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Review Paper

The profiles of Ascl2 Gene Expression in different Conditions of Cells

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The profiles of gene expressions are cellular responses to the several conditions varying from normal to pathologic. Although the Ascl2 expression increased in various cancers, however, its expression profiles are yet to be conclusively understood, such as changes in trophoblastic disease, nasopharyngeal, and ovarian cancer. By analyzing 3'end Ascl2 genes, both using the Sac-II restriction enzyme, RT-PCR, and single strand conformation polymorphism (SSCP), and DNA sequencing, we found that the 3'end of the Ascl2 gene also plays a role in gene expression in trophoblastic disease. It suggested, the expression of the Ascl2 gene is regulated by two regulators, one site from the 5'end and the other from 3'end. By using the experimental animals that were given treatment supplementation of soybean extract, resulting in the shortened villi and thicker crypt, suggesting Ascl2 had two different molecular targets. To implement the Ascl2 expression profile in medicine, we tested that gene in 258 healthy women (15-59 years). The results show that Ascl2 is a strong expression and the expression in adult women is stronger than in the young female. The expression of the Ascl2 gene in pathological conditions has been shown using nasopharyngeal cancer and ovarian cancer. As a comparison, we used a Hela cell. The results show that the expression of the Ascl2 in nasopharyngeal is stronger than in ovarian cancer (moderately expressed). In Hela cells, Ascl2 is expressed very strongly. To complete the profile, we also reported the miR302b, cMyc, and KLF4 gene expression. The different expression of Ascl2 on various cancer cells showed that cell cancers have their own mechanism dealing with the Ascl2 pathway. Therefore, it needs other genes such as cMyc, KLF4, and miR302b that completed the Ascl2 analysis.

Keywords: Threshold cycle, Ascl2, miR320b, Housekeeping gene.

INTRODUCTION

Analysis of gene expressions is very important for the detection of differential expressions of the gene as gene between different tissue types or between normal and disease status can provide leads for early detection of disease and thus for predictive medicine. The cellular functions including cell proliferation and differentiation are the result of the activation of multiple signaling pathways, for which in this paper we report Ascl2 activities in trophoblastic disease, nasopharyngeal and ovarian cancer.

Trophoblast derived from trophectoderm provides the functional bridge between the fetus and the mother. One of growth disturbances of the trophoblast is hydatidiform mole or complete mole that has a high predisposition to malignant trophoblastic tumor (choriocarcinoma) in human [1,2]. This mole is originated from incomplete conception, where the growing embryonic tissue does not have the genome from the mother, but containing androgenetic component. Epidemiological study has shown that half of choriocarcinoma follow complete mole, one-fourth follow normal pregnancies, and the remaining follow abortions. We have analyzed the genetic origin of choriocarcinoma by using sequence taq site (STS) polymorphic marker that most of the choriocarcinoma was originated from complete mole [3].

In this review, we report one of microRNAs that has been known to have a relationship with the Ascl2 expression that is miR302b in colon cancer [4]. However, it has not yet been

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known in nasopharyngeal and ovarian cancer. In this paper, we also report another profile of Ascl2 mRNA expression in a healthy woman. We also reported the response of Ascl2 gene to soybean supplementation in intestinal mouse associated with villi and crypt sizes to explain that Ascl2 has two different molecular targets. The mRNA expressions were determined by a conventional RT-PCR and by a quantitative real-time PCR system (Bio-Red) and calculated by formula $2^{-\Delta\Delta ct}$ [5].

ASCL2 GENE HAS TWO GENE REGULATORS PLAYING A ROLE IN TROPHOBLASTIC DISEASE

The human achaete-scute2 (Ascl2) or Hash2 (Human achaete-scute homolog 2) gene is a member of the basic helix-loop-helix (bHLH) of the human transcription factor. The product of Ascl2 gene has been reported to be involved in genomic imprinting with maternal allele expression in mouse [6]. These imprinted genes are clearly involved in the development of trophoblast tissues, which derivate from the trophectoderm of the blastocyst [7]. If the embryonic tissue derived from non-paternal genomes, it will have a placental failure and the embryo die or is aborted [6,7]. Interestingly, the mechanisms loss of imprinting involves many molecular networks, including molecular interaction within 5'UTR [8,9,10] and might be between 5' and 3' UTR in choriocarcinoma [11]. We explain the Ascl2 function in trophoblastic disease associated with SacII restriction profile in a 3'UTR segment [11].

