the exist of new splicing variant of MYBL2 (MYBL2c) at mRNA level.

Both MYBL2a and MYBL2b are protein-coding genes, for further analysis, we fused the HA tag upstream to MYBL2a and MYBL2c within the ORF respectively, and detected HA fusion protein expression by fluorescent microscope and Western blotting (Fig. 2A). Consistent with the results of sanger-sequencing, western blotting showed a higher band in HA fusion MYBL2a than HA fusion MYBL2c (Fig. 2B). To our amazement, when detecting the cellular distribution of MYBL2a and MYBL2c, MYBL2a is mainly located in the nucleus

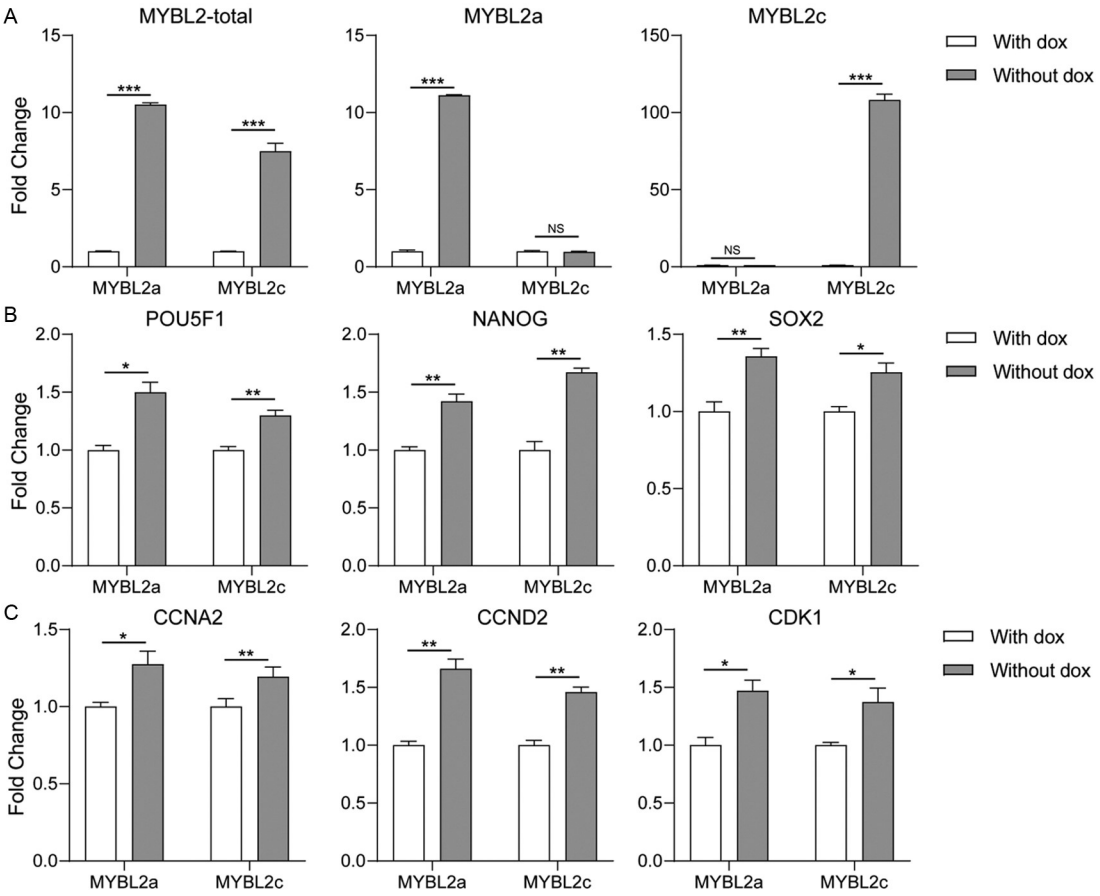

Fig. 3 — MYBL2c increases self-renew ability and proliferation of hESCs. (A) qPCR analysis shows the induction efficacy of HA-MYBL2aand HA-MYBL2c; (B) qPCR analysis of pluripotent genes, POU5F1, NANOG and SOX2 when overexpression of MYBL2a and MYBL2c; and (C) qPCR analysis of cell-cycle genes, CCNA2, CCND2 and CDK1 when overexpression of MYBL2a and MYBL2c. (*P<0.05; ** P< 0.01; *** P< 0.001 show statistical significance)

