AN HSPB1-NULL MOUSE TO DEPICT THE CONTRIBUTION OF HSP27 IN BEEF TENDERNESS

I. Cassar-Malek[1], M. Kammoun[1], T. Astruc[2], C. Chambon[3], C. Barboiron[1], A. Delavaud[1], B. Picard[1]

[1] UMR1213 Herbivores, INRA – VetAgro Sup, 63122 Saint Genes Champanelle, France

[2] QuaPA UR 370, INRA, 63122 Saint Genes Champanelle, France

[3]INRA UR 370 PFEM-Plateau Protéomique, 63122 Saint-Genès-Champanelle, France

Abstract – In order to examine the role of Hsp27 in the molecular mechanisms of Beef tenderness, we generated an HspB1-null mouse. The mutant mouse was viable, fertile and showed neither apparent morphological nor anatomical alterations. The macroscopic or microscopic muscle phenotype was not altered. However, there were evidences for a muscle-type specific alteration of the molecular phenotype in relation to 1) apoptosis, Hsp status and anti-oxidant status in an oxidative muscle and 2) Hsp status and calcium homeostasis in a glycolytic muscle. Lastly, electron microscopy revealed ultrastructural abnormalities in the myofibrillar structure of mutant mice. These data suggest that Hsp27 could directly impact the organization of muscle cytoskeleton and contribute to tenderness at the molecular and ultrastructural levels.

Key Words – Hsp27, biomarker, tenderness, muscle ultrastructure

I. INTRODUCTION

Tenderness is a top priority attribute for consumers who seek for Beef regularity. Beef tenderness is a complex function of production, processing and meat preparation. A combination of molecular processes in the muscle in both the live animal and during the *post-mortem* period contributes to this quality. However information on tenderness is only obtainable after slaughter, which is a limitation to the delivery of a consistent quality meat. Examination of the muscle transcriptome and proteome has been used as a strategy to identify biomarkers for Beef tenderness, predict it and understand the underlying mechanisms.

Recent studies including ours identified the heat shock protein Hsp27 as a Beef tenderness biomarker [1] with differential expression in the muscle of animals giving high *vs* low meat tenderness. However the relationships of Hsp27 with tenderness are not fully understood. Hsp27 is present as a central node in a molecular network of biomarkers of tenderness [2-5]. This suggests that Hsp27 may play a crucial role for the conversion of muscle into meat [6]. Thus, understanding the role of Hsp27 in Beef tenderness is a research challenge for meat production and detection of favorable phenotypes. In this study, we have questioned the role of Hsp27 in the development and characteristics of muscles by generating an HspB1-null mouse and examining the associated phenotype.

II. MATERIALS AND METHODS

A mouse strain devoid of Hsp27 protein was generated by homologous recombination of the HspB1 gene on C57BL/6 background. This was done in collaboration with the Institut de Génomique Fonctionnelle de Lyon (Lyon, France), the Plateau de Biologie Expérimentale de la Souris (Lyon, France), and the experimental facility of nutrition and microbiology of the French National Institute for Agricultural Research (INRA-Theix, France). Experimental procedures and animal holding respected the French animal protection legislation including licensing of experimenters. They were controlled and approved by the French Veterinary Services and the Committee on the Ethics on Animal Experimentation in Auvergne (agreement number CE 12).

The length, weight, and growth of mice were followed from birth to 1-year old. The m. *Soleus* (oxidative) and the m. *Tibialis Anterior* (glycolytic) were collected at 12 weeks of age

and analyzed by immuno-histology and electrophoresis as described in [7].

The muscles were further analyzed by electron microscopy at the Cellular Imaging Center for Health (CICS, http://www.clermont-universite.fr/Centre-Imagerie-Cellulaire-Sante) at Clermont-Ferrand university campus (France) at 0h and 72h *post-mortem*.

