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Differential expression patterns of myogenic regulatory factors in the postnatal *longissimus dorsi* muscle of Jeju Native Pig and Berkshire breeds along with their co-expression with Pax7

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ABSTRACT

Background: Myogenic regulatory factors (MRFs) such as *MyoD*, *Myf6* and *Myf5* play a vital role in the growth and development of muscles. Jeju Native Pig (JNP) is the top ranker in Korea amongst the indigenous livestock reared for meat purpose. Few studies covering transcript abundance of the MRFs and related to their co-expression with *Pax7* in JNP have been conducted. Despite having better quality pork, JNP does not have a comparative growth rate with respect to western breeds. Therefore, the present study was designed with the objective to study the relative transcript levels of MRFs in the postnatal myogenesis of *longissimus dorsi* muscles in JNP and Berkshire breeds.

Results: Relative transcript levels were analyzed by qRT-PCR and blot expression analysis through Western blotting. Immunocytochemistry was performed to analyze their expressions at cellular levels. ToppCluster aided in the analysis of gene ontology of biological processes. The quantitative transcript levels of *MyoD* and *Pax7* were significantly ($P < 0.05$) higher in Berkshire than in JNP. Myotube formation was observed under the co-expression of *MyoD* and *Pax7*. ToppCluster helped in the understanding of the linking of biological processes of the MRFs with the different signaling pathways. *MyBPH* had significantly ($P < 0.05$) high transcript levels during the chosen age groups in JNP than Berkshire.

Conclusions: The current study can be helpful in understanding the genetic basis for myogenesis in postnatal stage. Moreover, it can act as stepping stone for the identification of marker genes related to body growth and meat quality in JNP.

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1. Introduction

Skeletal muscles comprise 20–50% of the total body mass in pigs and are one of the main tissues responsible for pork production in pigs [1]. An impacted research during previous years helped in understanding the development, physiology and metabolism of porcine muscle tissue [2].

Jeju Island (Republic of South Korea) has very typical weather conditions and because of such weather conditions, Jeju Island has very unique native flora and fauna. Meat from Jeju Native Pig (JNP) is tenderer, juicier and has higher marbling score than western breeds [3,4]. These qualities of JNP meat bring it on the preference list of consumers especially in China, Japan and Korea. Pork from JNP is regarded as of high quality and is the costliest pork in Korea [5]. Apart from being resistant to diseases, major snags faced by JNP are lesser rate of growth, lower feed efficiency and lesser prolificacy rate [6].

The JNP has been widely hybridized with Berkshire breed during 1930s–1940s. Moreover, Berkshire breed is expected to inter-

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vene in paternal linages of JNP and is preferred for meat production [7,8]. Due to better meat quality, pork from the crossbreds of JNP and Berkshire occupies a unique share in meat industry of tropical Asian countries. Therefore, these two breeds are frequently chosen for the breed improvement programs with an aim to improve the meat quality and quantity [9].

Muscularity and growth rate are among the prime economic traits for meat animals. Clear lines of evidence have established that size of muscle fiber; number of fibers; fiber area and density of fibers are in close vicinity with the traits related to meat quality [10]. Prospec for the growth is interlinked with the number of the secondary myotubes of muscles. Further, it has been observed that the postnatal growth and regeneration of skeletal muscles are linked with the existence and activities of the satellite cells [11]. The satellite cells are reported to surround myotubes, which have significant role in hyperplastic and hypertrophic growth of skeletal muscles [12].

Muscle regulatory factors (MRFs), are member of basic helix-loop- helix (bHLH) family. It has been reported that, myogenesis and transcription of the genes specific for muscles is controlled by bHLH transcription factors [13]. Myogenic determination factor (*MyoD*), myogenic factor-6 (*Myf6*) and myogenic factor-5 (*Myf5*) are the versatile members of MRFs. *MyoD* is involved in the differentiation of the skeletal muscles. *MyoD* and paired box transcription factor-7 (*Pax7*) are reported to co-express in the activated satellite cells [14]. *Pax7* is reported to induce self-regeneration of satellite cells and can be one of the candidate genes to affect the spirited stages of post-natal muscle growth in the pig [15].

Myf6 is known for coding for the bHLH transcription factors and significantly affect the differentiation of muscle fibers [16]. *Myf6* has the tendency to express more in the adult skeletal muscle tissue of lean breeds [17,18]. Moreover, inactive Myf6 protein has been reported to be associated with the retarded muscle growth [19]. Therefore, *Myf6* is considered as one of the promising genes affecting skeletal growth and meat quality– related traits in adult pigs [20,21].

Earlier studies have suggested that anomaly in the cleavage of MRFs can have deep effects on the growth of skeletal muscles [22]. Similarly, the myosin filaments of striated muscles consist of a family of enigmatic myosin-binding protein (MyBP) with its two isoforms, myosin-binding protein-C (MyBPC) and myosin-binding protein-H (MyBPH) [23]. Although the relationship between the weight of carcass, characteristics of muscle fiber and traits related with pork quality is not fully understood, still the muscle mass and the weight of the pigs are related to fiber composition [10].

During the past decade to improve quantity and quality of pork, JNP has been crossbred with Berkshire. Few reports are on record related to the characteristics of muscle fibers of JNP. Despite better tenderness, juiciness and higher marbling score in the pork, JNP has a lesser rate of growth. Therefore, it has been hypothesized that there may be some difference at the transcriptomic levels of MRFs in JNP and western breeds.

