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Expression pattern of Rheb gene in Jabal Barez Red goat

AZRA NAJMI NOORI[1], MOHAMMAD REZA BAHREINI BEHZADI[2] and MOHAMMAD REZA MOHAMMADABADI[3]

Yasouj University, Yasouj 75918–74831 Iran

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ABSTRACT

According to importance of Rheb gene on growth, cell cycle and cancer, expression of this gene for the first time was studied in Jabal Barez Red goat. Rheb belongs to Ras family that encodes a carboxylterminal CAAX box indicating that the protein may undergo post-translational farnesylation. Over-expressions of Rheb simulate cell growth while knockdown of Rheb expression, inhibits protein synthesis and cell growth. In this study, expression of Rheb gene was investigated by Real-Time PCR and Pfaffl method in various tissues including brain (medulla), brain (cortex), heart, kidney (cortex), kidney (medulla), testis, lung, liver and spleen. For analyzing the data of Pfaffl method, SAS software was used. Results showed that the Rheb gene was expressed in all the tested tissues and the highest level of expression was observed in spleen and the lowest level was detected in lung. Therefore, this gene is expressed in all the tissues and physiological effects of this gene needs to be investigated in different tissues and different animals.

Key words: Expression, Jabal Barez Red goat, Rheb gene, Tissue

According to the growth of population in developing countries, using new methods to meet the needs of this huge population seems necessary. In developed countries, scientific methods of animal husbandry became alternative to traditional methods. This could cause a great change in livestock production. Jabal Barez Red goat with population of over 450,000 head breeds in Jiroft and Kahnoj area, Kerman Province, Iran. Seeing a colour range of red to brown, the dominant colour of this animal is red (Abbaszadeh Mehrabadi *et al.* 2011). This goat is important for the production of fluff, red meat and dairy products. The advantages of raising Jabal Barez Red goat include no need for major investments, low fat meat production, high ratio of twinning and proper milk production (Abbaszadeh Mehrabadi *et al.* 2011). Since 1997, the huge investment is done to expand agricultural productions by fluff trait in Europe (Hermann *et al.* 1997). Rheb gene has diverse role in different organisms. In general, this gene has function in growth and cell cycle. TSC is a genetic disease caused by mutation in Rheb gene and causes tumor. These tumors caused nervous disorders in brain (Inoki *et al.* 2003, Ma *et al.* 2008). Different research shows that Rheb gene inactivity time caused by mutations decreased cell size and Rheb gene overexpression caused increased cell size. Rheb proteins are new and unique family of Ras large family that is part of binding proteins to guanosine triphosphate. Rheb gene is originally identified as immediate early gene in 1994, encoding 184 amino acids and Rheb has an Arg at the position 12 instead of a Gly (Yamagata *et al.* 1994). The mRNA frequency of Rheb gene in mammalian cell culture is greatly controlled by growth factor (Buerger *et al.* 2006). Two polypeptide of Rheb are similar in 50% of amino acid sequences in mammals. Rheb proteins are critical in regulating growth and cell division cycle. This effect is, due to Rheb role in signaling pathway (Insulin/TOR/S6K) (Saucedo *et al.* 2003). Rheb is an upstream regulatory factor in mTOR signaling pathway and the Rheb-GTP can activate mTOR (Long *et al.* 2007). mTOR is a central protein that control cell growth and proliferation through mechanism of transcription and translation in response to amino acids and growth factors. After considering Rheb gene relative expression levels in different tissues including brain, heart, testes, liver, kidneys, spleen, lungs, and pancreas in Mongolian Cashmere goat using Semi-Quantitative RT-PCR, it was found that Rheb gene expressed in all tested tissues and the highest level of mRNA accumulation was detected in brain (Zheng *et al.* 2001). Yamagata *et al.* (1994) showed that Rheb gene in mice is expressed at high levels in brain. The experiments indicated that Rheb gene is expressed in all tissues of the adult human including heart, brain, placenta, lung, liver, heart muscle, kidney and pancreas. These results are similar to data obtained from rat and the highest level of mRNA accumulation was

Present address: [1]MSc Student (azra.najmi@gmail.com), [2]Assistant Professor (bahreini@yu.ac.ir), Department of Animal Science. [3]Professor (mmohammadabadi@yahoo.ca), Department of Animal Science, Shaid Bahonar University, Kerman, Iran.

observed in heart and skeletal muscles. Tohidinezhad *et al.* (2014) showed this gene expressed in tissues including brain, heart, lung, pancreas, spleen, kidney, liver and testis of Raini Cashmere goat. In exact study of Rheb gene expression in different tissues using Gene Tools software, highest level of expression of this gene was observed in kidney and the lowest level in pancreas and testis tissues.