In order to implement the list of Hsp27 interactors and target genes that may contribute to differences in muscle structure, a bioinformatic analysis was performed using the software Pathway Studio and was validated by Western-blotting [1]. A proteomic analysis was also done by 2DE coupled to mass spectrometry and protein identification at the Metabolism Exploration platform (http://www6.clermont.inra.fr/plateforme_exploration_metabolisme_eng/).

III. RESULTS AND DISCUSSION

The HspB1-null mouse was viable, fertile and born at the expected Mendelian distribution, and no fertility or other obvious health defects were detected. It showed no apparent morphological or anatomical alterations compared to homozygous control. However the HspB1-null mutant was smaller and lighter than control mainly due to lower birth weights but with no obvious changes in its growth rate. From the bibliography, the invalidation of the gene has little effect [8, 9] except in [10] where it was found lethal.

Although Hsp27 is expressed at the highest levels of any tissues in muscle, there was neither specific macroscopic nor microscopic muscle phenotype associated with HspB1 invalidation. We did not observed any impact of HspB1 disruption on the relative weight of muscles and the size or types of muscle fibers. Fiber typing, cross-sectional areas, shapes and perimeters of fibers did not differ between the mutant mouse and the wild-type (control) one in the m. *Soleus* and the m. *Tibialis anterior*. However, distinct electrophoretic MyHC profiles were observed between the HspB1-null mouse and the control in the m. *Soleus*. A third MyHC was detected in HspB1-null mouse comparatively to control that could be a developmental isoform as detected at 4 weeks of age in the *Soleus*. Thus, the absence of Hsp27 could be associated with a delay in the acquisition of muscle contractile properties.

Proteomics combined to bioinformatics revealed a muscle-type specific alteration of molecular phenotype in the absence of Hsp27. In the m. *Soleus*, the data were in favor to the stimulation of the apoptotic pathway, changes in the Hsp status (increase in α-B crystallin, Hsp90aa1, Hsp70a1a, decrease in Hsp20 and Hsp70-8), anti-oxidant status and the abundance of cytoskeletal proteins including desmin [1]. In the m. *Tibialis Anterior*, changes were recorded in the abundance of other Hsp (decrease in Hsp70-8 and Hsp70-9) and in proteins involved in calcium homeostasis (increase in Casq1 and Srl) (Table 1).

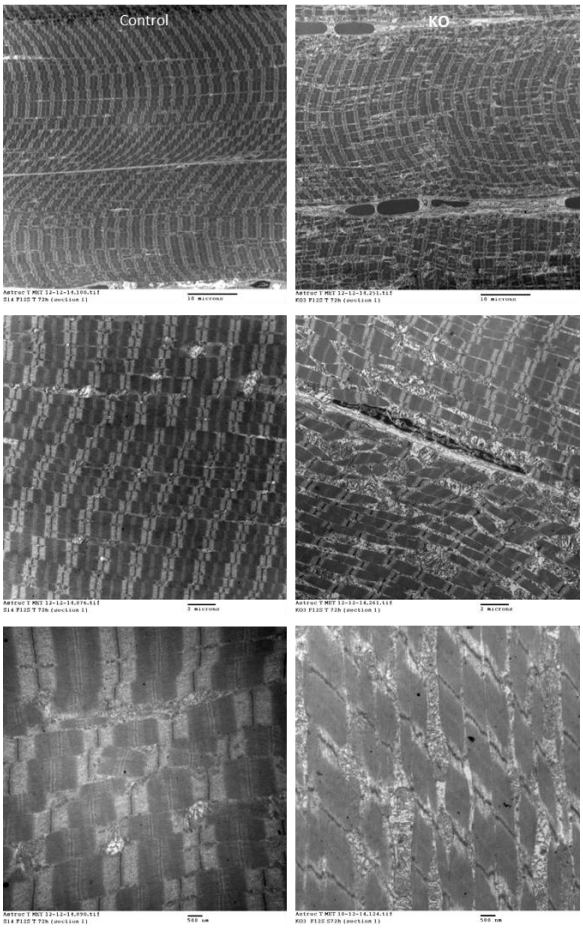
Table 1. Changes in the abundance of proteins targeted by bioinformatics or revealed by 2DE in the muscles of the HspB1-null mouse compared to control ($P \leq 0.05$; ~: ns)