whereas MYBL2c is located both in the nucleus and cytoplasm (Fig. 2C). In summary, approximately 45% of MYBL2a is solely found in the nucleus, while 20% is present in both the nucleus and cytoplasm. On the other hand, only a small portion of MYBL2c is localized in the nucleus, with the majority being found in both the nucleus and cytoplasm (Fig. 2D). Altogether, western blotting and fluorescent microscope suggest this new splicing variant (MYBL2c) is also a protein-coding gene. And it locate in both the nucleus and cytoplasm other than its isoform MYBL2a only located in the nucleus.

We constructed inducible MYBL2a and MYBL2c cell lines in hESCs derived by TetON promoter to further study its function. With the help of dox, we could detect its expression. Specific primers were designed to distinguish MYBL2a from MYBL2c. With dox addition, total MYBL2 expression

were elevated both in MYBL2a and MYBL2c inducible hESCs. The specific increment of either MYBL2a or MYBL2c verified the correctness of the inducible hESCs (Fig. 3A). We further investigated whether overexpression of MYBL2a and MYBL2c affect the expression of pluripotent core factors. Overexpression of MYBL2c for 5 days significantly increased the expression of core factors including NANOG, POU5F1 and SOX2, as well as MYBL2a (Fig. 3B). In addition, Overexpression of both MYBL2a and MYBL2cfor 5 days could also elevate the cell-cycle genes, such as CCNA2, CCND2 and CDK1 (Fig. 3C). Altogether, consistent with MYBL2a, MYBL2c could accelerate the self-renew and proliferation of hESCs. hESCs hold unlimited self-renewal ability and the potential to differentiate into all three germ layers *in vitro* under proper culture conditions, which can be used for drug screening, transplantation and *in vitro* model system. MYBL2c act as core factors promoting the self-renew and proliferation of hESCs, which expand the application of hESCs.

