ITRAQ ANALYSIS OF SKELETAL MUSCLE PROVIDES AN INSIGHT INTO YAK PROTEOME RELATED ITS OXIDATIVE STABILITY

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Abstract – Yak (*Bos grunniens*) meat has been increasingly becoming popular among the consumers in China in recent years for its game-like flavor. In order to reveal important proteins associating with oxidative stability of yak meat, qualitative and quantitative differences in proteomes between yak and cattle *Longissimus dorsi* muscle were analyzed by proteomic approach using isobaric tag for relative and absolute quantification (iTRAQ) and LC-ESI-MS/MS. The results of the iTRAQ analysis demonstrated that there were 52 differentially expressed proteins in yak and cattle muscles, among which, 20 proteins were up-regulated and 32 down-regulated in yak muscle. The results from LC-ESI-MS/MS analysis and mascot database searching showed that these proteins were classified into following categories: metabolic proteins, myofilament and contractile proteins, cellular defense and stress response, signal transduction and miscellaneous. Prostaglandin reductase 1, an enzyme participating in reduction of lipid oxidation products, with expressed amount of 5 times higher in yaks than in cattle, was regarded as a main redox enzyme protecting yak from cellular stress. Overall, our investigation has provided an insight into the changes in yak proteome and this can be used as molecular marker assistant yak breeding for better meat quality.

Key Words –Yak, iTRAQ, Proteomic, High altitude, Hypoxia

I. INTRODUCTION

Yaks (*Bos grunniens*) inhabit the Qinghai-Tibetan Plateau for hundreds of generations. Yak meat has become increasingly popular among the consumers in China in recent years for its organic feature and game-like flavor. Oxidative stability is critical to the quality of fresh and processed meats. Recent genomic comparisons between yaks and cattle identified an expansion of gene families related to hypoxic response and energy metabolism in yaks, and the related genes were different or expressed at varied levels in yaks and cattle [1]. This suggests that the oxidative stability of yak meat may be possibly influenced by genetic as well as environmental factors. Previous studies reported that color of yak meat was preferred by consumers to cattle of the same age[2,3].

In order to reveal important proteins associating with oxidative stability of meat, proteomics analysis was utilized. Previous proteomic literatures on animals in response to high altitude were limited to two-dimensional polyacrylamide gel electrophoresis (2DE) and two-dimensional fluorescence difference gel electrophoresis (2D-DIGE) analysis, in which high acidic/basic and high/low molecular weight proteins as well as low abundant proteins are difficult to be observed on 2DE. Recent advances in proteomic techniques make it possible to overcome these shortcomings of 2DE by non-gel-based methods. The isobaric tag for relative and absolute quantification (iTRAQ) is a quantitative proteomic approach that can simultaneously identify and quantify proteins with high throughout by measuring the peak intensities of reporter ions with MS/MS [4]. In the present study, we compared qualitative and quantitative differences in proteomes in order to discover important proteins associating with oxidative stability of yak meat thus help on screening the meat quality related molecular markers to assist better yak breeding.

II. MATERIALS AND METHODS

Longissimus dorsi were obtained from three adult male yaks from Hongyuan County, and three adult male bovines from Chengdu Plain of Sichuan (n = 3). The muscles were removed quickly from slaughtered animals within 1 hour and kept frozen at -20°C until use.

iTRAQ labeling. iTRAQ analysis was implemented at Beijing Genomics Institute (BGI, Shenzhen, China). The iTRAQ labeling of peptide samples derived from yak and cattle muscles were

performed using iTRAQ Reagent 8-plex Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. Two independent biological triplicates (yak labeled with reagents 113, 114, 115 and cattle labeled with reagents 116, 119, 121) were applied. After incubation at room temperature for 2 h, labeled samples were mixed before being dried by centrifugal evaporation.

LC-ESI-MS/MS proteomic analysis based on TripleTOF 5600. The mass spectroscopy analysis was performed with a TripleTOF 5600 System (AB SCIEX, Concord, ON), which was fitted with a Nanospray Ⅲ source (AB SCIEX, Concord, ON) and a pulled quartz tip as the emitter (New Objectives, Woburn, MA) as described before [5].

Database search and iTRAQ quantification. The MS raw data recovery to MGF was processed via pFind (http://pfind.ict.ac.cn/downloads.html). Protein identification and quantification were performed using Mascot 2.3.02 (Matrix Science Inc, Boston, MA).