Therefore, the relative analysis of transcript levels of MRFs in the postnatal myogenesis of *longissimus dorsi* muscle in JNP and Berkshire breeds has been targeted in the current study. With this aim the deliberation in the present study has been done for MRFs as candidate genes for muscle growth during the breed improvement programs. Further, the biological functions and pathways involving MRFs have been targeted in the current study to understand the molecular mechanism involved in the regulation of the expression of the genes required for muscle growth.

2. Materials and methods

2.1. Collection of tissue samples and statement of ethics

In the current study, pure bred animals from JNP and Berkshire breeds were chosen. Five one day old and adult females each from JNP and Berkshire breeds were taken for the present study. Adult females were chosen due to their significant importance in the breeding programs. Animals used for the current study were reared by birth under uniform climatic and nutritional conditions. Pens for the animals were equipped with the drinking bowl having a nipple and feeder. Animal pens were of concrete flooring. Pigs were housed at the ambient temperature of 25 ± 1°C.

The animal bio-ethics committee of the Department of the Animal Biotechnology, Jeju National University, Jeju approved the experiment and the animals were slaughtered following the approved protocols of Jeju National University. The *longissimus dorsi* muscle tissue samples between 12th and 13th rib spaces were harvested instantly following the slaughter. Tissue samples were rapidly stored in dry ice and were later kept at −80°C till their further use for RNA extraction.

2.2. Isolation followed by quality analysis of RNA and protein

The RNA was extracted from the fragmented frozen *longissimus dorsi* muscle (120 mg) of one day old piglets and adult animals of both the breeds. TRIzol™ (Invitrogen, USA) reagent was used to isolate RNA from *longissimus dorsi* muscle. The tissue was homogenized by mixing with 2.0 ml of TRIzol and 400 μl of chloroform. The isopropanol (Junsei Chemical Co. Ltd., Japan) was subsequently used to precipitate the homogenized tissues. 1 ml of 75% ethanol was used to wash the precipitated pellet.

Immediately, after extraction, isolated samples of RNA were stored at −80°C. To eliminate the genomic DNA impurities, 25 μg of RNA samples were treated with the RNase-free DNase set (QIAGEN, Hilden, Germany) and RNA was purified with the RNeasy mini kit according to the manufacturer's guidelines (QIAGEN, Hilden, Germany).

The quantity of RNA was assessed by the Bioanalyzer 2100 with RNA 6000 Nano Labchips and quality was judged by automated capillary gel electrophoresis according to the manufacturer's guidelines (Agilent Technologies Ireland, Dublin, Ireland). The ratios of 28S/18S for the RNA samples were between 1.8 and 2.0 and the values for the integrity of RNA ranged from 8.0 to 10.0.

Protein from the homogenized *longissimus dorsi* muscle was extracted by "Radio Immuno Precipitation Assay (RIPA) buffer" and concentration of protein was quantified using "Pierce™ BCA" protein assay kit (Thermo Scientific, USA) in Bio-Rad Micro-plate Reader (Model-680) following the manufacturer's guide lines. RNA and protein samples were stored at −80 °C till their further use.

2.3. Quantitative real time polymerase chain reaction CR (qRT-PCR)

Primers for the qRT-PCR were constructed by the online Primer-3 software [24] and the information of the primers has been enlisted in Table 1. The quantitative evaluation of mRNA transcript levels of *MyoD*, *Myf6, Myf5, Pax7* and *MyBPH* genes in JNP and Berkshire was performed through real-time qRT-PCR. qRT-PCR was performed using an Applied Biosystems, Step-One Real Time PCR system. To determine the quantity of transcripts of target genes EvaGreen (Biotium, USA) dye was used.

Triplicate samples were used for the quantification by setting the following amplification conditions i.e. 95°C for 10 min initially (activation), and then 40 cycles of 95°C for 15 s (denaturation) and

Table 1
List of primers used for quantitative real time PCR to analyze the relative quantity of transcripts in the one day old piglets and adult animals of Berkshire and JNP.

Gene name	Primer sequences	T_m	Product size	Gene bank ID
MyoD	F TGCAAACGCAAGACCACTAA R GCTGATTCGGGTTGCTAGAC	55°C	127	NM_001002824.1
Pax7	F GGCAGAGGATCTTGGAGACA R TGGGTGGGGTTTTCATCAAT	55°C	144	AY653213.1
Myf6	F ATCTTGAGGGTGCGGATTTC R CAATGTTTGTCCCTCCTTCCT	62°C	108	XM_003481764
Myf5	F CCGACACAGCTTGTGGAATA R GCCAATCAACTGATGGCTTT	55°C	128	XM_001924362.2
MyBPH	F AGTGCAGAAGGCAGACAAA R AAGACCCGGAAGGAGTAAGA	62°C	117	NC_010451
β- actin	F GACATCCGCAAGGACCTCTA R ACACGGAGTACTTGCGCTCT	60°C	157	XM_003124280

60°C for 1 min (annealing/extending). The efficiency of real-time PCR was defined by the standard curve method. The amplified transcript levels of the target genes were compared with that of the *β-actin* as an endogenous control [25]. mRNA transcript levels were quantified by the comparative C_T method. The results in terms of relative expressions have been expressed after normalizing with the transcript levels of the endogenous reference [26,27].

