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A novel method for isolation and culture of primary swine gastric epithelial cells

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Abstract

Background: Culturing primary epithelial cells has a major advantage over tumor-derived or immortalized cell lines as long as their functional phenotype and genetic makeup are mainly maintained. The swine model has shown to be helpful and reliable when used as a surrogate model for human diseases. Several porcine cell lines have been established based on a variety of tissues, which have shown to extensively contribute to the current understanding of several pathologies, especially cancer. However, protocols for the isolation and culture of swine gastric epithelial cells that preserve cell phenotype are rather limited. We aimed to develop a new method for establishing a primary epithelial cell culture from the fundic gland region of the pig stomach.

Results: Mechanical and enzymatic dissociation of gastric tissue was possible by combining collagenase type I and dispase II, protease inhibitors and antioxidants, which allowed the isolation of epithelial cells from the porcine fundic glands showing cell viability > 90% during the incubation period. Gastric epithelial cells cultured in RPMI 1640, DMEM-HG and DMEM/F12 media did not contribute enough to cell adhesion, cluster formation and cell proliferation. By contrast, William's E medium supplemented with growth factors supports confluency and proliferation of a pure epithelial cell monolayer after 10 days of incubation at 37 °C, 5% CO2. Mucin-producing cell phenotype of primary isolates was confirmed by PAS staining, MUC1 by immunohistochemistry, as well as the expression of *MUC1* and *MUC20* genes by RT-PCR and cDNA sequencing. Swine gastric epithelial cells also showed origin-specific markers such as cytokeratin cocktail (AE1/AE3) and cytokeratin 18 (CK-18) using immunohistochemical and immunofluorescence methods, respectively.

Conclusions: A new method was successfully established for the isolation of primary gastric epithelial cells from the fundic gland zone through a swine model based on a combination of tissue-specific proteases, protease inhibitors and antioxidants after mechanical cell dissociation. The formulation of William's E medium with growth factors for epithelial cells contributes to cell adhesion and preserves functional primary cells phenotype, which is confirmed by mucin production and expression of typical epithelial markers over time.

Keywords: Cell culture, Swine gastric epithelium, Tissue engineering, Animal models, Biotechnology

Background

Swine are considered to be one of the most valuable animal models used in preclinical studies due to their physiological and anatomical similarities to those of humans [1, 2]. The swine model has been extensively used for understanding the pathophysiology of diabetes and coronary artery disease (CAD) associated with atherosclerosis and hypercholesterolemia, considering their

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