In silico bioinformatics analyses reveals the structural and functional characterization of protein G3N0V0 differentially abundant in dark-cutting beef

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Introduction: Proteomics analysis helps to understand protein changes in a biological system. The peptide sequences are matched with a protein database to identify proteins. However, proteomic analysis results in unidentified proteins. Previous research from our laboratory has shown that G3N0V0 protein is one of the differentially abundant stress-related proteins reported in dark-cutting beef with a ≥ 2-fold change. However, G3N0V0 protein is not characterized in bovine with no known three-dimensional structure (3D).

Purpose: The objective of the current study was to utilize in silico bioinformatics approaches to characterize the structure and functional annotation of uncharacterized G3N0V0 protein in dark-cutting beef proteomes analyzed in our laboratory.

Materials and Methods: Normal-pH and dark-cutting longissimus lumborum muscles were purchased from a USDA-approved slaughter facility (n = 6 replications). A gel-free LC/MS-MS-based proteomics analysis was conducted to characterize protein expression profile differences. The amino acid sequence of the uncharacterized G3N0V0 protein identified in dark-cutting beef proteomes analyzed in our laboratory (Kiyimbaet al., 2021) was obtained from UniProtKB in FASTA format using the accession number G3N0V0. The detailed protein physicochemical characterization was determined using the ProtParam tool of ExPasy, and the secondary structure composition was predicted using the SABLE web software. The 3D homology structure modeling of G3N0V0 protein was done using the Swiss-Model server, and the final 3D model quality assessment was done using the Swiss-Model interactive workplace. The 3D structural visualization was done using PyMol V.2.4, and the conserved protein domains were determined using the conserved domain database. The disordered and flexibility protein regions were predicted using the protein web tool, and the goPredSim prediction of gene ontology (GO) terms was employed to predict the molecular and cellular localization of the uncharacterized G3N0V0 protein differentially abundant in dark-cutting compared with normal-pH beef.

Results: The in silico bioinformatics analyses revealed that G3N0V0 protein consists of three immunoglobulin C1-set domains including; the CH4 domain (fourth constant Ig domain of heavy chain), CH1 domain (first constant Ig domain of heavy chain), and the immunoglobulin domain, which belong to the CI11960 superfamily. These C1-set domains are classical Ig domains exclusively present in molecules involved in immune system. Molecular functional characterization using the predicted protein web tool showed that the G3N0V0 protein is involved in antigen and immunoglobulin receptor binding. These results suggest that the uncharacterized G3N0V0 protein is an immunoglobulin heavy chain protein involved in the major histocompatibility complex (MHC) class 1 and II. Thus, up-regulation of the G3N0V0 protein in dark-cutting beef might be associated with pre-slaughter adaptive stress responses.

Conclusion: Our study shows that the differentially abundant G3N0V0 protein in dark-cutting beef is an immunoglobulin protein involved in immune and stress adaptive responses. Further understanding the role of G3N0V0 protein in meat color development may provide insights into the occurrence of the dark-cutting phenotypes in beef.

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Literature:

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