Bioinformatics analysis. The GO (Gene Ontology) (http://www.geneontology.org) and COG (Cluster of Orthologous Groups of proteins) (http://www.ncbi.nlm.nih.gov/COG/) analysis were conducted according to method reported in early literature. The metabolic pathway analysis of differentially expressed proteins was conducted according to KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway database (http://www.genome.jp/kegg/pathway.html).

III. RESULTS AND DISCUSSION

Primary data analysis and protein identification. A total of 307,326 spectra were generated from the iTRAQ experiment using yak and cattle LD muscle as materials. The data collected from these samples were analyzed using Mascot software (version 2.3.02, Matrix Science Inc, MA, USA). Mascot identified a total of 29,108 spectra matched to known spectra, 19,757 spectra matched to unique peptides, 5795 peptides, 5119 unique peptides and 1121 proteins. A false positive peptide discovery rate of 0.416 % was detected.

Differentially expressed protiens by iTRAQ. As far as we known, we are the first group to report the proteome of yak skeletal muscle. Moreover, the number of proteins we identifying was larger than that was reported of 542 in pigs with the same method [6]. Differentially expressed proteins were selected when the proteins had both a 1.4 or 0.714-fold change cut-off and a P-value of less than 0.05. Based on the two criteria, 52 differentially expressed proteins were identified, 20 (38%) of which were found to be up-regulated in yak and 32 (62%) were up-regulated in cattle samples (Table 1). According to the biological function and pathway involved, these proteins were classified into following categories: metabolic proteins (14, 26.9%), myofilament and contractile proteins (12, 23.1%), cellular defense and stress response (5, 9.6%), signal transduction (2, 3.8%) and miscellaneous (19, 36.5%)(Fig. 1). Detailed information can be found in Table 1. Prostaglandin reductase 1, with expressed amount of 5 times higher in yaks than in cattle, was regarded as a main redox enzyme protecting yak from cellular stress.

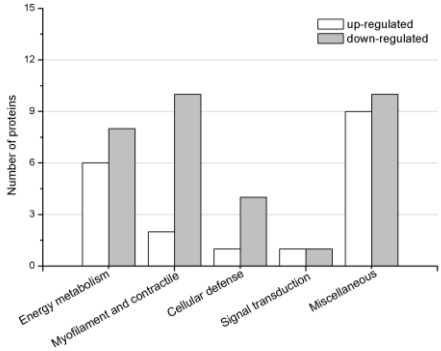

Fig. 1 Function categories of differentially expressed proteins in yak *Longissimus dorsi* (LD) muscles as compared with cattle.

Table 1 Differentially expressed proteins in yak *Longissimus dorsi* (LD) muscles as compared with cattle.

Uniprot Accession	Protein name	Yak:cattle ratio	*P* value
Metabolic proteins			
P20004	Aconitate hydratase, mitochondrial	1.484	0.011
Q3ZBU2	CDGSH iron-sulfur domain-containing protein 1	1.639	0.012
P23004	Cytochrome b-c1 complex subunit 2, mitochondrial	1.817	0.049
P13621	ATP synthase subunit O, mitochondrial	3.554	0.012
P00570	Adenylate kinase isoenzyme 1	2.863	0.029
P23109	AMP deaminase 1	1.654	0.028
Q5E9B1	L-lactate dehydrogenase B chain	0.473	0.005