2.4. Western blot analysis

The protein extract (60 μg) and 2× loading buffer (4% sodium dodecyl sulfate (SDS), 20% glycerol, 0.004% bromophenol blue, 25% 0.5 M Tris, and 5% β-mercaptoethanol) were mixed in the proportion of 1:1. The denaturation of the protein extracts was performed by boiling the extracts for 10 min prior to loading on a 12% SDS-PAGE gel. After completion of electrophoresis, proteins were transferred to 0.45 μm nitrocellulose membrane (Invitrogen, USA) and the blocking of membrane was performed at room temperature for two hours using 5% milk in 1X PBS containing 0.05% Tween-20 (TBS-T). Later, the primary and secondary antibodies specific for MyoD, Myf6, Myf5, Pax7, MyBPH and β-actin proteins were used to block the membranes (Table 2).

The "Luminescent Image Analyser (LAS-4000 mini)" was used to analyze the membranes for specific chemiluminescent signals. The blot expressions have been represented in terms of relative intensities of bands. The relative band intensities of the targeted proteins were normalized with the area densities of endogenous β-actin bands through image-J software (National Institute of Health, Bethesda, Maryland, USA).

Table 2
List of primary and secondary antibodies used in the expression analysis of proteins, isolated from the *longissimus dorsi* muscles of one day old piglets and adult animals of Berkshire and JNP through Western blotting.

Protein	Molecular weight	Primary antibody*	Secondary antibody*
MyoD	45 kDa	Rabbit polyclonal 1:50	Goat anti-rabbit, 1:1000
Pax7	56 kDa	Goat polyclonal 1:50	Donkey anti-goat, 1:1000
Myf6	30 kDa	Rabbit polyclonal 1:50	Goat anti-rabbit, 1:1000
Myf5	32 kDa	Rabbit polyclonal 1:50	Goat anti-rabbit, 1:1000
MyBPH	52 kDa	Goat polyclonal 1:50	Donkey anti-goat, 1:1000
β-Actin	43 kDa	Mouse monoclonal 1:1000	Goat anti-mouse, 1:1000

* All antibodies were from Santa Cruz.

2.5. Cell culture of stable C2C12 cell line

The C2C12, mouse myoblast cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM). 20% fetal bovine serum (FBS), 100 unit/ml penicillin, 100 μg/ml streptomycin and 100 μg/ml gentamycin were added in the DMEM. The cells were seeded at the rate of 1×10^5 cells/25 cm^2 (i.e. 4000 cells/cm^2) in the tissue culture flask. The cells were sub-cultured, once they attained 70% confluence in the flask.

2.6. Immuno-cytochemistry

At 70% confluency, the C2C12 cells were seeded in 24 well plate for immunostaining. The fixation of the cells with 4% paraformaldehyde at 4°C was performed after 24 h of their seeding. Later the fixed cells were treated with 0.1% Triton X-100 (pH 7) at room temperature. The cells were washed twice with PBS and non-specific binding was prevented with the use of washing buffer (5% v/v FCS in PBS with 0.05% v/v Triton X-100) for 30 min. The fixed cells were incubated at 4°C overnight in the dark with primary antibodies (Table 2), which were unique for the respective proteins. The next day after washing twice with PBS, cells were incubated with the secondary antibodies specific for the respective proteins (Table 2). Later, the cells were counterstained with 4,6-diamindino-2-phenylindole (DAPI, @0.3 μg/ml) (Sigma, USA) for 5 min for the visualization of nuclei of cells. Subsequent image analysis of the labeled cells was done using Olympus, IX 70 fluorescent microscope.

2.7. Gene ontology (GO) analysis

To analyze the functional study of genes of interest, GO analysis has emerged as a candidate approach of interest. In the present study, GO analysis has been performed to analyze the biological processes linked with the functioning of the *MyoD, Myf6, Myf5, Pax7* and *MyBPH* genes in *Sus scrofa*. The shared and specific features of the genes associated with the biological processes have been visualized in the current study. A cutoff *P*-value of 0.05 was chosen in the ToppCluster and no false discovery correction procedure was practiced.

2.8. Statistical analysis of the differential expression patterns of the MRFs

The gene expression data were analyzed for fold change in JNP with respect to Berkshire using $2-\Delta\Delta CT$ method [28]. The statistical analysis of differential expression patterns of MRFs was done by ANOVA. The values have been expressed as mean ± SEM. $P < 0.05$ was set as statistical level of significance and subsequently Tukey's b-test was applied for analyzing the level of significance.

3. Results

3.1. MRFs express differentially in one day old and adult animals of JNP and Berkshire

Relative quantitative mRNA transcript levels of *MyoD*, *Myf6*, *Myf5*, *Pax7* and *MyBPH* normalized with the transcript levels of the endogenous reference *β-actin*, were investigated through Real Time qRT-PCR. In the current study, *MyoD* exhibited significantly ($P < 0.05$) higher quantitative transcript level in Berkshire during both the stages than in JNP (Fig. 1a). Similarly, significantly ($P < 0.05$) higher transcript levels of *Pax7* have been recorded in the adult and one day old piglets of both breeds (Fig. 1b).

The blot expressions of proteins also complimented the transcript levels of the genes under study. Blot expressions of MyoD and Pax7 proteins are significantly ($P < 0.05$) higher in Berkshire as compared to JNP during the post natal development (Fig. 2).

Major protein in the thick filaments which influences the type of muscle fiber is myosin. Significantly higher transcript levels of *MyBPH* have been observed during both age groups in JNP than Berkshire (Fig. 1c, Fig. 2). The type of the muscle fibers is reported to be responsible for the color, stability and tenderness of meat [29]. The higher transcript levels of *MyBPH* in JNP during the current study indicated that it may contribute to the meat quality of JNP.