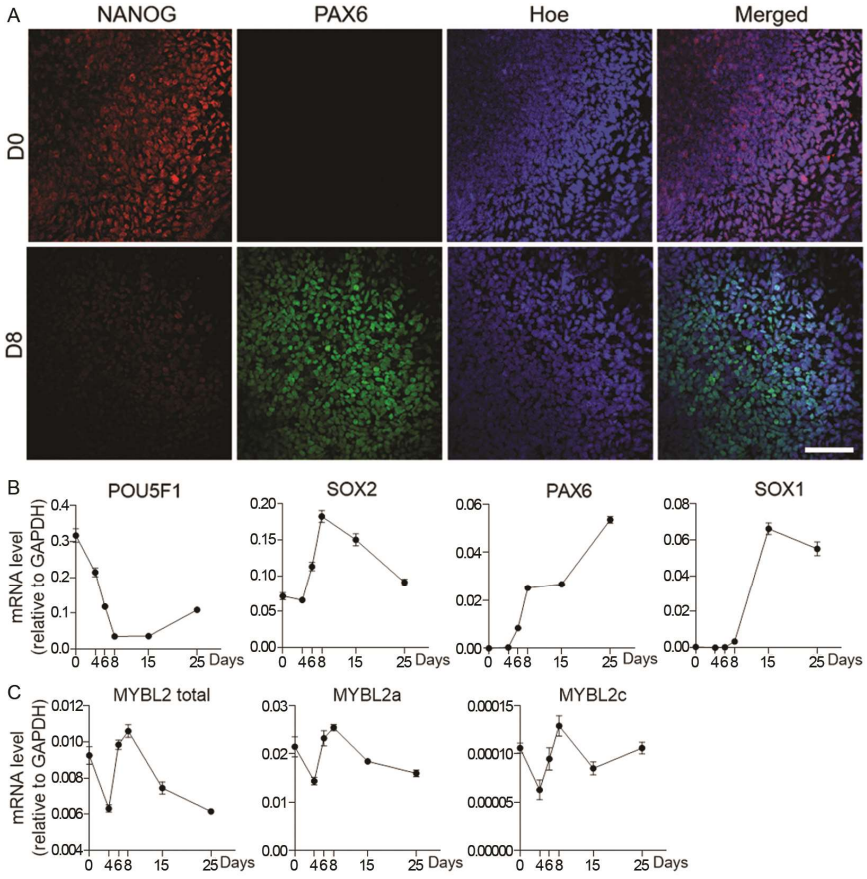

Fig. 4 — MYBL2c shows a similar expression as SOX2 during neural differentiation. (A) 8 days post neural differentiation of hESCs, pluripotent genes, NANOG is decreased, while neural genes, Pax6 is increased. Scale bar = 50 μM; (B) qPCR analysis of POU5F1, SOX2, PAX6 and SOX1; and (C) qPCR analysis of MYBL2, MYBL2a and MYBL2c

We next differentiated hESCs to neural lineages by the neural differentiation system described. Human ESCs were first suspended as embryoid bodies (EBs) for 4 days to initiate gastrulation. EBs were then transferred to the neural differentiation medium for another 2 days to promote neural differentiation. At day 6 post differentiation, cells were plated down onto laminin-coated culture surfaces and continue cultured for 4 days. Around this time, the cells were committed to the neural fate and verified by immunostaining of NANOG and PAX6 (Fig. 4A). Next, we detected the expression of pluripotent and neural genes, such as POU5F1, SOX2, PAX6, and SOX1, during the neural differentiation. As expected, the expression of POU5F1 decreased dramatically, whereas the expression of PAX6 and SOX1 increased as neural differentiation continued (Fig. 4B). When detecting the expression of MYBL2a and MYBL2c, we found the expression patterns of both MYBL2a and MYBL2c were similar to SOX2, indicating their complex role in hESCs and neural stem cells (NSCs) (Fig. 4C). During the neural differentiation, the expression trends of MYBL2a and MYBL2c are consistent, but from day15 to day25 of neural differentiation, MYBL2c shows an uptrend as opposed to MYBL2a, indicating its unique role in neurogenesis.

Discussion

MYBL2 is a highly conserved member of the MYB family of transcription factors that is crucial in controlling several cellular processes such as cell cycle, proliferation, differentiation, apoptosis, and senescence[25]. Previous studies identified two main splice variants of MYBL2 known as MYBL2a and MYBL2b[26,27]. Among them, MYBL2a has a sequence length of 355bp, and its gene sequence contains multiple exons. MYBL2b, which is 24 amino acids shorter than MYBL2a, isdeficient in exon 3. In our study, a new splicing variant of the MYBL2 gene was cloned and named MYBL2c. Sequencing results showed that the sequence length of MYBL2c was 190

bp, and its gene structure was different from that of MYBL2a (which lacked exon 3 and exon 4) and MYBL2b (which lacked only exon 3). Therefore, MYBL2c may be the novel isoform of MYBL2.

Previous studies have found that MYBL2 is involved in cell cycle regulation in a variety of cell types and is capable of maintaining self-renewal and differentiation of pluripotent stem cells[28,29]. In our study, we found overexpression of MYBL2c affects the expression of pluripotent core factors and cell cycle genes of human embryonic stem cells, as well as MYBL2a. However, Owing to the partial absence of a DNA-binding domain, MYBLc exhibits localization in both the nucleus and cytoplasm. Its nuclear function resembles MYBLa promoting the self-renew and proliferation, whereas its cytoplasmic function may regulate metabolism which need to be further explored. Furthermore, the expression pattern of MYBL2c in NSCs is identical to that of MYBL2a. Thus, our findings indicate that MYBLA2c may function similarly to MYBL2a, participating in cell cycle regulation while playing a role in stem cell stemness maintenance.

It has been reported that Transcriptional cofactors including CBP/P300, cycinD1, PARP1, p107, and p57kip2 enter the nucleus and bind to the MYBL2 promoter region, thereby facilitating the transition from the G1 to the S phase, where the cell undergoes autonomous division[30]. Our results indicate that MYBL2a is mainly located in the nucleus, whereas MYBL2c is localized in both the nucleus and cytoplasm. Thus, MYBL2c is not only a protein-coding gene but also has a different distribution in the nucleoplasm, which confers a complex biological function to MYBL2c. All in all, its potential functions and mechanisms need to be further investigated.

