2016 Reciprocal Meat Conference – Muscle and Lipid Biology and BioChemistry

Meat and Muscle Biology™

Transfecting SK6 Cells with the Porcine SCD1 Increases the Production of Monounsaturated Fatty Acids

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Keywords: acid, Palmitoleic acid, SK6 kidney cells, Stearoyl-CoA desaturase
Meat and Muscle Biology 1(2):159 **doi:10.221751/rmc2016.152**

Objectives

Fatty acid composition is an important component of foods derived from livestock species, as it contributes to both the healthfulness and the functionality of beef, lamb, pork, and dairy products. The most highly regulated and most abundant fatty acid in animal tissues and dairy products is oleic acid (18:1n9). Oleic acid is synthesized by the Δ9 desaturase, stearoyl coenzyme A desaturase (SCD1), which also is responsible for the synthesis of *cis*-9, *trans*-11 conjugated linoleic acid. The objective of this study was to clone the porcine SCD1 (pSCD1) cDNA and generate a porcine SK6 transgenic cell line for sustained overexpression pSCD1 in an inducible manner by utilizing a novel All-in-One Tet-On Lentiviral expression system.

Materials and Methods

The Tet-inducible bidirectional lentiviral (tetracycline-controlled) vector and the vector encoding the pSCD1 gene under the influence of a tetracycline-responsive promoter element (TRE) were combined together to generate an inducible all-in-one lentiviral vector system. Swine Kidney 6 (SK6) cells were transduced with recombinant lentiviral stocks, induced with doxycycline (4 µg/mL), then cultured. Doxycycline was replenished in the media every 48 h to continue the induction of the vector. Non-transgenic as well as transgenic SK6 cells, which overexpress SCD1, were exposed to 50µM palmitic acid or ethanol (control). After 6 h of palmitic/ethanol treatment, cells were harvested for RNA quantification of SCD1. The harvested cells were also used in a protein analysis of SCD1 and a fatty acid composition analysis. RNA and protein data were analyzed and compared to negative controls using Student's *t* test and one-way analysis of variance followed by Tukey's Multiple Comparison Test (Graph Pad Prism 5.0, Graph Pad Software, La Jolla, CA). Means for fatty acid percentages were compared by analysis of variance and, means were separated by Fisher's Protected LSD method. All experiments were conducted in triplicates with at least two independent runs. Significance was accepted at $P < 0.05$.

Results

The pSCD1-transfected cells overexpressed pSCD1 mRNA over 1000-fold ($P < 0.0001$). Non-transduced SK6 cells incubated with 50 µM palmitic acid contained (per well) 0.67 µg palmitoleic acid (16:1n-7), 4.51 µg stearic acid (18:0), 6.14 µg oleic acid (18:1n-9), and 0.94 µg cis-vaccenic acid (18:1n-7). The pSCD1-transfected SK6 cells induced with doxycycline and incubated with 50 µM palmitic acid contained 2.41 µg palmitoleic acid, 3.94 µg stearic acid, 6.28 µg oleic acid, and 1.50 µg cis-vaccenic acid. Therefore, pSCD1 transfection of SK6 cells caused the Δ9 desaturation of palmitic acid to palmitoleic acid, which subsequently was elongated to cis-vaccenic acid. Additionally, total cellular lipid was increased from 22.1 to 27.8 µg/well ($P < 0.05$).

Conclusion

These results indicate that this all-in-one pSCD1 lentiviral overexpression system effectively increased the Δ9 desaturation of the saturated fatty acid, palmitic acid. We predict that this system can be used in future studies to increase the concentration of healthful fatty acids such as oleic acid in pork by increasing SCD1 activity in porcine muscle and adipose tissue.