3.2. Clustering of MyoD, Pax7, MyBPH, Myf6 and Myf5 genes and annotation of the pathways

The result in the form of graphical network was obtained from the ToppCluster. In the current study, the biological processes related to the skeletal muscle cell differentiation, skeletal muscle tissue development, skeletal muscle organ regeneration and skeletal muscle satellite cell commitment were selected. The gene ontology of the biological process common between the genes

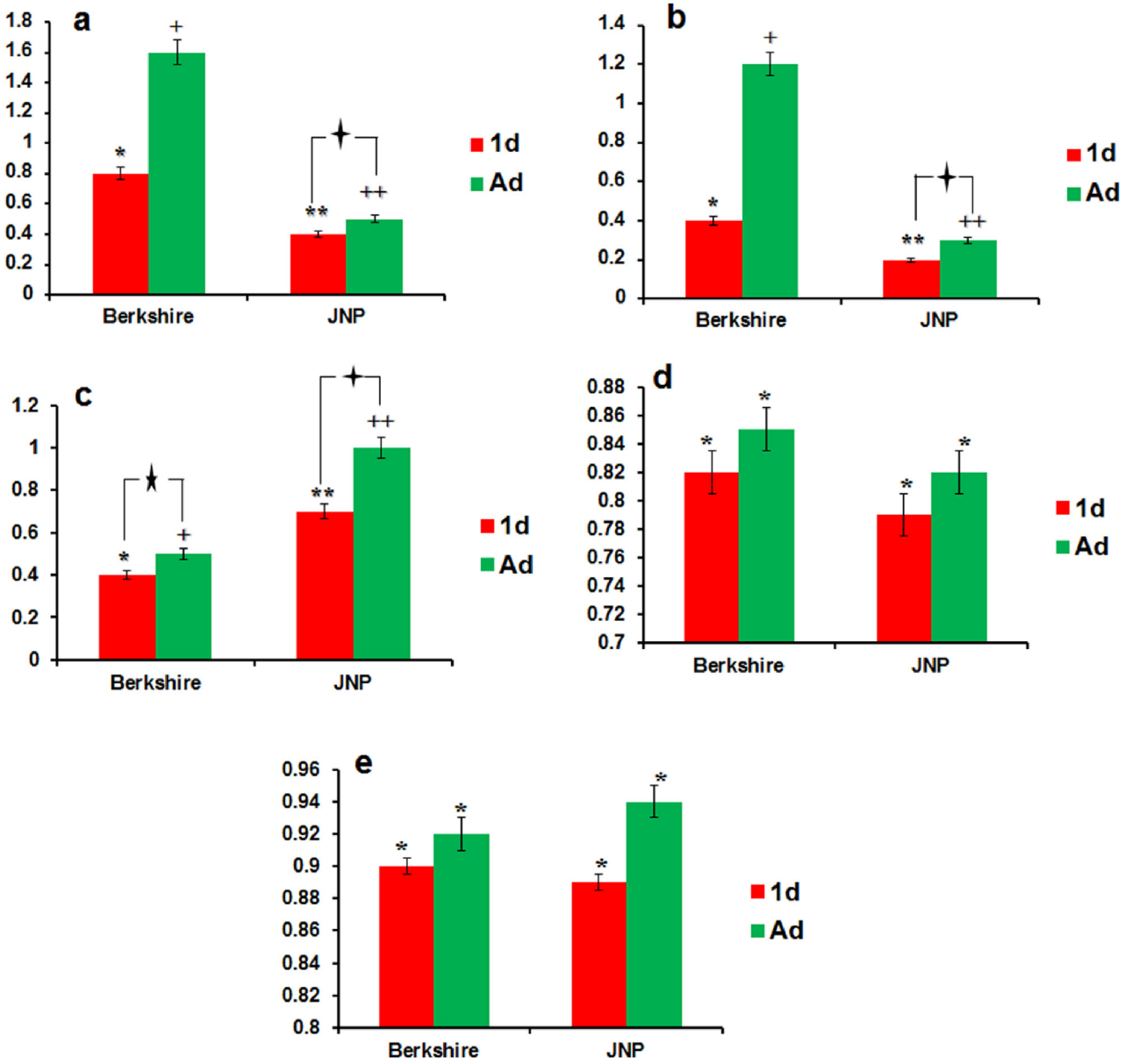

Fig. 1. Relative Quantity (RQ) of transcripts in Berkshire and Jeju Native Pig (JNP). RQ of (a) *MyoD*, (b) *Pax7*, (c) *MyBPH*, (d) *Myf6* and (e) *Myf5* has been presented in the one day old piglets and adult animals from both the breeds. Bars with different superscripts are significantly different ($P < 0.05$).

