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Analysis of oxidative stress, apoptosis and necrosis in myocytes from aging porcine muscles by flow cytometry (#650)

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Introduction

The transformation of muscle into meat involves numerous biochemical changes that determine to a great extent the quality of the resulting meat. The understanding of the precise molecular mechanisms underlying these biochemical changes is essential to manage meat aging properly, minimize undesirable changes and promote those contributing positively to meat quality. Many previous studies have investigated the postmortem glycolytic metabolism given the relevant role of glycogen depletion and pH decline on several major quality traits. The ocurrence of oxidative stress is known to take place in postmortem muscles as a result of the collapse of the endogenous antioxidant defenses, the release of pro-oxidant factors and the exposure of meat to oxygen and light. Yet, the nature of the reactive oxygen species formed and their role in the death of muscle cells after slaughter is not well understood. The study of postmortem oxidative stress in aging muscle is complex given that the underlying reactions and mechanisms are complex and the methodological approach can actually modify the nature and intensity of the oxidative reactions.

Flow cytometry has been extensively used in molecular biology since its introduction in the 70s of the past century; initially focused on the analysis of sperm DNA. Later on, the applications of flow cytometry expanded to the study of the integrity of the cell membranes and mitochondrial function. While the costs and complexity of initial flow cytometers limited their use to specialized laboratories and mainly to research applications, nowadays more affordable, reliable and user friendly systems allow to implement flow cytometry in assorted fields of cell biology.

This study is the first approach to apply a simple flow cytometry protocol to assess generation of ROS, and apoptotic and necrotic changes in myocytes from porcine muscles during aging.

Methods

Porcine muscles were obtained from a local slaughterhouse. Oxidative (psoas major) and glycolytic (longissimus thoracis) muscles were allowed to age for 10 days. At sampling times, samples were finely minced using sterile scalpels until it resembles a fine paste. Samples were treated with 5 mg/ml of collagenase D and 1.2 U/ml of dispase II. The total digestion time took 1,5 h and the digested slurry was filtered through a 100 µm cell strainer over a 50 ml conical tube. Myocytes were collected after centrifugation in a conical tube and resuspended in PBS for subsequent staining with stained with Hoechst 33342 (H42) to identify cells and discard debris, CellRox Deep Red for yhe detection of ROS, Cell Event Caspase-3/7 Green for the assessment of apoptotc changes and ethydium homodimer (Eth-1) for necrotic events. Flow cytometry analyses were conducted using a Cytoflex® flow cytometer (Beckman Coulter, CA, USA) equipped with violet, blue and red lasers. The instrument was calibrated daily using specific calibration beads provided by the manufacturer. A compensation overlap was performed before each experiment, however due to emission and excitation characteristics of the combination of probes used, spectral overlap was negligible. Files were exported as FCS files and analyzed using FlowjoV 10.5.3 Software for Mac OS (Ashland, OR, USA). Unstained, single-stained, and Fluorescence Minus One (FMO) controls were used to determine compensations and positive and negative events, as well as to set regions of interest. In addition to flow cytometry analyses, samples were analyzed for lipid oxidation (TBARS), protein oxidation (protein carbonyls by the DNPH method) and reduced and oxidized glutathion (LC-MS).

Results

The application of flow cytometry to myocytes extracted from postmortem muscles enabled a detailed study of moleculat events ocurred during aging at individual cellular level (Figure 1). Events plotted against H42 and Eth-1 fluorescence showed three populations, live (H-42 +), apoptotic (H-42 +/Eth-1 +) and dead myocytes (Eth-1 +). Confronting caspase 3 positive vs. H42 fluorescence enabled the detection of three subpopulations: live myocytes (H-42 positive events), early apoptotic (Caspase 3 dim) and late apoptotic (Caspase bright events). The progression of apoptosis during aging concurr with generation of ROS, oxidative damage to proteins and depletion of endogenous antioxidant resources. Myocytes from oxidative muscles suffered more intense oxidative stress and apoptotic and necrotic changes occured earlier than in glycolytic ones.

Conclusion

The present results show the feasability of using flow cytometry to assess precise biological changes in intact postmortem myocytes. The application offers a broad range of studies aimed to clarify the biochemical changes occured during transformation of muscle into meat and the influence of those events on meat quality.

Notes

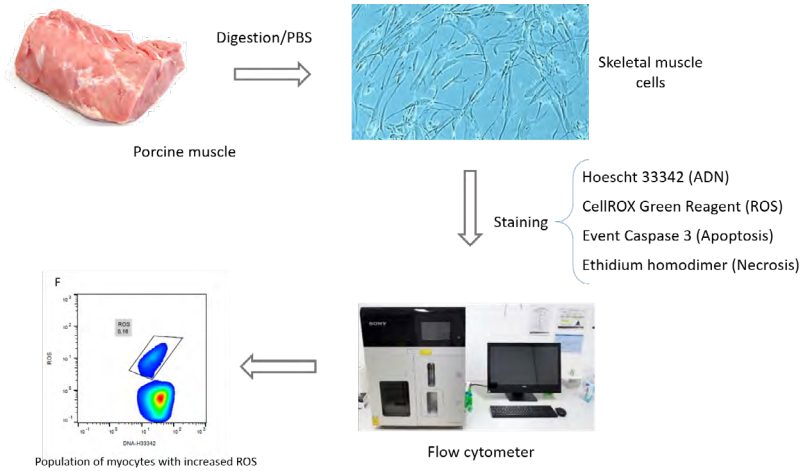

Figure 1
Work flow for the analysis of postmortem events in myocytes from porcines muscles using flow cytometry

Notes