RNA-seq REVEALS CONTRIBUTION OF HISTONE UBIQUITINATION TO POST-TRANSLATIONAL REGULATION OF HEAT SHOCK PROTEINS AND CYTOKINES OF CATTLE AFTER EXPOSURE TO HEAT SHOCK

Gbolabo O. Onasanya[1,2,3*], Sunday Peters[7], Tirumurugaan K. Gopalan[3], Aranganoor K. Thiruvenkadan[2], Nagarajan Murali[2], Ramasamy Saravanan[2], George M. Msalya[4], Angamuthu Raja[5], Itodo J. Iyoyo[9] and Christian O. Ikeobi[8]
[1]Department of Animal Science, Federal University Dutse, Dutse, Nigeria
[2]Department of Animal Genetics and Breeding, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India
[3]Translational Research Platform for Veterinary Biologicals, Central University Laboratory, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India
[4]Department of Animal, Aquaculture and Range Sciences, Sokoine University of Agriculture, Morogoro, Tanzania
[5]Department of Veterinary Microbiology, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India.
[6]Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, Nigeria.
[7]Department of Animal Science, Berry College, Georgia, USA.
[8]Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, Nigeria
[9]Department of Animal Science, Federal University of Lafia, Lafia, Nigeria
*Corresponding author: onasanya.gbolabo@gmail.com; +2347069209207

ABSTRACT

Epigenetics is the study of post translational changes in gene expression/function without corresponding alteration in the DNA sequence of a particular body phenotype. Ubiquitin, Histone and DNA methylations are the evidence of epigenetics involving gene modulations in eukaryotes. In this study, we isolated peripheral blood monoclonal cells (PBMCs) from twelve (12) India Gir zebu cattle and exposed them to different doses of in vitro assault of thermal condition. RNAs isolated from the heat-shocked PBMCs were processed for production of enriched mRNA library for RNA-seq. We performed RNA-seq analyses followed by transcriptional expression of mRNAs for differential gene expression and gene ontology term enrichment analyses. Our findings showed that thermal stress stimulation of our PBMCs impinged heat shock on the bovine cellular system thereby activating expression of histones, ubiquitin and heat shock protein genes. Further to the above, we found direct relationship between the expression of histone (H2A1), ubiquitin and heat shock protein genes. We concluded therefore that, ubiquitination of histone protein (H2A1) plays an epigenetic role in post-translational regulation/overexpression of heat shock proteins (HSPs) including HSPs 70, 90, small HSPs (HSPB11, HSPB6, HSP22, HSP1L1, HSP27) and cytokines (IL1A, IL18) in zebu cattle and this may permit joint regulation of expression and function of these target proteins (HSPs and cytokines) for their thermotolerance and immune-competence potentials in bovine species upon exposure to heat shock/thermal assault.

Keywords: Ubiquitin, Histone, Heat shock, HSPs. PBMCs, Epigenetics, Zebu cattle, Bovine

INTRODUCTION

Heat shock proteins (HSPs)are molecular chaperone that perform essential functions of providing cellular protection/cyto-protection, immune response, protein synthesis, cyto-skeletal protection, protein folding and unfolding, protein translocation and regulation of steroid hormone receptors, inhibit apoptosis, adaptation during and after stressful conditions including disease infection and environmental conditions (Onasanya *et al.,* 2017; Kapila *et al.,* 2013; Sodhi *et al.,* 2013).

The expression of HSPs is activated by physiological conditions such as pathogenic infections, assault of thermal conditions/heat shock, physiological condition including epigenetics of histones and ubiquitin genes. Nucleosome is subunits/chains/beads of chromatin (DNA) wrapped around protein called histones (Iberts *et al.,* 2002). These histones such as *H2A, H2B, H3, and H4* play significant roles in DNA replication and regulations of gene expression. Histone protein modification of gene expression which include histone acetylation, methylation, phosphorylation, and ubiquitination represents classical epigenetic mechanisms that alter gene expression/function without altering the traditional/underlining nucleotide sequence (Alaskhar *et al.,* 2018; Dupont *et al*., 2009).