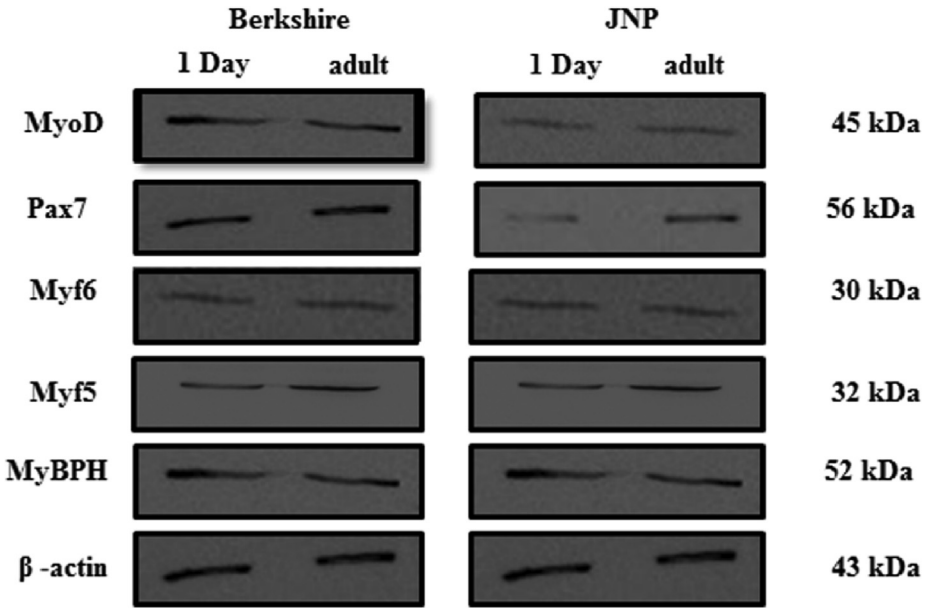

Fig. 2. Relative expression levels of MyoD, Pax7, Myf6, Myf5, MyBPH and β- actin proteins isolated from the *longissimus dorsi* muscles of one day old piglets and adult animals of Berkshire and Jeju Native Pig (JNP). Protein levels were normalized with endogenous control β- actin.

expressing in the *longissimus dorsi* was chosen to show the dissected gene level view (Fig. 3). The distinct functional segregation has been viewed in the abstracted network analysis. Higher significance for the group of genes bestowing the phenotypes is noticeable by the cluster analysis. The MRF gene family consists of *MyoD, Myf6* and *Myf5*. MRFs play a significant role in the maturation of

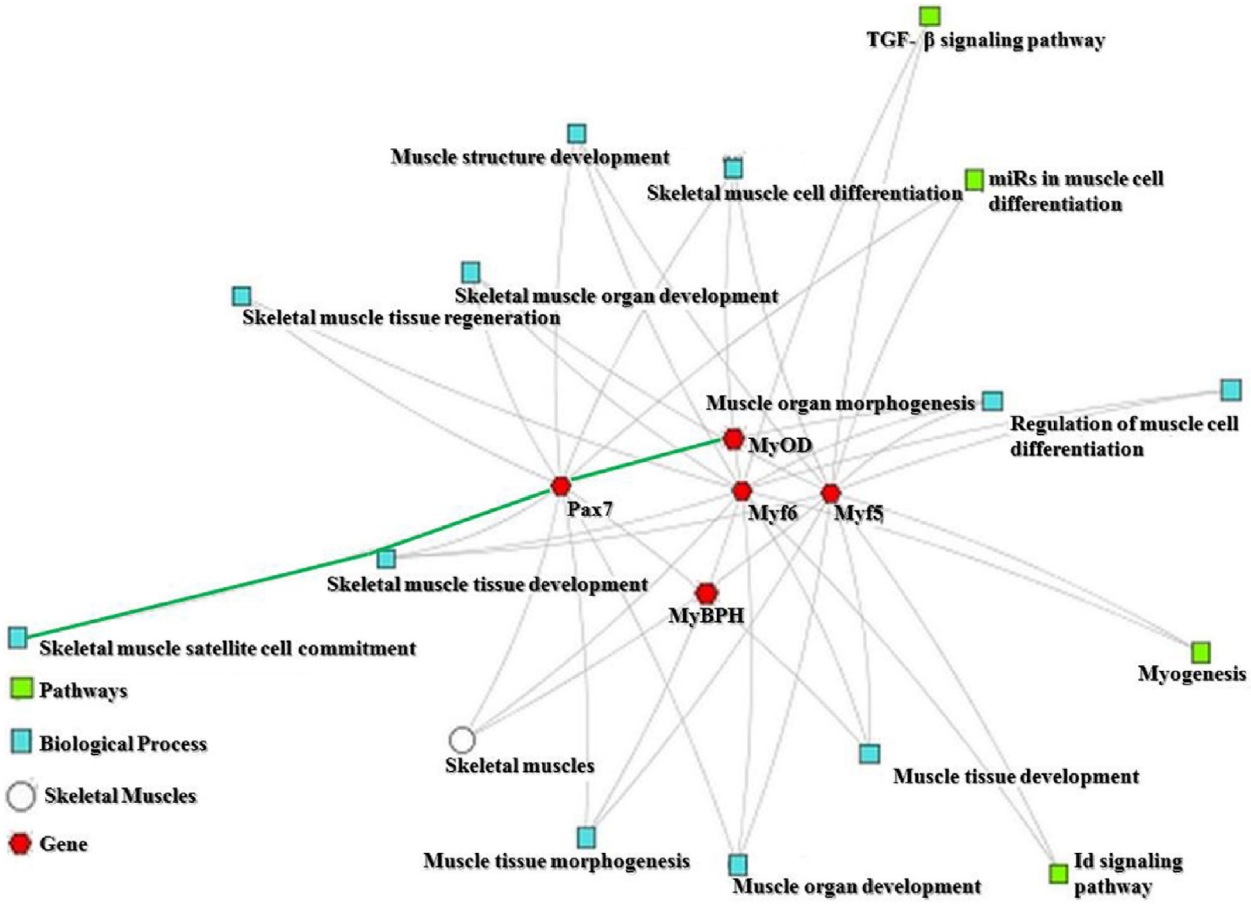

Fig. 3. Dissected gene level view sharing GO of biological process shared by *MyBPH* and MRFs in *longissimus dorsi* muscle. An abstracted network is showing biological pathways enriched with the cluster of genes specific for skeletal muscle.