	Bioinformatics and Western-Blot		Proteomics	
Protein	*Tibialis A*	*Soleus*	*Protein*	*Tibialis A*
Hsp27	-100%	-100%	HSP70.8	-15%
Hsp20	~	-45%	Hsp70.9	-22%
Cryab	~	+37%	Alb	+25%
Hspbap1	~	~	Tnnt3	+14%
Hsp40	~	~	Casq1	+38%
Hsp70a1a	~	+33%	Srl (5 spots)	+16-43%
Hsp90aa1	~	+102%	Napa	+16%
Fas	~	+76%	Gmpr	+24%
Chuk	~	~	Pdhb	+8%
Sod1	~	+24%	Myh4	-22%
Casp3(17kDa)	-22%	+54%	Ckm	-42%
Cycs	~	-22%	Tpi1	-46%
Eif4E	~	+36%	Park7	-13%
Eif4G1	~	+29%	Ak1	-24%
Desmin	~	-38%	Aldh2	+21%
			Mdh1	+16%
			Pygm	+ 97%
			Dusp3	-18%

Transmission electron microscopy revealed ultrastructural abnormalities in the myofibrillar network of the HspB1-null muscle as shown by

disturbances of the cross-striated band pattern at two times *post-mortem* (0h and 72h). In the m. *Soleus* of the HspB1-null mouse, we observed myofibril destructuration, higher intermyofibrillar space and the presence of some altered mitochondria with grouping of subsarcolemal mitochondria in favor to a weakening of the cytoskeletal network (Figure 1). There was a heterogeneity in fiber alteration, with altered and non-altered regions, suggesting that there could be an interaction with the fiber type.

Figure 1. Electron micrographs of longitudinal sections illustrating muscle damage following Hsp27 disruption at 72h *post- mortem*.



Hsp27 is part of the chaperone network. It plays a canonical role in the cellular response to stress including heat shock, oxidative stress and chemical stress through its involvement in protein quality control, folding, and degradation [11]. It is one of the most induced Hsps, reaching 1% of the total cellular protein, but accumulates in a slower kinetics than that of Hsp70 the other highly stress responsive Hsp. To date the role of constitutively expressed Hsp27 is not fully understood. Since Hsp27 is highly abundant in skeletal muscle, the moderate phenotype of the HspB1 null-mouse was quite unexpected. Thus, it may have adapted to the loss of Hsp27 through compensatory changes in the expression of cognate members of the Hsp family which was partly detected. The phenotype was very different from the muscle phenotype of the Hsp70 null-mouse [12] which is characterized by reduction in fiber cross section area and specific force, and increase in the amount of extracellular tissue when compared to controls.

Although there are only few reports that Hsp27 contributes to the integrity of muscle ultrastructure in basal conditions, our results suggest that depleting its constitutive expression results in myofibril ultrastructural abnormalities. They support the idea that the Hsp27 chaperone (together with αB-crystallin) is crucial for the assembly/maintenance and remodelling of myofibrillar structures. Several hypotheses can be formulated to account for the ultrastructural phenotype of mutant mouse including misfolded proteins, abnormal myofibrillogenesis and disorganization / loss of costameric proteins, activation of proteolysis.

Lastly, our data showed muscle-specific consequences for the inactivation of Hsp27. This could partly explain the differences between the abundance of biomarkers, including Hsp27, and Beef tenderness observed between muscles [13]. The HSPs (constitutive and induced *post-mortem*) undoubtedly play a role in the installation of tenderness in oxidative muscles (where their abundance is higher) through their anti-apoptotic action and protection of structural proteins.

IV. CONCLUSION

The absence of Hsp27 did not impair mouse development or the macroscopic characteristics of skeletal muscles. In-depth phenotyping indicated that invalidation of the HspB1 gene affects the organization of the myofibrillar cytoskeleton and plays an important role for the

regulation of its dynamics in a fiber-type specific manner. Thus, HspB1 could contribute to tenderness both at the molecular and ultrastructural levels.