Ascl2 has reported that it was expressed in human placenta [11], controlling intestinal stem cell fate [12], high expression of Ascl2 in tumor tissues promotes the proliferation, invasion, migration and epithelial-mesenchymal transition in the colorectal cancer [13, 4], lung squamous cell carcinoma [14] and elevated Ascl2 expression in breast cancer [15]. The transcription factor Ascl2 formed a bimodal switch that activates a gene signature fundamental to the intestinal stem cell state, thus the Ascl2 formed and auto-activating loop that leads to an on/off expressions pattern [16].

The exact reason of moles for the higher propensity to develop malignancy is unknown. The complete mole arises from fertilization, by one or two sperm, of an ovum with no maternal chromosomes. Thus, only paternal chromosomes are present in complete mole [1, 2]. Both expressions of paternally transcribed genes and loss of expression of maternally transcribed genes are to play a role in molar development. Our previous result showed that Ascl2 gene was the positive expression in normal placenta but the negative expression in complete mole, and positive in choriocarcinoma [11]. The further experiment, we examined a 3'end segment of Ascl2 gene, including a SacII restriction site in two different clinicopathological forms of the trophoblast; complete mole and choriocarcinoma compared to the normal placenta.

This segment which has a size of 68 bp extended from nt 2903 to 2970 (NCBI) was performed by using the primers described by Miyamoto et al; F;5'-CGGCCCAGCCTGACCAATG-3' and R;5'-GAAGCCGCCAGCCCTTATG-3' [17]. The results showed that the expected band was observed in different condition of trophoblast development, both in normal placenta and in pathologic trophoblast (complete mole and choriocarcinoma) [3]. The products generated following amplification of DNA from choriocarcinoma were larger than those seen in normal placenta and complete mole (Fig 1a). This result was reconfirmed by Single-Strand Conformation Polymorphism (SSCP) by labeling the PCR product with (γ^{32}P) dATP. In figure 1b, the 68 bp segment was found both in normal placenta and complete mole. After digestion with SacII, there were two bands detected in normal placenta, but only one both in the complete mole and the malignant tumor, suggesting the segment was undigested in mole, PSTT, and choriocarcinoma (fig 1c and 1d).

We concluded that 3'UTR might be important in regulating the expression of Ascl2 in trophoblast development. However, the molecular mechanism is not clear yet. The further experiment, we continued to analyze DNA sequence of 3'end Ascl2 gene extended from 2651 to 3081 (430 nucleotides) containing 68 segments including a SacII restriction site. Our data showed that the sequence of 3'end Ascl2 gene both in mole and normal placenta was no mutation, including the SacII segment (fig 2).

This means that the segments targeted at the mole and normal placenta are identical to each other, and there are no mutations, we suggested the difference of restriction segment might be because of DNA methylation. In choriocarcinoma, those segments were larger than mole and normal placenta and uncut by SacII. We suggested, there is a difference in 3'UTR Ascl2 gene in the form of mutations and methylation changes in choriocarcinoma compared to the normal placenta or mola

The further experiment, we tested the expression of miR302b associated with Ascl2 expression. According to Zhu, et al., (2012) the expression of Ascl2 was blocked by increasing miR302b expression in colon cancer [4]. Relevant with this data, we demonstrated our result concerning miR302b expression in normal placenta and complete mole. The result showed that in eight normal placentas (ct value = 26.23±1.95) and in fourteen complete moles (ct value = 21.44±3.01), suggesting the expression of miR302b were moderately in normal placenta and strongly expressed in mole respectively. The expression of miR302b was high in complete mole, but non-expression of Ascl2, meaning our result was probably identical with the previous mechanism [4].

Based on our data, we hypothesize there are four possibilities of the Ascl2 gene regulation: methylation, mutation, microRNA attachment to Ascl2 mRNA, and or to mRNA repressor blocking the interaction between the promoter and the 3'end component. We concluded that Ascl2 gene probably has two gene regulators playing a role in trophoblastic disease, one from the 5'end and the other from the 3'end of the gene.

