DISTRIBUTION AND DEGRADATION OF PORK FILAMIN DURING POSTMORTEM AGING

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I. INTRODUCTION

Degradation of cytoskeletal proteins could contribute to structure change and quality improvement of pork during postmortem aging [1]. Filamin C (FLNC), an actin binding protein, is crucial for forming networks of actin filaments and connecting the actin cytoskeleton to cell membranes [2]. The objective of this study was to investigate the localization and degradation of pork FLNC during postmortem aging, thus laying the groundwork for future studies on the effects of FLNC changes to water holding capacity of pork.

II. MATERIALS AND METHODS

2.1 Sample collection

Crossbred Duroc × Landrace × Yorkshire pigs (100 ± 10 kg) were slaughtered at a meat processing factory (Jiangsu Sushi Meat Product Co., Ltd., China). Thirteen *longissimus dorsi* (LD) muscles from the left side carcasses were collected. Each muscle was divided equally into 6 portions and aged at 4 °C for 1 h, 6 h, 12 h, 24 h, 72 h and 168 h, separately.

2.2 Intracellular localization

The distribution of pork FLNC was determined by immunofluorescence method referring to Liu *et al.* [3]. Digital images were obtained by confocal laser scanning microscopy.

2.3 FLNC content

The changes of FLNC content in pork LD muscles during postmortem storage were detected by western blotting with the approach of Hou *et al.* [4].

2.4 Caspase-3 inhibitor treatment

The Ac-DEVD-CHO (specific inhibitor to caspase-3) was used following the methods of Tian *et al.* [5].

2.5 Statistical analysis

All data were presented as the means ± standard errors. T-test was applied for significance analysis ($P < 0.05$) of caspase-3 inhibitor treatment and one-way analysis of variance (Duncan's multiple-range tests, $P < 0.05$) was performed for other parameters using IBM SPSS Statistics 26.0.

III. RESULTS AND DISCUSSION

3.1 FLNC localization

In fluorescent images, a large amount of FLNC signals were observed at the plasma membrane and weaker signals were dispersed at the intracellular domains (Figure 1a, A-B). The signals displayed a punctate pattern on cross-sections, while a long strip pattern with the varied intensities around the membrane on longitudinal sections (Figure 1a, C-D).

3.2 FLNC degradation during postmortem aging

Results showed that intact FLNC bands of 290 kDa were rapidly degraded within 72 h (Figure 1c), while after which only a small portion of degradation occurred.

3.3 Caspase-3 inhibitor treatment

Among each group, immunoblotting bands in CAS groups were significantly higher than those of CON groups within 168 h (Figure 1e), indicating that caspase-3 could contribute to the degradation of FLNC during postmortem aging.

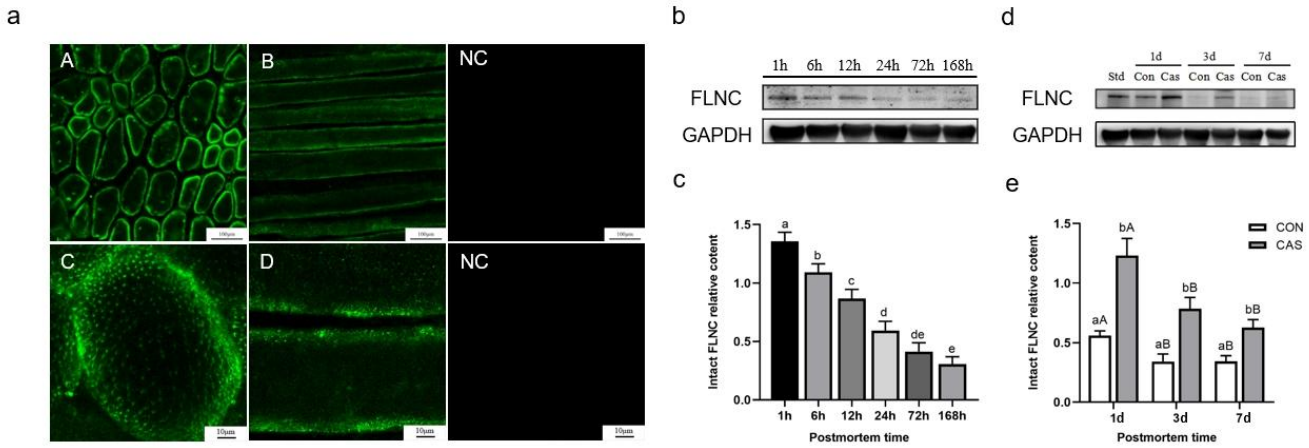

Figure 1. (a) Immunofluorescent staining of FLNC in porcine *longissimus dorsi* muscle cells. Negative control (NC) was incubated with PBS rather than primary antibody. (b, c) Western blotting of changes in FLNC content (n = 13) during postmortem aging (b), and the relative contents of intact FLNC at different time points (c). Means with different superscripts differ significantly ($P < 0.05$). (d, e) Western blotting of FLNC degradation in different treatment groups (n = 6) during postmortem phase (d) and the relative contents of intact FLNC within different groups (e). For Fig. e, different lowercase letters indicate significant difference between treatment groups at the same time, while different capital letters indicate significant difference among the same treatment at different postmortem time ($P < 0.05$). CON: control groups; CAS: caspase-3 inhibitor treated groups.

IV. CONCLUSION

The FLNC was primarily distributed around the sarcolemma where it played a supporting role for anchoring the actin cytoskeleton to the cell membrane. The content of FLNC decreased gradually within 3 d, and caspase-3 was proven to contribute to FLNC degradation during postmortem aging.

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