Ubiquitination (a post translational modification of gene function) is critical to epigenetics in eukaryotes, ubiquitin genes belong to the family of structurally conserved proteins that epigenetically regulate a host of processes in eukaryotic cells including gene expression. Ubiquitin proteins are regarded as ubiquitous genes because of their widespread existence or being found everywhere within the eukaryotic genome. The addition of ubiquitin to a substrate protein such as histone is called ubiquitylation/ubiquitination (Pickart and Eddins, 2004). This study investigated how epigenetics of histones and ubiquitin modulate the expression of some selected HSPs and cytokines of bovine species under assault of thermal conditions/heat shock.

MATERIALS AND METHODS

Twelve Indian zebu bulls between the ages of 5–6 years were randomly sampled for blood collection (5 mL per animal) to generate peripheral blood mononuclear cells (PBMCs). The procedures for blood collection, PBMCs isolation, thermal assault conditions (Control: 37°C Vs Test: 45°C for 30 minutes each), *in vitro* thermal stress stimulation (TSS) of PBMCs, quantitation of PBMC count, processing and preservation of PBMC, total RNA extraction from PBMC pellets, RNA quality check and Integrity test, RNA quantitation, preparation and mRNA enrichment for mRNA Library including quantitation, enriched mRNA quality check along with integrity test and validation of mRNA Library were performed according to earlier published works by Onasanya *et al.* (2023, 2024) and Tirumurugaan *et al.* (2020).The enriched mRNA samples were transported in cooled iced-packed carrier for next generation sequencing (NGS) of transcripts/transcriptome (Tirumurugaan *et al.,* 2020). Transcriptome annotation, gene ontology term enrichment analysis, differential gene expression and protein-protein-interaction (PPI) were performed according to earlier published procedures of Tirumurugaan *et al.* (2020). The aligned transcripts were mapped against indexed *Bos indicus* GCF_000247795.1 reference genome and gene model downloaded from NCBI database (https://www.ncbi.nlm.nih.gov/data-hub/genome/GCF_000247795.1/) using STAR v2.7 aligner (Love *et al.,* 2014), and were compared with NCBI non-redundant protein database using BLASTX program (Love et *al.,* 2014). Differential expression analysis of the transcriptome was performed using DESeq2 software package after normalizing the data using relative log expression normalization method (love *et al*., 2024). Gene level expression values were obtained as read counts using Feature Counts software version 2.0.0 (Liao, et al., 2014) and read counts of differentially expressed genes were evaluated. Reads counts are total numbers of transcripts/exons belonging to a particular gene (Liao *et al.,* 2014).

RESULTS AND DISCUSSION

Our finding demonstrated that the detected Ubiquitin (*UBE30, UBALD1, UBTD2, UBE25, UBER2, UBE212, UBR1, UBE4B, UBNX7, UBQLN4, UBN2*) and histone (*H2A1*) regulate overexpression of *HSPs* such as *HSPs* 90, 70, and Small HSPs including, *HSPB11, HSPILI, HSP22, HSP27* etc. and cytokines (*IL1A*, *IL18* and *CXL12*) as shown in Figure 1. Addition of substrate protein such as histone to ubiquitin in modifying expression of a target protein is called histone ubiquitination (Pickart and Eddins, 2004).