ASCL2 HAS TWO DIFFERENT MOLECULAR TARGETS

The mucous of the small intestine consists of two compartmental structure, the first is the proliferation zone (crypts) and the second is the differentiation zone (villi). Our experimental study was done using 18 male mice, that were four weeks old of Swiss Webster strain (The ethical clearance number: 141/FKUP-RSHS/KEPK/Kep/EC/2011). In this study, we tested the effect of soybean on the structure of villi and crypts in the mouse that has been given soybean supplement for four weeks (140 mg/ml/day soybean infusion supplement). The data were obtained by observing and measuring microscopically villus height and thickening of the crypt for intestinal mucosa, and the other samples were analyzed to see the profile of Ascl2 gene expression.

Data was then analyzed statistically with One Way ANOVA and Kruskal Wallis test. Here, we reported our study concerning the ability of Ascl2 to change the height of intestinal villi of mice. After administration of soybean extract (140 mg/ml per day, for 30 days), villi appeared to be shortened ($p < 0.05$) and positive Ascl2 expression (there was Ascl2 gene expression).

(c)

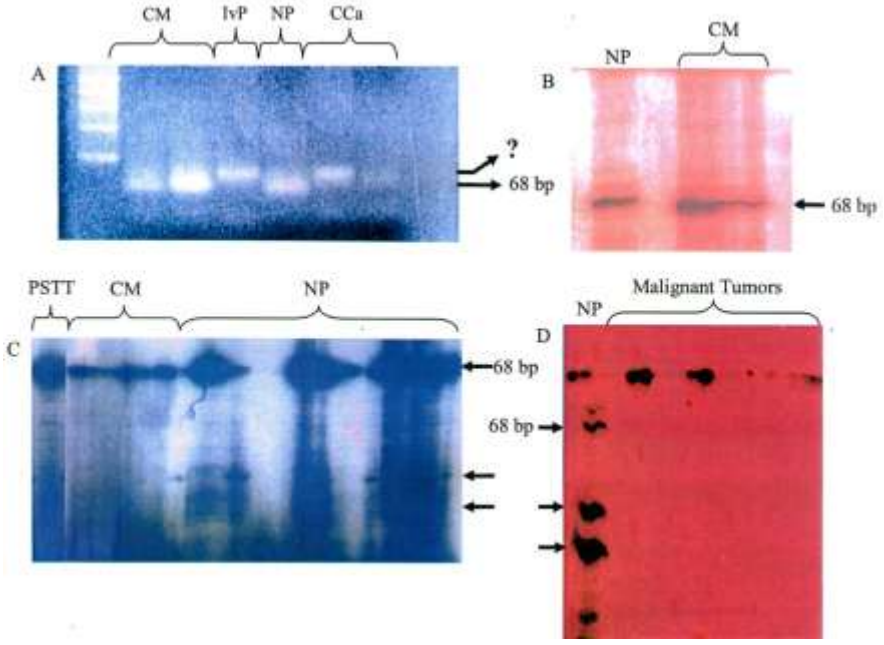


Figure 1. Amplification of 3'UTR. (a) PCR products identified in CM, IvM, normal placenta (NP) and ChCa following amplification of 3'UTR of HASH2. A 68-bp band is identified in NP and CM, while a larger band is seen in IvM and ChCa. (b) SSCP of PCR products from NP and CM. A 68-bp band is observable in all samples. (c) and (d) showed the malignancies bands did not cut.