So far, no report is available about expression of this gene in Jabal Barez Red goat. The aim of this research was to study expression of Rheb gene in different tissues of Jabal Barez Red goat and comparison of expression level in studied tissues.

MATERIALS AND METHODS

Tissues including brain, heart, kidney (cortex), kidney (medulla), testis, lung, liver, spleen were collected from Jabal Barez Red goat after slaughter while the goat was bred on a natural diet in Jiroft city, Iran. Tissue samples were immediately frozen in liquid nitrogen and stored at –80°C. Total RNA of studied tissues was isolated using Topaz gene kit. The quality and quantity of extracted RNA were studied using agarose gel electrophoresis and UV spectrophotometry. Extracted RNA was stored until later testing at –80°C. Fermentas kit was used for cDNA synthesis. It should be mentioned that in all process of cDNA synthesis, the entire materials were stored on ice. Primers were procured commercially from Takapouzyst Company (Table 1).

Polymerase chain reaction was performed to amplify Rheb gene and determine the temperature of binding primers to target DNA sequences. In this study, Beta-actin gene was used as a control gene. Amplification of Rheb and Beta-actin genes were conducted with initial denaturation at 94°C for 4 min, secondary denaturation at 94°C for 1 min, annealing at 57°C for 1 min, initial synthesis at 72°C for 1 min, 35 repeat cycles for steps 2–4 and ultimate synthesis at 72°C for 10 min.

To study relative Rheb gene expression, Real-Time PCR reaction by SYBR Green method was used. Real-Time PCR reaction mixture contained DEPC treated water (4.7 µl), SayberPermixTaq II (7.5 µl), ROX (0.3 µl), forward primer (0.5 µl), reverse primer (0.5 µl) and template cDNA (1.5 µl). Real-Time PCR reaction of Rheb and Beta-actin genes were conducted with initial denaturation at 95°C for 45 sec, secondary denaturation at 95°C for 30 sec, annealing at 57°C for 60 sec, 45 repeat cycles for steps 2–4, extension at 72°C for 40 sec and melting step 72–95°C. To analyze the data from the Real-Time PCR, Pfaffl method was used (Mahdian *et al.* 2014). In this method, after standard curve and obtaining Rheb and Beta-actin gene efficiencies, following formula was used to calculate amount of Rheb gene expression in each sample. E_{target} and E_{ref} are PCR efficiency of studied and housekeeping genes, respectively.

$$\text{Ratio} = \frac{(E_{target})\Delta Ct_{target\ (control\text{-}sample)}}{(E_{ref})\Delta Ct_{ref\ (control\text{-}sample)}}$$

ΔCt is the result of subtracting Beta-actin gene Ct and Rheb gene Ct. The data which was obtained from pfaffl method representing the relative gene expression in various tissues of Jabal Barez Red goat were analyzed using SAS software. For determining significant differences between groups Duncan's test was utilized (Saucedo *et al.* 2003).

RESULTS AND DISCUSSION

Absorption numbers of extracted samples were between 1.77–1.9 at a wavelength of A260/A280, which indicates the suitable quality of the extracted RNA. Two bands 18S and 28S in rRNA indicate the integrity of RNA and no additional band indicate its purity (Fig. 1). To find the proper fusing temperature of target gene (Rheb) and control (Beta-Actin) primers, a temperature gradient PCR reaction was done and the most suitable temperature was chosen for the connection-specific primers (temperature 57°C). After reaction, the PCR products were investigated using agarose gel electrophoresis (2%). Observation of single band in the range of 555 bp for Rheb primer and in the range of 229 bp for Beta-actin in all samples confirmed the accurate amplification of target fragment (Figs 2, 3).