skeletal muscle fibers and postnatal growth hypertrophy [30]. The *MyoD* and *Pax7* genes showed extensive relationship enrichments in the differentiation of skeletal muscle cells, skeletal muscle satellite cell commitment, development of skeletal muscle organs and fundamental role during myoblast proliferation. Similarly, *MyBPH, Myf6* and *Myf5* genes have extensive relationship enrichments in muscle tissue morphogenesis, muscle organ development and muscle tissue development (Fig. 3).

3.3. Over and co-expression of MyoD and Pax7 in the activated cells cause myogenesis by significant formation of myotubes

MyoD and *Pax7,* co-express in the activated satellite cells [14]. *Pax7* is reported to induce self-regeneration of satellite cells and can be one of the candidate genes to affect the spirited stages of post-natal muscle growth in the pig [15]. Within breed analysis has shown that *MyoD* and *Pax7* have significantly higher transcript levels in adult animals than the one day old piglets of Berkshire whereas no significant difference was observed in JNP (Fig. 1a, Fig. 1b). A significant co-expression of *MyoD* and *Pax7* was also observed under the fluorescence in the C2C12 mouse myoblast cells by immunocytochemistry (Fig. 4).

The *Pax7* is reported to play a double role in the regulation of muscle formation by propelling commitment to the myogenic program. Such mechanism would allow muscle progenitor development and/or maintains discreet differentiation (Fig. 5). At the same time, it has been reported that knockout mice with *Pax7* and *Myf5* have shown total loss of trunk muscles and expression of *MyoD*. This finding suggests that expression of *MyoD* depends either on *Pax7* or *Myf5*. Therefore, lesser expression of *MyoD* and *Pax7* genes in JNP once again indicates the probable reason for its retarded growth performance.

4. Discussion

Being an economical source of animal proteins, the demand of pig meat is increasing day by day. In the tropical Asian and Southern European continents, pigs also play a fundamental role in the social and economic status of meat industry [6]. Muscle growth rate is the most important economic trait for meat production. Genes for skeletal muscle development are potentially functional candidates in livestock production and meat quality [31]. Therefore research on the relationships between the characteristics of skeletal muscle and meat quality is important to improve our understanding of the molecular basis of the phenotypic expressions in skeletal muscle [32].

Recent studies in neonatal and adult skeletal muscles of pig have revealed the developmental patterns of gene expression including gene associated with the myogenesis [33]. Our extended work also focused on the co-expression profiles of the other MRFs, paired box transcription factors and bone morphogenetic proteins with *MyBPH* gene in both the breeds.

Both *Myf6* and *Myf5* did not indicate any statistically significant difference between the breeds during postnatal development (Fig. 1d, Fig. 1e, Fig. 2). The results through knockout mouse with *MyoD* and *Myf6* or *Myf5* in an earlier study also supported the concept [34]. It is a well-documented fact that postnatal muscle growth in pigs is a collective result of association of satellite stem cells and hypertrophic mechanisms [35]. In single, double and triple mutant mice an up-stream activity of *Myf6* with *MyoD* gene has been reported [11]. *Myf6* is the plentifully expressed myogenic factor in the postnatal muscles and it quantitatively dominates over the transcripts of *MyoD* family [36]. A raised *Myf6* mRNA and protein levels are related with the bigger myofiber size [37] and mean fiber area [38]. *Myf6* has dominant expression levels in postnatal mature fibers [20,39,40]. Significantly similar transcript levels of *Myf6* in both the breeds may help in explaining the presence of fast growing fiber types in Berkshire and better meat quality related properties of JNP. Polymorphism in the promoter region and exon 1 of *Myf6* is reported to significantly relate with the weight of *longissimus dorsi* muscle and daily weight gain [17]. Significantly similar transcript levels of *Myf5* and *Myf6* genes in both the breeds indicate towards their close vicinity on SSC5.

Relative transcript levels and blot expressions of *MyBPH* have shown different results to relative expression analysis of *MyoD* and *Pax7* in JNP. *MYBPH* is a vital segment of myosin protein present in the skeletal muscles and is found at the cross bridges of C zones of striated muscle sarcomere. Myofibrils encompass actin and myosin as the main proteins in thin and thick filaments [41].

Higher muscularity in Pietrain pigs is suggestive of higher expression of *MyoD* and *Pax7* [11]. Such findings are parallel to our results, showing that Berkshire showed higher transcript levels of *MyoD* and *Pax7* than JNP which may support higher skeletal muscle regeneration potential of Berkshire pigs. That is why these