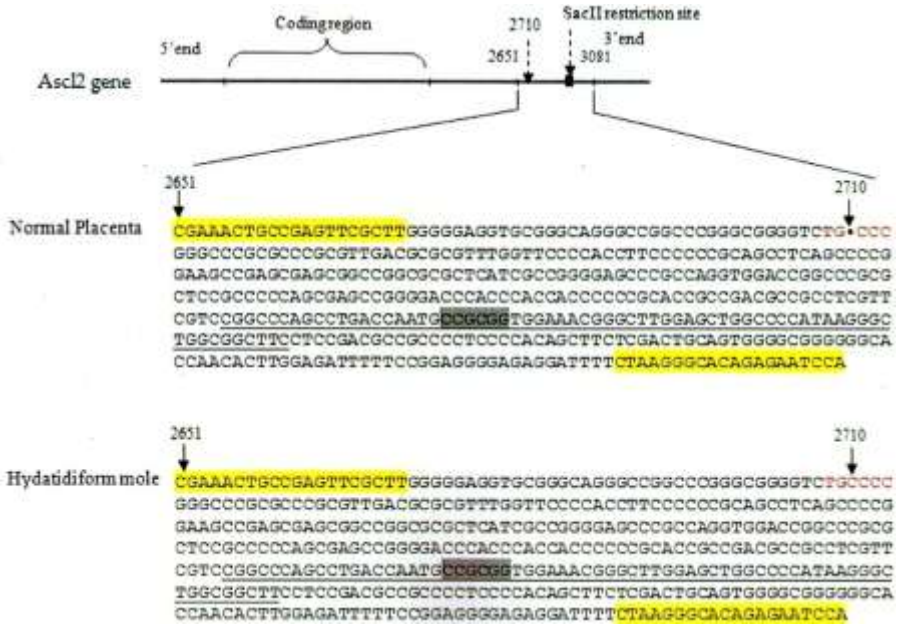
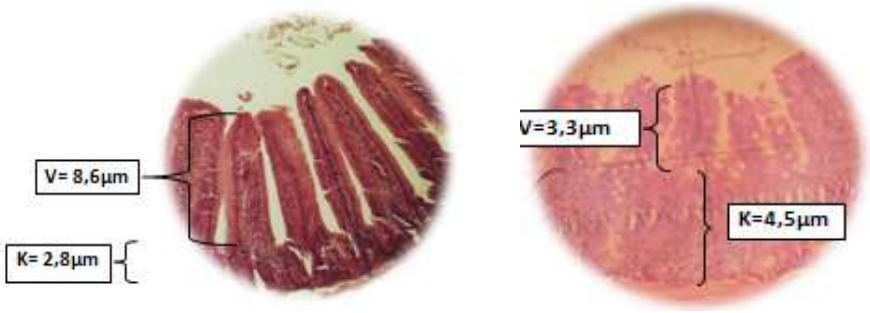


Figure 2. The nucleotides sequences extended from 2652 to 3081 (NCBI:AF442769,2001) and the underlined region is the 68bp segment, the grey area is SacII restriction site, and the yellow area is primers location.



(a) (b)

Figure 3. Comparison of the villi and the crypts between group control (a), soybean supplementation of 140 mg/ml (b).

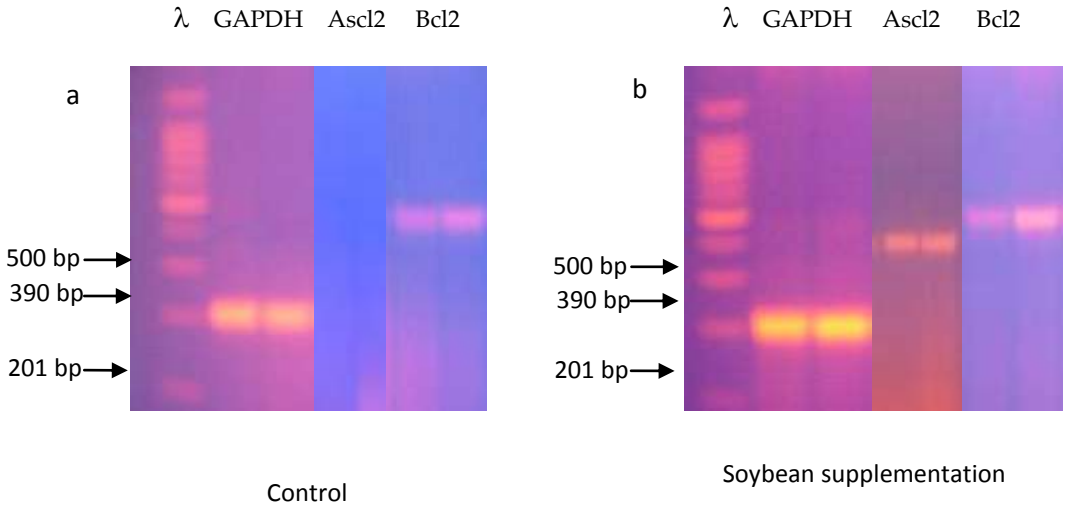


Figure 4. The effect of soybean on genes expression. The Ascl2 was a negative and bcl2 was positive expressions (a), the Ascl2 and bcl2 were positive expressions at 140 mg/ml (b).