During the reaction, Real Time PCR device showed fluorescent lighting changes at each cycle as proliferation curve (Fig. 4). Therefore whatever increases the product produced, the colour connected between two DNA strands is more, so the amount of fluorescent lighting emitted would

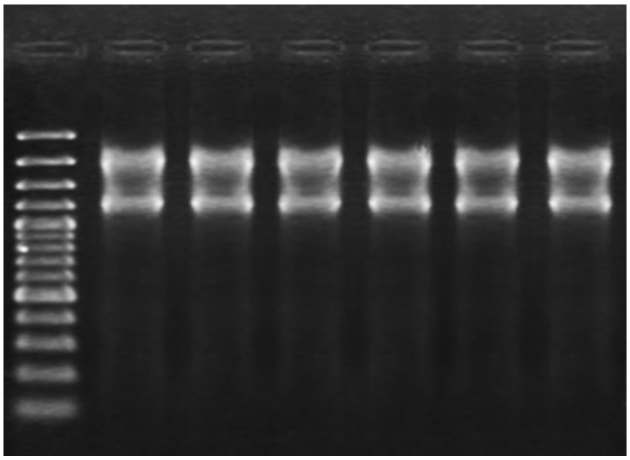

Fig. 1. Samples of the extracted RNA.

Table 1. Sequence (5'–3') of primers used in semi-quantitative RT-PCR for Rheb and beta-actin genes

	Rheb gene	Beta-Actin gene
Forward primer	ATGCCGCAGTCCAAGTCC	TGGCACCACACCTTCTACAACGAGC
Reverse primer	TCACATCACCGAGCAG	CGTCCCCAGAGTCCATGACAATG

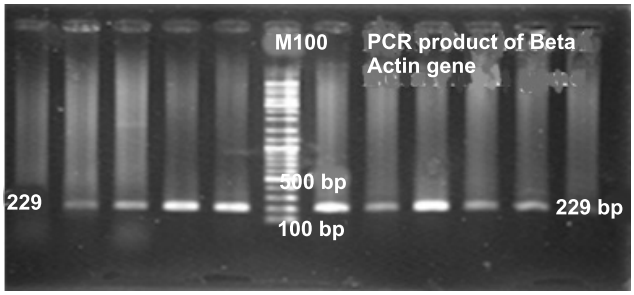

Fig. 2. Electrophoresis of samples using Beta-Actin primer. M100; size marker.

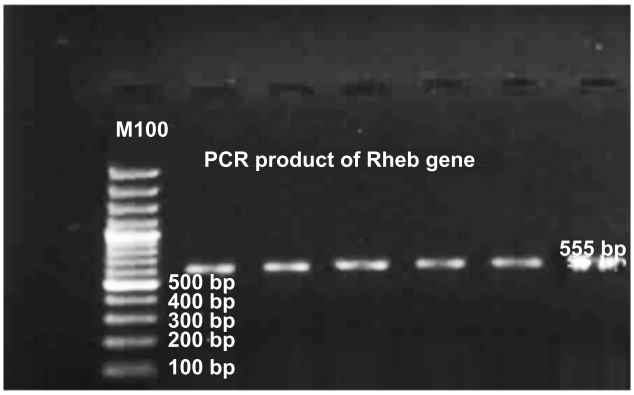

Fig. 3. Electrophoresis of samples using Rheb primer. M100; size marker.

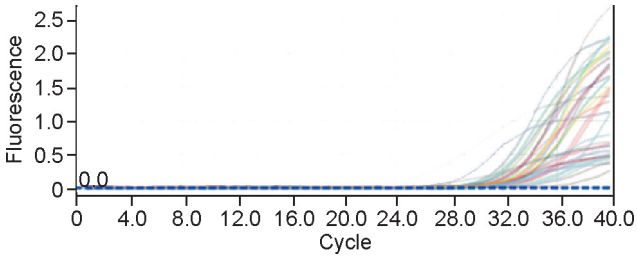

Fig. 4. Logarithmic curve of Rheb gene amplification using Real Time PCR device.

be more. For proliferation curve of all samples, a threshold in the exponential phase was defined. A Ct was obtained which indicated in which cycle intensity of the fluorescent lighting emitted of proliferation reaction reached the threshold.

In order to obtain the efficiency of PCR for Rheb and control (Beta-Actin) genes, 3 dilutions of 1, 10, and 100 of reference cDNA was prepared and after the reaction, the efficiency of Rheb and control genes was 100 and 99%, respectively (Figs. 5 and 6).