Q32PA8	Mth938 domain-containing protein	2.643	0.007
P10790	Fatty acid-binding protein, heart	0.462	0.021
Q96Q06	Perilipin-4	0.616	0.001
P68401	Platelet-activating factor acetylhydrolase IB subunit beta	0.705	0.018
P11586	C-1-tetrahydrofolate synthase, cytoplasmic	0.643	0.042
Q5T481	RNA-binding protein 20	0.566	0.024
Q8WTS6	Histone-lysine N-methyltransferase	0.643	0.005
Myofilament and contractile proteins			
Q14324	Myosin-binding protein C, fast-type	1.446	0.041
Q8TDC0	Myozenin-3	2.318	0.032
Q5VTT5	Myomesin-3	0.637	0.003
Q5E9E1	PDZ and LIM domain protein 1	0.614	0.032
P97447	Four and a half LIM domains protein 1	0.697	0.019
Q3SZE5	Myosin regulatory light chain 2, ventricular/cardiac muscle isoform	0.494	0.013
P13533	Myosin heavy chain 6 (Fragment)	0.605	0.033
Q9BE39	Myosin heavy chain 7	0.571	0.035
P63315	Troponin C, slow skeletal and cardiac muscles	0.618	0.002
P02645	Troponin I, slow skeletal muscle	0.450	0.016
Q8MKH6	Troponin T, slow skeletal muscle	0.644	0.004
Q3ZBD4	Small muscular protein	0.308	0.018
Cellular defense and stress response			
Q3SZJ4	Prostaglandin reductase 1	4.735	0.012
Q3SZX4	Carbonic anhydrase 3	0.429	0.034
Q9N0V4	Glutathione S-transferase Mu 1	0.124	0.016
Q148F8	Heat shock protein beta-6	0.501	0.025
P54149	Mitochondrial peptide methionine sulfoxide reductase	0.324	0.002
Signal transduction			
P31415	Calsequestrin 1	9.998	0.001
P02639	S100 A1	0.326	0.005
Miscellaneous			
P02769	Serum albumin	4.050	0.001
Q5RBC8	Aralar1	1.484	0.033
Q02357	Ankyrin-1	1.463	0.001
Q13884	Beta-1 syntrophin	1.426	0.020
P61283	Barrier-to-autointegration factor	1.416	0.021
P55268	Laminin subunit beta-2	1.638	0.013
Q17QS0	Secernin-3	1.757	0.025
O02739	Serpin B6	1.726	0.033
A2I7N3	Serpin A3-7	1.538	0.019
Q9GZV1	Ankyrin repeat domain-containing protein 2	0.498	0.029
O00499	Myc box-dependent-interacting protein 1	0.657	0.001
Q9H444	Charged multivesicular body protein 4b	0.694	0.032
Q9TTE1	Serpin A3-1	0.590	0.025
Q13951	Core-binding factor subunit beta	0.693	0.031
A6QLJ8	Phosphotriesterase-related protein	0.497	0.009
P69678	Protein CutA	0.686	0.047
Q9BSL1	Ubiquitin-associated domain-containing protein 1	0.636	0.000
Q2YDF7	Junctional sarcoplasmic reticulum protein 1	0.212	0.001
Q4U0T9	Cysteine and glycine-rich protein 3	0.593	0.007

IV. CONCLUSION

We first used a robust and reliable comparative proteomic technique (iTRAQ) to compare the qualitative and quantitative differences of proteomes from yak and cattle muscles. In yak, 20 up-regulated and 32 down-regulated proteins were identified. These proteins were classified into following categories: metabolic proteins, myofilament and contractile proteins, cellular defense and stress response, signal transduction and miscellaneous. Prostaglandin reductase 1 was regarded as a main redox enzyme in yak.

ACKNOWLEDGEMENTS

This work was supported by Sichuan Yak Production Program (2012NZ0047-03, 04, 05), the National Nature Science Foundation of China (J1103518), and the National Beef Cattle Industrial Technology System (CARS-38).

REFERENCES

1. Qiu, Q., et al., (2012). The yak genome and adaptation to life at high altitude. Nature Genetics 44(8): 946-949.
2. Gu, S., Chen, D., Yin, S., Tang, K., & Sun, Q. (2007). Analysis of cDNA sequence of yak myoglobin and its oxidation in muscles. In Proceedings of 53rd international congress of meat science and technology, Beijing, China. 223-224.

3. Yang, M., Wen, Y. L., Wang, J. W., Wu, X. Z., Ma, L., Yang, R. S., & Zhang, J. L. (2009). Color-difference analysis of Biceps femoris and Longissimus dorsi in saughtered yak and yellow cattle. Food Science (in Chinese), 30(19), 104-108.
4. Wu, W.W., et al., (2006). Comparative study of three proteomic quantitative methods, DIGE, cICAT, and iTRAQ, using 2D gel-or LC-MALDI TOF/TOF. Journal of Proteome Research 5(3): 651-658.
5. Andrews, G.L., et al., (2011). Performance characteristics of a new hybrid quadrupole time-of-flight tandem mass spectrometer (TripleTOF 5600). Analytical chemistry 83(13): 5442-5446.
6. Hakimov, H.A., et al., (2009). Application of iTRAQ to catalogue the skeletal muscle proteome in pigs and assessment of effects of gender and diet dephytinization. Proteomics 9(16): 4000-4016.