Table 1. The profile of Ascl2 and bcl2 gene-expressions after soybean supplementation associated with villi and crypt development

	Villi		**Crypts**	
	(C)	**(T)**	**(C)**	**(T)**
Ascl2 expression	-	+	-	+
bcl2 expression	+	+	+	+
Size (µm)	8.6 µm	3.3 µm	2.8 µm	4.5 µm

C= Control

T= Treatment (140 mg/ml/day)

Table 2. Ct, Δct, $2^{-\Delta\Delta ct}$ value of genes expression in Hela cells, Nasopharyngeal, and ovarian cancer

	GAPDH	Ascl2	cMyc	KLF4	miR302b
Hela cell (n=3)					
ct value	22.21±3.22	17.19±3.11	24.37±4.32	30.73±1.46	26.96±0.90
Δct		-5.03±1.80	2.15±1.10	8.51±4.48	4.74±2.68
$2^{-\Delta\Delta ct}$		47.8±37.05	0.26±0.15	0.03±0.05	0.11±0.17
Nasopharyngeal cancer tissue (n=20)					
ct value	18.88±2.87	18.81±6.10	19.90±3.22	31.81±2.55	20.69±4.09
Δct		0.15±4.59	1.38±1.68	13.19±2.77	1.81±2.18
$2^{-\Delta\Delta ct}$		4.61±7.15	0.70±0.89	0.00±0.00	0.64±1.02
Ovarium cancer (n=15)					
ct value	19.12±3.90	27.22±7.22	32.87±3.6	33.6±2.58	18.36±1.58
Δct		7.93±5.30	13.74±5.84	15.31±5.33	-1.44±3.47
$2^{-\Delta\Delta ct}$		1.39±4.83	0.00±0.00	0.00±0.00	118.82±350.25

On the contrary, crypt appears thickened ($p < 0.05$), this means soy extract can inhibit cell differentiation in villi and induce cell proliferation in crypt (table 1 and fig. 3). Cells undergoing proliferation are in the crypt section, whereas the differentiated cells, mostly located in the intestinal villi. Electrophoretic data showed that GAPDH (a house-keeping gene) did not change to soy supplementation. Interestingly, the bcl2 gene was moderately expressed both in control and in supplement animal (fig. 4).

The bcl2 expression in control was positive and did not change after feeding the soybean extract, suggesting the shortening of villi was not because of apoptotic pathway activity. Surprisingly, the crypt grew even higher than the control (2.8 μm to 4.5 μm), suggesting bcl2 remaining active in crypt cells but not in villi (figure 4). From our data, we concluded that soybean extract in soybean extract supplementation did not inhibit cells proliferation in the crypt but inhibit cells differentiation in villi. Furthermore, we concluded that the shortening of the villi might be not because of apoptosis but inhibition of villi cells differentiation. We added another experiment by using Hela cells culture we found that the Ascl2 gene showed a high expression in Hela cells (ct value= 17.19±3.11), suggesting Ascl2 play a role in cell cycle for cell proliferation, this biological phenomenon validated to crypt cells. It means that Ascl2 has two molecular targets; first, on the cell cycle for cell proliferation; the second, on the transcription process for cell differentiation (fig. 3.)

It has been known that soybean contains dietary components influencing the morphology of the villus height and crypt depth [18,19], but their relationship with Ascl2 and bcl2 gene expressions have not yet been documented. Another group has reported that soy saponins exhibit inhibitory effects on neoplastic cells [20]. It is a milestone to create the soybean in cancer dietetic therapy

THE PROFILES OF mRNA ASCL2 IN HEALTHY WOMAN AND MALIGNANCIES

Gene expression is a cellular response expressed in the form of proteins and or RNAs. The proteins both functional and structural proteins were used to explain the pathogenesis or mechanism of disease, diagnostic, and therapy. The biological impact of gene expression depended on tissues types, times, and amount of RNAs. Although gene expression and gene regulation are thought to contribute to the development of diseases, little is known about the clinical implementation of the mRNA level as the targeted therapy or diagnostic.