Our study indicated that MYBL2c is a novel splice variant of the MYBL2 gene that is localized in both the nucleus and cytoplasm. MYBL2c has the same function as MYBL2a in the regulation of the cell cycle and the maintenance of stem cell stemness.

Conclusion

In summary, we report here that MYBL2c is a novel alternative splicing variant of the MYBL2 gene. Its distribution throughout the cell implies that its role may not be limited to the nucleus, which is different from MYBL2a. Moreover, the high expression of MYBL2cin hESCs and NSCs is similar to the expression pattern of SOX2, demonstrating its potential role in pluripotency maintenance of embryonic stem cells.

Acknowledgment

This work was supported by the National Natural Science Foundation of China (Grant No: 81900287) , National Natural Science Foundation of China (Grant No: 82200901).

Conflict of interest

All authors declare no conflict of interest.

References

1 Roussel M, Saule S, Lagrou C, Rommens C, Beug H, Graf T & Stehelin D, Three new types of viral oncogene of cellular origin specific for haematopoietic cell transformation. *Nature*, 281 (1979) 452.

2 Ness SA, Myb protein specificity: evidence of a context-specific transcription factor code. *Blood Cells Mol Dis*, 31 (2003) 192.

3 Shin DH, Lee HW, Jeon GS, Lee HY, Lee KH & Cho SS, Constitutive expression of c-myb mRNA in the adult rat brain. *Brain Res*, 892 (2001) 203.

4 Zorbas M, Sicurella C, Bertoncello I, Venter D, Ellis S, Mucenski ML & Ramsay RG. c-Myb is critical for murine colon development. *Oncogene*, 18 (1999) 5821.

5 Toscani A, Mettus RV, Coupland R, Simpkins H, Litvin J, Orth J, Hatton KS & Reddy EP, Arrest of spermatogenesis and defective breast development in mice lacking A-myb. *Nature*, 386 (1997) 713.

6 Tanaka Y, Patestos NP, Maekawa T & Ishii S, B-myb is required for inner cell mass formation at an early stage of development. *J Biol Chem*, 274 (1999) 28067.

7 Ushakiranmayi M, Naveen K & Sudhakar P, Isolation, structure elucidation and bioactivity of secondary metabolites produced by marine derived *Streptomonospora arabica* VSM-25. *Indian J Biochem Biophys*, 59 (2022)73.

8 Fischer M, Quaas M, Steiner L & Engeland K, The p53-p21-DREAM-CDE/CHR pathway regulates G2/M cell cycle genes. *Nucleic Acids Res*, 44 (2016) 164.

9 Lorvellec M, Dumon S, Maya-Mendoza A, Jackson D, Frampton J & García P, B-Myb is critical for proper DNA duplication during an unperturbed S phase in mouse embryonic stem cells. *Stem Cells*, 28 (2010) 1751.

10 Sadasivam S, Duan S & DeCaprio JA. The MuvB complex sequentially recruits B-Myb and FoxM1 to promote mitotic gene expression. *Genes Dev*, 26 (2012) 474.

11 Sadasivam S & DeCaprio JA, The DREAM complex: master coordinator of cell cycle-dependent gene expression. *Nat Rev Cancer*, 13 (2013) 585.

12 Saldivar JC, Hamperl S, Bocek MJ, Chung M, Bass TE, Cisneros-Soberanis F, Samejima K, Xie L, Paulson JR, Earnshaw WC, Cortez D, Meyer T & Cimprich KA, An intrinsic S/G2 checkpoint enforced by ATR. *Science*, 361 (2018) 806.

13 Zhan M, Riordon DR, Yan B, Tarasova YS, Bruweleit S, Tarasov KV, Li RA, Wersto RP & Boheler KR, The B-MYB transcriptional network guides cell cycle progression and fate decisions to sustain self-renewal and the identity of pluripotent stem cells. *PLoS One*, 7 (2012) e42350.