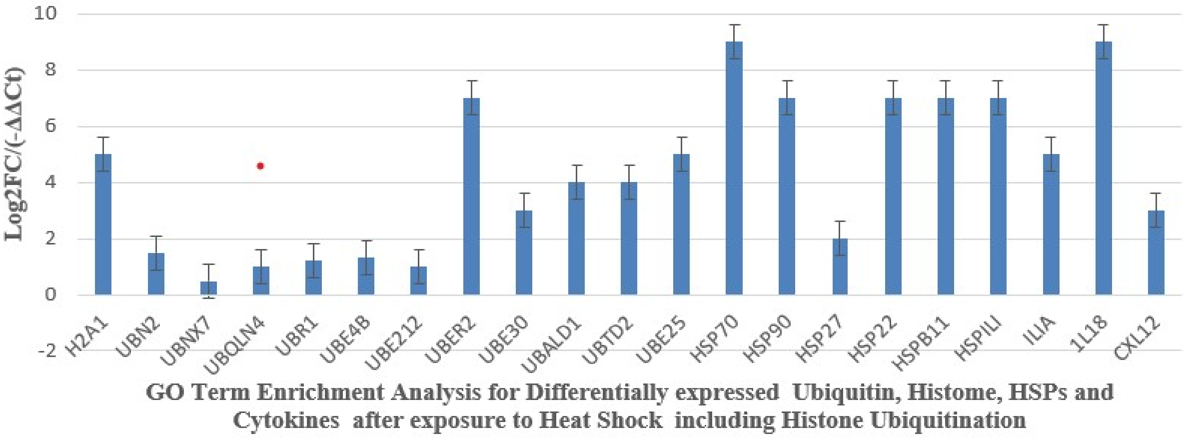

Figure 1: Gene Ontology Term Enrichment Analysis for Differentially Expressed Ubiquitin, Histone, HSPs and Cytokines after exposure to Heat Shock and Histone Ubiquitination

Corresponding overexpression detected in Ubiquitin, Histone including HSPs, and cytokines might be evidence of post-translational modulation of these target proteins by Ubiquitin and Histone. Alaskhar *et al.* (2018) and Dupont *et al.* (2009) reported that modification of gene expression by Histone protein include histone acetylation, methylation, phosphorylation, and ubiquitination; these biological processes representing classical epigenetic mechanisms that alter gene function/expression without altering the traditional/underlining nucleotide sequence.

Ubiquitination is an enzymatic post-translational modification involving the addition of ubiquitin proteins onto substrate proteins such as Histone (Morgan and Shilatifard, 2020). Histones are often ubiquitinated with one ubiquitin molecule and this process is called monoubiquitination while those inquinated with more than one ubiquitin molecules are called polyubiquitination (Morgan and Shilatifard, 2020). Histone ubiquitination is an essential epigenetic mechanism regulating DNA replication transcription and repair of damaged DNA especially during heat shock (Jeusset and McManus, 2019). Jeusset and McManus (2019) reported that aberration that occurs during histone ubiquitination can permit oncogenesis by changing the expression of tumour suppressors and oncogenes, mis-regulating cellular differentiation, thereby leading to cancer cell proliferation. Ubiquitin is a family small protein that is ubiquitously present in all eukaryotic cells and performs myriad functions through conjugation to a large range of target proteins (Sharp and Li, 1987).

CONCLUSION

In this study, we demonstrated how histone ubiquitination takes part in the modulation of overexpression of HSPs and cytokines of zebu cattle under assault of thermal conditions. We observed that the roles of histone ubiquitination during the exposure of bovine cellular system to assault of thermal condition may enhance fidelity in the DNA replication and permit DNA repair of HSPs, cytokines including other target proteins. Therefore, ubiquitination of histone protein (*H2A1*) plays essential epigenetic role in post-translational regulation of HSPs including *HSPs* 70, 90, small HSPs (*HSPB11, HSPB6, HSP22, HSP1L1, HSP27*) and cytokines (*IL1A*, *IL18*) in zebu cattle and this may permit regulation of expression and function of these target proteins (HSPs and cytokines) for their thermotolerance and immune-competence potentials in bovine species upon exposure to heat shock/thermal assault conditions.

ACKNOWLEDGEMENT

The Authors Acknowledge the funding of this project through TWAS-DBT Post-Doctoral Research Fellowship awarded to Dr Onasanya Gbolabo Olaitan, we are equally thankful to Tamil Nadu Veterinary and Animal Sciences University, Chennai, India for post-doctoralhosting support offered to Dr Onasanya Gbolabo Olaitan, we are grateful them.

REFERENCES

Alaskhar A. B., Khalaila, R., Wolf, J. *et al.* (2018). Histone modifications and their role in epigenetics of atopy and allergic diseases. *Allergy Asthma Clin Immunol* 14: 39 https://doi.org/10.1186/s13223-018-0259-4

Dupont, C., Armant. D. R., Brenner, C. A. (2009). Epigenetics: definition, mechanisms and clinical perspective *Seminars in Reproductive Medicine.* 27 (5): 351–7. doi:10.1055/s-0029-1237423. PMC 2791696. PMID 19711245

Iberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K. and Walter, P. (2002). Chromosomal DNA and Its Packaging in the Chromatin Fiber. Garland Science.