It is important to note that fluctuation of mRNA and microRNA level contribute to fluctuation in protein levels. These fluctuations of mRNA and protein numbers result in the cell to cell fluctuation than the variation of organ functions, leading to the earlier detected the stage of an abnormalities. Besides the mRNA and protein numbers, **still,** have another molecular regulation of transcription that is microRNA molecules [21]. One of the microRNA (miRNA) in human associated with Ascl2 gene expression is miR302b [9]. According to the previous result, the expression of Ascl2 has a connection with miR302b activity in which the miR302b activates cyclinD2 to maintain the cell cycle in the Human Embryonal Carcinoma (22).

Examinations at the molecular level including gene expression examination and its regulation have not been applied yet in community health in Indonesia. The lack of standardized protocols for gene expression or its combination with a variety of miRNA, make the molecular networks of them was difficult to be implemented in the community. In this study, we determined the expression of Ascl2 in normal blood circulation collected from 258 healthy subjects. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Health Research Ethics Committee Padjadjaran University (number 222/UN6.C1.3.2/KEPK/PN/2016). The use of the mean expression value of several expressed RNA and miRNA in a healthy person might be useful as a reference for knowing the changes in genes expression in unhealthy persons. In the past, it had no effect of genetic information on the practice of medicine. Recently, several countries have entered a transition period in which specific knowledge such as molecular genetics is important to the healthcare of patients. In this paper, we analyzed Ascl2 and miR302b gene expressions in healthy females (15-59 years) for supporting the community health.

The Ascl2 gene has widely reported as a gene that encodes transcription factors that are important for cell differentiation but never implemented on a large scale. If we consider the miR302b a suitable partner of Ascl2, we have to look at their profile expression in several different conditions. In this paper, we analyzed the Ascl2 gene expression in different condition, such as in Hela cell, nasopharyngeal, and ovarian

cancer. miRNA is a small RNA molecule that plays a role in post-transcription or translation leading to protein synthesis inhibition. In human cells, there are various microRNAs, and each miRNA can regulate up to 200 mRNA molecules. In this study, isolation of RNA from the blood of 258 healthy females aged 15-59 years, consisted of 103 young females (15-19 years old) and 155 adult females (20 – 59 years old). RNAs were isolated from blood or tissues by their corresponding method according to the normal instruction of the company using the trisol reagent (Total RNA mini kit blood/culture cells, Gene aid) and for tissues (Invitrogen, USA) respectively. The normal tissue of ovarium was obtained during hysterectomy of non-ovarium and non-cancer patients. Total RNA purity, quantity, and quality were checked by NanoDrop spectrophotometer ND.1000 (Thermo Scientific, USA).

The RNA solution was ready for Real-time PCR (qRT-PCR) (Kappa Biosystem, USA). The RNA samples with absorbance ratio less than 1,8 were excluded from subsequent analysis. The result can be seen in the form of threshold cycle (ct value) by applying the relative changes in targeting genes expression. Threshold cycle (ct) is reported as the arbitrary placed signal threshold. The average Ct was calculated for both target gene and PCR cycle number that crosses an internal control (GAPDH) and the ∆ct was equal to the difference in threshold cycles for the target and GAPDH genes (ct target–ct GAPDH). The ∆∆ct is calculated from ∆ct of target gene minus ∆ct of GADPH [13]. All genes were normalized to GAPDH expression. We categorized the ct values varying from 15 to 40; 15-20 was a very high expression; >20-25 was a high expression: >25-30 was a moderate expression; >30-35 was a low expression, and >35-40 was very low or no expression. We also adapted the possibilities of genes expression under 15 of ct value, called over-expression. All these categories were confirmed by gel electrophoresis data [23, 24]. The calculation of genes expression was exposed by relative fold changes of the expression (either fold increased (↑) or fold decreased (↓)) in normal blood circulation (13).