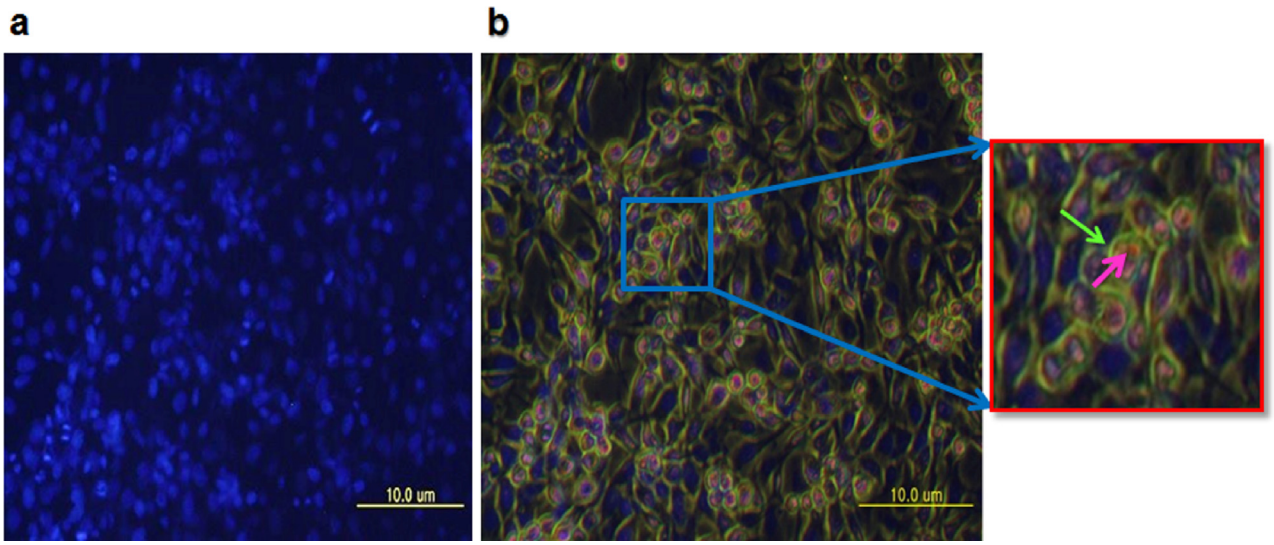

Fig. 4. C2C12 mouse myoblast cells showing the co-expression of MyoD and Pax7. The cells have been immuno-stained with primary antibodies of these respective proteins with DAPI nuclear staining (a) and merged with co-expression of MyoD (pink in nucleus) and Pax-7 (green in membrane) (b).

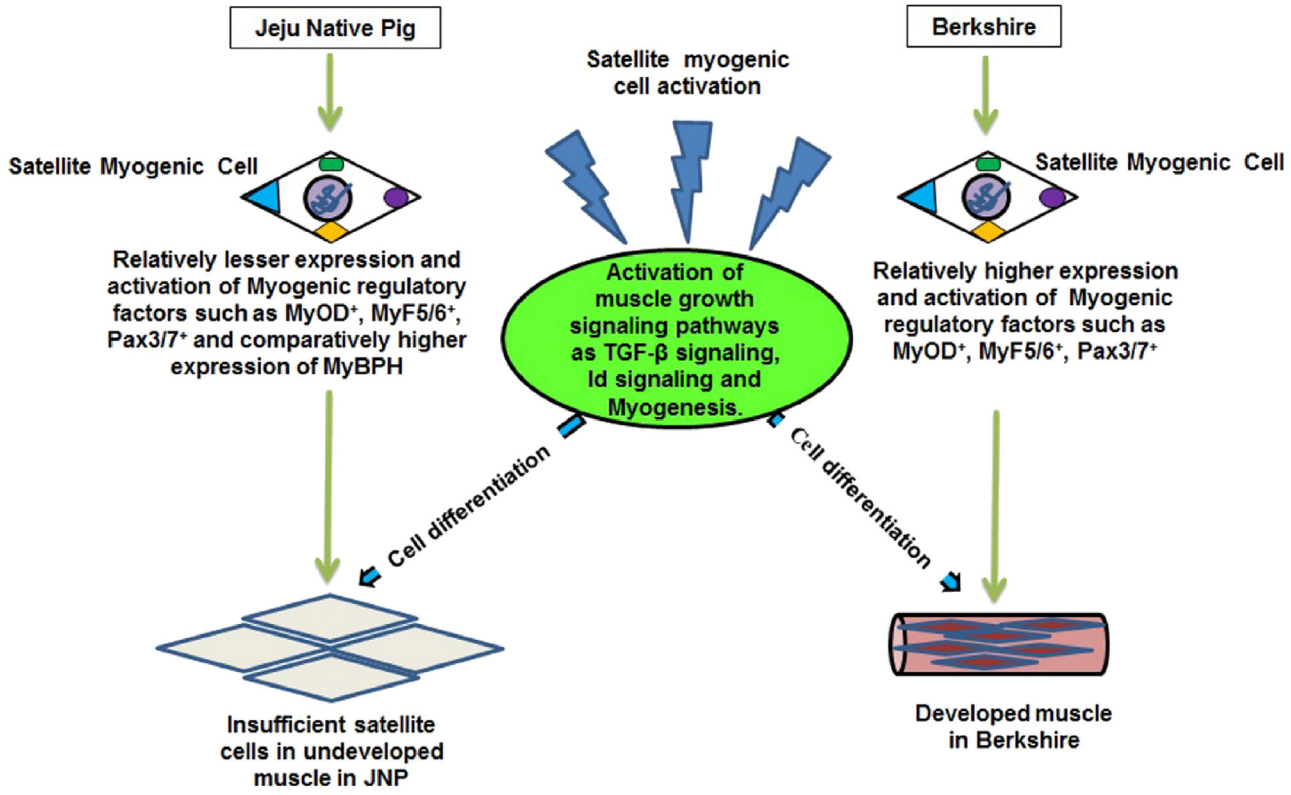

Fig. 5. A bridging avenue for the understanding of the pattern of myogenesis in JNP and Berkshire.

genes are targeted as candidate genes for meat production in pigs. Moreover, study shows that expression of *Myf5* gene is overridden by *MyoD* that can be the cause for the reduction of deleterious effects due to mutations in any of these genes [42]. While constructing the network through ToppCluster, we choose the terms of our interest to be incorporated in the network. It may be very beneficial for the researchers working in this field who are further interested in the exploration of some specific enriched terms in the results [43]. The output from ToppCluster showed that, the MRFs were primarily involved in the TGF- β signaling pathway, myogenesis, miRs in muscle cell differentiation and Id signaling pathways. These are also reported to be related with meat quality and body growth in JNP [44,45,46].