ACKNOWLEDGEMENTS

The authors thank F. Guillou (UMR INRA 0085 - UMR CNRS 7247, Tours, FRA), D. Aubert (Plateau de Biologie Expérimentale de la Souris, Lyon, FRA), J. Henry-Berger (CNRS UMR 6293 - INSERM, Aubière, FRA), V. Blanquet (UMR 1061, Limoges, FRA) and M. Bonnet (UMR 1213 Herbivores, Theix, FRA) for helpful discussions, and the Animal Physiology and Husbandry department of INRA for funding the production of the HspB1-null mouse.

REFERENCES

1. Kammoun, M., Picard, B., Henry-Berger, J. and Cassar-Malek, I. (2013). A network-based approach for predicting Hsp27 knock-out targets Computational and Structural Biotechnology Journal. 6(7): e201303008.
2. Bernard, C., Cassar-Malek, I., Le Cunff, M., Dubroeucq, H., Renand, G. and Hocquette, J.-F. (2007). New indicators of beef sensory quality revealed by expression of specific genes. Journal of Agricultural and Food Chemistry. 55: 5229 - 5237.
3. Morzel, M., Terlouw, C., Chambon, C., Micol, D. and Picard, B. (2008). Muscle proteome and meat eating qualities of Longissimus thoracis of "Blonde d'Aquitaine" young bulls: A central role of HSP27 isoforms. Meat Science. 78(3): 297-304.
4. Kim, N., Cho, S., Lee, S., Park, H., Lee, C., Cho, Y., Choy, Y., Yoon, D., Im, S. and Park, E. (2008). Proteins in longissimus muscle of Korean native cattle and their relationship to meat quality. Meat Science. 80(4): 1068-1073.
5. Hocquette, J.-F., Bernard-Capel, C., Vidal, V., Jesson, B., Leveziel, H., Renand, G. and Cassar-Malek, I. (2012). The GENOTEND chip: a new tool to analyse gene expression in muscles of beef cattle for beef quality prediction. BMC Veterinary Research. 8(135).
6. Guillemin, N., Bonnet, M., Jurie, C. and Picard, B. (2011). Functional analysis of beef tenderness. Journal of Proteomics. 75(2): 352-365.
7. Kammoun, M., Cassar-Malek, I., Meunier, B. and Picard, B. (2014). A reliable immunohistochemical classification of skeletal muscle fibres in mice. European Journal of Histochemistry. 58(2): 2254.
8. Huang, L., Min, J.-N., Masters, S., Mivechi, N. F. and Moskophidis, D. (2007). Insights into function and regulation of small heat shock protein 25 (HSPB1) in a mouse model with targeted gene disruption. genesis. 45(8): 487-501.
9. International Mouse Phenotyping consortium. https://www.mousephenotype.org/.
10. Eroglu, B., Min, J.-N., Zhang, Y., Szurek, E., Moskophidis, D., Eroglu, A. and Mivechi, N. F. (2014). An Essential Role for Heat Shock Transcription Factor Binding Protein 1 (HSBP1) During Early Embryonic Development. Developmental biology. 386(2): 448-460.
11. Arrigo, A.-P. (2012). Pathology-Dependent Effects Linked to Small Heat Shock Proteins Expression: An Update. Scientifica: 19 pp.
12. Senf, S. M. (2013). Skeletal muscle heat shock protein 70: diverse functions and therapeutic potential for wasting disorders. Frontiers in Physiology. 4: 1-6.
13. Picard, B., Gagaoua, M., Micol, D., Cassar-Malek, I., Hocquette, J.-F. and Terlouw, E. M. C. (2014). Inverse relationships between biomarkers and beef tenderness according to contractile and metabolic properties of the muscle. Journal of Agricultural and Food Chemistry. 62(40): 9808-9818.