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PORCINE MUSCLE CELLS EXPRESSED MUSCLE SPECIFIC PROTEINS BUT NOT FORM MYOTUBES I
DIFFERENTIATION-INDUCING SERUM-FREE MEDIUM

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Background

Both quality and quantity of meat largely depend upon the property of muscle cells. During muscle development, the proliferate, differentiate and grow for the maturation. At the myotube formation in differentiation stage, the muscle cells fuse each other and their morphology drastically change. Although muscle development have been studied by many workers mechanism of myotube formation, an important step in muscle construction, is poorly understood in chicken, rat and mouse (Ku et al., 1995; Muroya et al., 1994; Rosen et al., 1992), much less in meat animals. Studies for myotube formation of meat anima necessary to elucidate the molecular mechanism of meat production.

Objectives

We aimed to elucidate how muscle cells fuse and form myotubes in meat animals. In the first place, we attempted to make m cells form myotubes efficiently in vitro to make further analysis easier, using porcine and bovine muscle cells.

Methods

Cell Preparation and Culture. The muscle cells were obtained according to the method designed by Yamaguchi and Kita (1991) with a few modifications. In brief, small pieces of *M. Longissimus thoracis* from 58 d fetus and 6 mo-aged adult pig, mo-aged calf were minced, digested with 0.1% collagenase (Sigma), 0.1% hyaluronidase (Wako) and 500 u/ml dispase (Godosy and then filtrated to prepare porcine and bovine muscle cells. The prepared cells were maintained on plastic dish coated with col type I (Sumitomo Bakelite) in the growth medium, Dulbecco's modified Eagle's medium (DMEM; Gibco) containing 10% feta serum (FCS; Biological Industries), renewing the medium every other day. After 3 d from seeding, they reached confluence and used for experiments within 5 passages from preparation except for immunofluorescence experiment. They were induc differentiate by switching the growth medium to the differentiation media, serum-free medium containing 1 µg/ml insulin (Cosme Cosmobio) or DMEM combined various concentration of FCS, porcine serum (PS; Sigma) and chicken embryonic extract (Gibco).

Detection of MHC and Desmin by Immunofluorescence. Muscle cells induced to differentiate were cultured for 3 days, fixed 4% formaldehyde for 1 h, washed with phosphate buffered saline (PBS) three times, and exposed in 0.2% Triton X-100 (Wako) for 20 min. Then the cells were incubated in blocking buffer, 1% bovine serum albumin, 0.2% NaN_3 in PBS for 1 h and reacted anti-myosin monoclonal antibody (Sigma) or anti-desmin rabbit antiserum (Chemicon International) for 1 h, accordi manufacturer's recommendations. After three washes with PBS, the cells were reincubated in blocking buffer and reacted biotinylated anti-mouse goat immunoglobulin (IgG; Vector) followed by incubation with fluorescein-isothiocyanate (conjugated streptavidin (Calbiochem) or FITC conjugated anti-rabbit goat IgG (Nordic Immunological Industries) for 30 1/1000 dilution, in combination with each primary antibody. The cells were washed with PBS three times and then observed epifluorescent illumination. All steps in the procedure were performed at room temperature.

Results and Discussions

Porcine cells did not form myotubes in Cosmedium which encouraged myotube formation of bovine cells. Five media were to induce both porcine and bovine muscle cells to differentiate. At the concentration of 5% or 10%, FCS and PS were not effecti

myotube formation in both cell cultures. In these cultures, the cells continued to proliferate and were possibly prevented from differentiation. Bovine cells formed myotubes in 2% FCS/DMEM and Cosmedium, a serum-free medium, promoted to bovine myotube formation further. However, both adult- and fetus-derived porcine cells, did not form myotubes in these conditions, though they seemed to quit to proliferate. PS also failed to encourage porcine myotube formation when used at the concentration of 10% or 2%, or in combinations of 5% FCS and 5% PS or 2.5% FCS and 2.5% PS.

MHC and desmin expressed and increased in porcine cells as well as bovine cells. It is important to define which stage porcine cells lost the myotube formation step during the differentiation. Immunofluorescent studies showed that both porcine and bovine cells expressed muscle specific MHC and desmin before the induction of differentiation. The expression of these markers was increasing as differentiation proceeded. They were strongly expressed in some mononucleated porcine cells and multinucleated bovine myotubes after 3 d in Cosmedium.

The observation of bovine muscle cells showed that Cosmedium was the most effective to induce myotube formation, possibly because no serum content and insulin caused effective suspension of cell cycle. On the other hand, Cosmedium can induce porcine muscle cells to differentiate but not to form myotubes, which suggest that, at least in this system, the myotube formation and the expression of muscle cell structural proteins proceed separately and simultaneously from the beginnings: porcine cell differentiation in Cosmedium proceeded in a step-specific manner. This system is useful for further analysis of the mechanism of myotube formation. In this experiment, Cosmedium succeed to form myotubes in bovine cell culture but not in porcine culture. Murine and chicken muscle cells formed myotubes efficiently in Cosmedium (Muroya et al., 1994), only porcine cells failed myotube formation. We speculate that there is some species specificity in the beginning mechanism of myotube formation among these animals. It is also beneficial to compare this system with the system in which porcine cells form myotubes (Hembree et al., 1991; Yamaguchi and Kitazawa, 1992) . Further analyses of the mechanism possibly contribute to more effective muscle construction and meat production.

Conclusions

Cosmedium, a serum-free medium, was the most effective to induce bovine myotube formation. However, porcine muscle cells failed to form myotubes in the same conditions. Both porcine and bovine cells expressed muscle specific MHC and desmin after 3 d in the medium. A species-specific mechanism of myotube formation possibly exists in porcine cell culture. The system of muscle cell development without myotube formation is useful for further analysis of the mechanism.

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