Jeusset, L. M. P. and McManus, K. J. (2019**).** Developing Targeted Therapies That Exploit Aberrant Histone Ubiquitination in Cancer. *Cells*, 8: 165. https://doi.org/10.3390/cells8020165

Kapila, N., Kishore, A., Sodhi, M., Sharma, A. and Mohanty, B. et al. (2013). Temporal changes in mRNA expression of heat shock protein genes in mammary epithelial cells of riverine buffalo in response to heat stress *in vitro*. *International Journal of Animal Biotechnol*ogy, 3: 5-9.

Liao, Y., Smyth, G. K. and Shi, W. (2014). Feature Counts: An efficient general-purpose program for assigning sequence reads to genomic features. *Bioinforma. Oxf. Engl.,* 30:923–30.

Love, M. I, Huber, W. and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.,* 15:550

Morgan, M. A. and Shilatifard, A. (2020). Reevaluating the roles of histone-modifying enzymes and their associated chromatin modifications in transcriptional regulation *Nature Genetics*. 52 (12): 1271–1281. doi:10.1038/s41588-020-00736-4. PMID 33257899. S2CID 227242638.

Onasanya G. O., Murali, N., Saravanan, R., Thiruvenkadan, A. K., Tirumurugaan K. G., Msalya, G., M., Raja, A., Yisa, A. A., Ikeobi, C. O. N. and Peters S. (2024). RNA-seq and Cytoscape Analyses Detected Network of Bio-Molecular Species Intervening Immuno-Competence of Indian Zebu Cattle Under Assault of Thermal Conditions. Proceedings of 29th Annual Conference of Animal Science Association of Nigeria Lagos, Nigeria.

Onasanya G. O., Thiruvenkadan, A. K., Yisa, A. A., Tirumurugaan, K. G, Murali, N., Saravanan, R., Raja, A., Msalya, G. M. and Ikeobi, C.O. (2023). Techniques of Using Peripheral Blood Mononuclear Cells as the cellular system to investigate "how "of the bovine species (Indian Zebu–Jersey Crossbreds) responds to *in vitro* thermal stress stimulation (Thermal Assault/Heat Shock). In X. Liu, X. and Li, H. (Eds.), Breeding Strategies for Healthy and Sustainable Development of Animal Husbandry. Veterinary Medicine and Science, Vol. 18, IntechOpen Publisher, SW7 2QJ, United Kingdom

Onasanya, G. O., Ikeobi, C. O., Ayotunde, A. S., Oke, F. O., Olorunisola, R. A. and Decampos, J. S. (2017) Thermo-regulatory functions of heat shock protein genes in some selected tropically stressed livestock animals. *International Journal of Applied. Research and Technol*ogy, 6(8): 37–43.

Onasanya, G. O., Msalya, G. M., Thiruvenkadan, A. K., Murali, N., Saravanan, R., Raja, A., Okpeku, M., Jeyakumar, M., and Ikeobi, C. O. (2022) Exposure to high thermal conditions for a long time induces apoptosis and decreases total RNA concentration in peripheral blood mononuclear cells among Indian Zebu–Jersey crossbreds, *Veterinary World*, 15(9): 2192–2201.

Pickart, C. M. and Eddins, M. J. (2004). Ubiquitin: structures, functions, mechanisms. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*. 1695 (1–3): 55–72. doi:10.1016/j.bbamcr.2004.09.019. PMID 15571809.

Sharp, P. M. and Li, W. H. (1987). Molecular evolution of ubiquitin genes. *Trends in Ecology* and *Evolution*. 2 (11): 328–32. doi:10.1016/0169-5347(87)90108-X. PMID 21227875.

Sodhi, M., Mukesh, M., Kishore, A., Mishra, B.P., Joshi, B. K.et al.(2013). Novel polymorphisms in UTR and coding region of inducible heat shock Trop Anim Health Prod protein 70.1 gene in tropically adapted Indian zebu cattle (Bos indicus) and riverine buffalo (Bubalus bubalis). *Gene*, 527: 606–615

Tirumurugaan, K. G., Pawar, R. M., Raj, G. D., Thangavelu, A., Hammond, J. A. and Parida, S. (2020). RNAseq Reveals the Contribution of Interferon Stimulated Genes to the Increased Host Defense and Decreased PPR Viral Replication in Cattle. *Viruses*, 12: 463; doi:10.3390/v12040463