Our results showed that there were significant differences in Ascl2 gene expression in teenage females (15-19 years old) with adult females (20-59 years old) ($p < 0.05$). However, there is no correlation with increasing ages. The mean of Ascl2 gene Ct value of young females (n=103) was 23.38±1.71, whereas the adult females (n=155) was 21.33±0.99, suggesting both genes were a strong expression. Although both of them were a high expression, the adult was stronger than younger ($p<0.05$). The expression of Ascl2 was significantly higher in adult than young females. The same profile of miR302b that was also significantly higher expression in adult than young females. However, there is no correlation between ages and gene expression both Ascl2 ($R^2 = 0.166$) and miR302b ($R^2 = 0.034$). It is a new normal value of Ascl2 gene expression in human blood for Indonesian females. It might be useful to distinguish between health and pathological. In a healthy state, the expression of the Ascl2 gene in the blood was in a strong category. Similarly, miR302b expression appears strongly expressed both in teenage and adult. Using our criteria of ct value levels of gene expressions we identified both Ascl2 and miR302b genes were high expressions in normal blood (ct value between >20-25). In normal placenta, the expression of Ascl2 and miR302b were moderately expressed.

Several studies have identified roles of Ascl2 in cancer development that its molecular network related to tumor initiation and progression, but the mechanism has not yet completely understood. To investigate a potential alteration in Ascl2 expression in a different type of tumor, we analyzed two types of cancer cell, including nasopharyngeal, and ovarian cancer. Previously, studies on Ascl2 and its interaction with miR302b has been reported [9], but its interaction with the others genes involving in cell proliferation and/or differentiation such as KLF4 [25] and cMyc [26] are not documented both in a healthy person and in pathologic. The decreased expressions of Ascl2 in HT-29 cell results in arrest at G-2/M cell cycle checkpoint through increasing of miR302b expression [9] showing us that this miR302b gene expressions play a role in cell growth and development. Lee, et al., reported that miR302b maintained “stemness” of human embryonal carcinoma cells by post-transcriptional regulation of cyclin D2 expression [22].

miR302b is a family of miR302 which is transcribed together as a non-coding RNA which other miR302 members such as miR302-367 and miR302b which quickly decrease after cell differentiation and proliferation. That is very interesting that miR302b is a potential molecular marker of esophageal squamous cell carcinoma and its function as a tumor suppressor by targeting an Erb B4 [27]. In addition, the mRNA expression profiling can be used to distinct adenocarcinoma sub-classes [28]. Our results showed that the increase of Ascl2 is not followed by decreasing of miR302b in nasopharyngeal cancer. It reinforces our hypothesis that the possibility of the expression of Ascl2 gene involving another protein might be a repressor regulated by miR302b in nasopharyngeal cancer. That is why, if miR302b expression increases the repressor inhibited and Ascl2 strongly expressed. However, this result was a different profile from those genes in ovarian cancer that the Ascl2 was moderate and the cMyc gene expression was low, whereas miR302b was strongly expressed in ovarian cancer. If we compared the Ct value of the Ascl2 expression in ovarium cancer (ct value = 27.22±7.22) to the normal ovary (ct value = 29.75±3.19), it showed that the Ascl2 expression in ovarian cancer was stronger than normal ovary, suggested it up-regulated in ovarian cancer.

The cellular functions including cell proliferation and differentiation are the result of activation of multiple signaling pathways, at which Ascl2, miR302b and the other genes including KLF4, cMyc, and many others genes might be involved. The transcription factor Ascl2 formed a bimodal switch that activates a gene signature fundamental to the intestinal stem cell state, thus the Ascl2 formed and auto-activating loop that leads to an on/off expressions pattern [12]. In the current study, we analyze Ascl2 and miR302b gene expression at the mRNA level associated with cMyc and KLF4 in the pathologic tissues such as nasopharyngeal cancer and ovarian cancer. In Indonesia, nasopharyngeal carcinoma (NPC) is frequent cancer, rating as the fourth for all malignancy and the most common malignancy in the head and neck. The prognosis remains poor with a 5-years survival of around 50% [29]. NPC patients generally come with an advanced stage at the time of diagnosis. Early diagnosis of NPC is still difficult to do because the symptoms are not typical of the initial examination.