The results of Real Time PCR showed that this gene is expressed in all the tested tissues. Different levels of Rheb gene expression in different tissues of Jabal Barez Red goat is shown in Fig. 7. Using SAS software for investigation of Rheb gene expression in different tissues of Jabal Barez Red goat, it was observed that this gene was expressed at highest level in spleen and at lowest level in lung tissue. In a similar study by Zheng *et al.* (2011) done on the Mongolian Cashmere goat, the highest level of expression

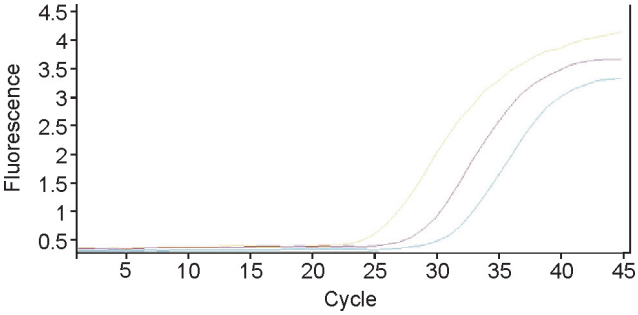

Fig. 5. Standard curve of Beta-Actin gene amplification in three dilutions.

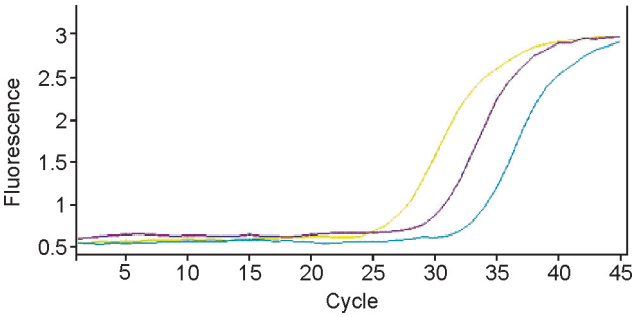

Fig. 6. Standard curve of Rheb gene amplification in three dilutions.

was observed in brain and its expression was restricted in heart and spleen. These results differed from the results of our research, probably due to differences in the genotype and environmental effects on the genotype. Yamagata *et al.* (1994) showed that this gene in mice is expressed at high levels in hippocampus, brain (cortex), lung, thymus, kidney and intestine. Tohidinezhad *et al.* (2014) showed this gene in Raeini goat is expressed in brain, heart, lung, pancreas, spleen, kidney, liver and testis. More investigation of Rheb gene expression in different tissues using Gene Tool software showed that this gene was expressed at the highest level in kidney and the lowest level in pancreas and testis (Tohidinezhad *et al.* 2014). The results of statistical

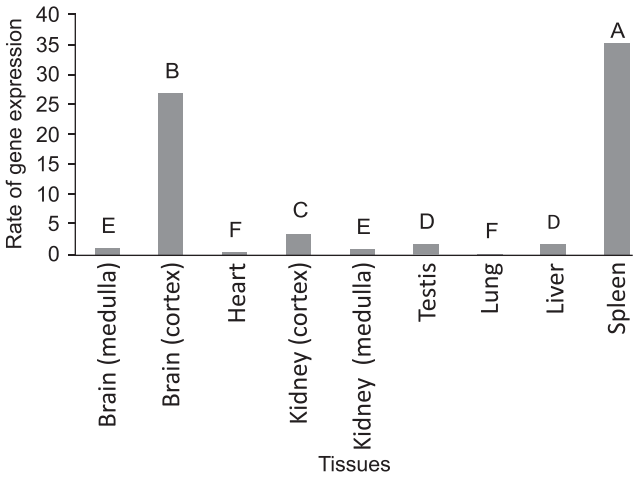

Fig. 7. Different levels of Rheb gene expression in different tissues of Jabal Barez Red goat.

analysis using SAS software showed that expression of Rheb gene in Jabal Barez Red goat was significantly ($P \leq 0.01$) different in various tissues. Furthermore, Tohidinezhad *et al*. (2014) presented significantly ($P \leq 0.01$) different expression of Rheb gene in various tissues in Raini Cashmere goat.

In general, the results of this study indicated that the Rheb gene was expressed in all the tested tissues and the highest level of expression was observed in spleen and the lowest level was detected in lung. The difference between the expression levels of RHEB gene in liver and testis and between tissues of the kidney (medulla) with brain (medulla) was not significant, but there was a significant difference between other tissues ($P \leq 0.01$). Therefore, we can say that Rheb gene plays an important role in goat cells and physiological effects of this gene in different tissues and different animals needs to be studied.

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