Earlier studies have enlightened that the satellite cells have a significantly important role in the hypertrophy of muscles. Satellite cells are one of the oldest adult stem cells. These play a vital role in the regeneration of muscles and their growth [47]. Moreover, in a study on knockout mice with *Pax7* gene, reported that knockout mice have lesser number of satellite cells and are poor in the regeneration of muscles [48]. In pig, it was observed that *Pax7* has a pivotal role in the physiology of satellite cells and is one of the candidate genes affecting the aggressive stages of early postnatal growth [15]. It has been reported that *Pax7* co-expresses with *MyoD* in both inactive and activated satellite cells. After proliferation, the activated satellite cells differentiate into myotubes (Fig. 6) [15]. Further, it is also observed that without *MyoD* gene transcripts activated cells return to their inactive state [12].

Various studies indicate that regulation of *MyoD* activity by *Pax7* represents a nodal point for the regulation of myogenic progression [49]. This finding suggests that lesser expression of *Pax7* in JNP may be one of the reasons for lesser growth and regeneration of muscles. Further, it has been reported that there is a significant increase in $Pax7^{+}/MyoD^{+}$ cells after one month of birth in porcine skeletal muscles [15]. Therefore, retarded growth performance in JNP targets the lesser quantity of RNA transcripts of *Pax7* and *MyoD* in adult animals. Such findings support our hypothesis for the current study.

5. Conclusions

Improvements in body growth rate and meat quality are among the top priorities of breeding plans. JNP which have good meat quality, high tenderness and juiciness but lags in body growth rate as compared to western breeds [3,4]. Continuous fall in the quality and carcass traits have directed the breeders to improve such parameters. Berkshire is a fast growing breed with muscular body and high muscle fiber content in the carcass with respect to JNP. Transcript levels of the genes and proteins under study have shown a significant ($P < 0.05$) increase with age in both the breeds.

In summary, our study on the relative quantitative transcript levels of MRFs in Berkshire and JNP presented them as candidate genes which are related with body growth and quality of meat. Till now very few studies covering transcript abundance of the MRFs in JNP have been conducted. Our experimental study is one of the pioneer expression studies in JNP. Our findings can be useful in understanding the genetic basis for myogenesis in postnatal adult muscles of JNP and in the breeds with low meat fat ratio. These findings can be used as the base to plan further studies to completely understand the precise signaling pathways for the transcriptional activation of target genes.

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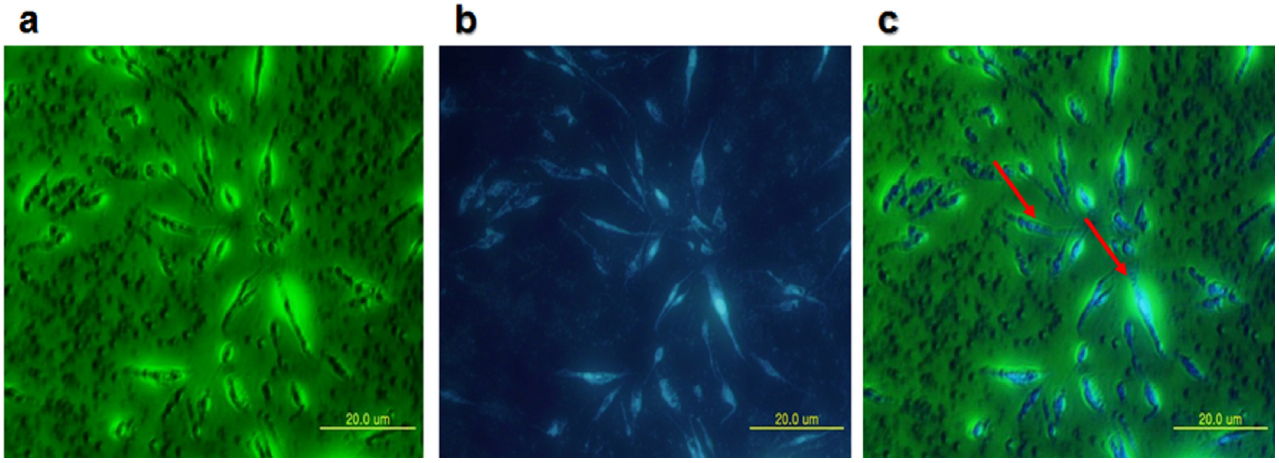

Fig. 6. Formation of myotubes in the C2C12 muscle myoblasts. The cells are showing green signals of FITC-conjugated MyoD (a), DAPI nuclear staining (b) and merged expression showing myotube formation (c) under the influence of the proteins under study.

Conflict of interest

There is no conflict of interest.

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