For the two cancer types might be the analysis of gene expression at mRNA level in the form multiplexed measurement [13, 30, 31] can be used to indicate the distinctive of molecular networks between health and malignancies. Our result suggested that the profile of Ascl2 and the other three genes (cMyc, KLF4, and miR3023b) may indicate the molecular differences between nasopharyngeal, ovarian cancer, and Hela cell. This study is to promote of supplying additional information about gene expression to a clinical-pathogenesis requirement for improved diagnostic. In table 2 the profile of Ascl2 and other gene expression obtained

from nasopharyngeal and ovarian cancers compared to Hela cells. Analysis of the Ascl2 gene expression revealed the Ct value of Ascl2 both in nasopharyngeal and in Hela cells were strong, whereas ovarian cancer was moderate. The Ct value of Ascl2 was 18.81±6.10 in nasopharyngeal and 27.22±7.22 in ovarian cancer. Probably ovarian cancer showing of different profile of those gene expressions. For quantitative comparison, the level of Ascl2, miR302b, and the other genes (cMyc and KLF4) in the different tissue including Hela cells, nasopharyngeal, and ovarian cancer compared to GAPDH as a housekeeping gene. In our result, the fold change increase more than 4.61for nasopharyngeal cancer and 1.39 for ovarian cancer may provide useful information for studying of ASCL2 gene in tumorigenesis.

In Hela cells, the expression of Ascl2 was very high (ct value= 17.19±3.11), and the fold changes to GAPDH was 47.8 times, it means this gene upregulated. On the other hand, miR302b was moderate (ct=26.96) which was 0.11 times GAPDH expressions. The other gene expressions such as cMyc (ct=24.37) which was high expressions might play an important role in cell division and cell proliferation. Based on the data, we can conclude that Ascl2, miR302b, cMyc, and GAPDH highly expressed in nasopharyngeal cancer but the KLF4 were low expressions. Suggested that those four genes were active in this cancer but loss or very low activities of KLF4. In Hela cells, the expression of Ascl2 was very high whereas the expression of miR302b was moderate. In ovarian cancer, the expression of Ascl2 and cMyc were lower than those in nasopharyngeal cancer. If we compare to the normal ovary, the Ascl2 up-regulated in ovarian cancer.

CONCLUSIONS

The human achaete-scute2 (Ascl2) or Hash2 (Human achaete-scute homolog 2) gene is a member of the basic helix-loop-helix (bHLH) of the human transcription factor. Ascl2 has been reported it expressed in the human placenta and it elevated in many types of cancers. Our previous result showed that Ascl2 gene was the positive expression in normal placenta but the negative expression in complete mole, and positive in choriocarcinoma [4]. We concluded that 3'UTR might be important in regulating the expression of Ascl2 of trophoblast development, suggesting the Ascl2 has two gene regulators. However, the molecular mechanism has not clear yet. The others experiment, we used soybean extract to induced Ascl2 gene expression. Soybean is one of the exogenous factors that can affect to histologic appearance of small intestinal mucosa such as shortening of villus mice after given soybean infusion as a food supplement. Interestingly, the thick of the crypt increased suggested the Ascl2 has two different molecular targets. Although we did not examine the protein level, we speculated that Ascl2 and bcl2 play a role in villi growth and development. Probably the shortening of villi did not through inhibition of bcl2, inducing apoptosis. Surprisingly, the crypt grew even higher than the control, suggesting bcl2 remaining active in crypt cells but not in villi. This result remained us to re-evaluate the use of soybean extract as a complementary therapy for inhibiting cell growth and development.

In the current study, we analyze Ascl2 and miR302b gene expression at the mRNA level associated with cMyc and KLF4 in the pathologic tissues such as nasopharyngeal and ovarian cancers. In Indonesia, nasopharyngeal carcinoma (NPC) is frequent cancer, rating as the fourth for all malignancy and the most common malignancy in the head and neck. Our result suggested that the profile of Ascl2 and the other three genes (cMyc, KLF4, and miR302b) may indicate the molecular differences between nasopharyngeal and Hela cell. In ovarian cancer, the Ascl2 was moderate expression whereas cMyc and KLF4 were the low expressions, but miR302b was a strong expression. The implementation of gene expression in the community conducted among 258 human females showed that Ascl2 was a strong expression. However, the expression of Ascl2 was significantly higher in adult than young females ($p<0.05$). The same profile of miR302b that was also significantly higher expression in adult than young females ($p<0.05$). However, there is no correlation between ages and gene expression both Ascl2 ($R^2 = 0.166$) and miR302b ($R^2 = 0.034$).

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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