14 Tarasov KV, Tarasova YS, Tam WL, Riordon DR, Elliott ST, Kania G, Li J, Yamanaka S, Crider DG, Testa G, Li RA, Lim B,

Stewart CL, Liu Y, Van Eyk JE, Wersto RP, Wobus AM & Boheler KR, B-MYB is essential for normal cell cycle progression and chromosomal stability of embryonic stem cells. *PLoS One*, 3 (2008) e2478.

15 Tarasov KV, Testa G, Tarasova YS, Kania G, Riordon DR, Volkova M, Anisimov SV, Wobus AM & Boheler KR, Linkage of pluripotent stem cell-associated transcripts to regulatory gene networks. *Cells Tissues Organs*, 188 (2008) 31.

16 Baker SJ, Ma'ayan A, Lieu YK, John P, Reddy MV, Chen EY, Duan Q, Snoeck HW & Reddy EP, B-myb is an essential regulator of hematopoietic stem cell and myeloid progenitor cell development. *Proc Natl Acad Sci USA*, 111 (2014) 3122.

17 Briegel K, Lim KC, Plank C, Beug H, Engel JD & Zenke M, Ectopic expression of a conditional GATA-2/estrogen receptor chimera arrests erythroid differentiation in a hormone-dependent manner. *Genes Dev*,7 (1993) 1097.

18 Akanksha V, Usha S, Anurag M & Paulraj R, Cytotoxic and genotoxic assessment of wastewater on HEK293 cell line. *Indian J Biochem Biophys,* 59 (2022) 558.

19 Joshi R, Buchanan JC, Paruchuri S, Morris N & Tavana H, Molecular Analysis of Stromal Cells-Induced Neural Differentiation of Mouse Embryonic Stem Cells. *PLoS One*, 11 (2016) e0166316.

20 Zhang X, Huang CT, Chen J, Pankratz MT, Xi J, Li J, Yang Y, Lavaute TM, Li XJ, Ayala M, Bondarenko GI, Du ZW, Jin Y, Golos TG & Zhang SC, Pax6 is a human neuroectoderm cell fate determinant. *Cell Stem Cell*, 7 (2010) 90.

21 Zhang XQ & Zhang SC, Differentiation of neural precursors and dopaminergic neurons from human embryonic stem cells. *Methods Mol Biol*, 584 (2010) 355.

22 Lynch J, Chung J, Huang Z, Pierce V, Saunders NS & Niu L, Enhancing transient protein expression in HEK-293 cells by briefly exposing the culture to DMSO. *J Neurosci Methods*, 350 (2021) 109058.

23 Zahra H, Azam AR, Bita M & Hamidreza MS, Association between the organic cation transporter3 methylation and hepatocellular carcinoma. *Indian J Biochem Biophys*, 60 (2023) 224.

24 Keshika D, Hrishita S, Largee B & Anita K, Nanotechnology in Forensic Science: Extensive Applications and New Perspective. *Indian J Biochem Biophys*, 59 (2022) 1144.

25 Bayley R, Ward C & Garcia P, MYBL2 amplification in breast cancer: Molecular mechanisms and therapeutic potential. *Biochim Biophys Acta Rev Cancer*, 1874 (2020) 188407.

26 Horstmann S, Ferrari S & Klempnauer KH, An alternatively spliced isoform of B-Myb is a transcriptional inhibitor. *Oncogene*, 19 (2000) 5428.

27 Kamano H, Burk B, Noben-Trauth K & Klempnauer KH, Differential splicing of the mouse B-myb gene. *Oncogene*, 11 (1995) 2575.

28 Bessa M, Joaquin M, Tavner F, Saville MK & Watson RJ, Regulation of the cell cycle by B-Myb. *Blood Cells Mol Dis*, 27 (2001) 416.

29 Blakemore D, Vilaplana-Lopera N, Almaghrabi R, Gonzalez E, Moya M, Ward C, Murphy G, Gambus A, Petermann E, Stewart GS & García P, MYBL2 and ATM suppress replication stress in pluripotent stem cells. *EMBO Rep*, 22 (2021) e51120.

30 Musa J, Aynaud MM, Mirabeau O, Delattre O & Grünewald TG, MYBL2 (B-Myb): a central regulator of cell proliferation, cell survival and differentiation involved in tumorigenesis. *Cell Death Dis*, 8 (2017) e2895.