Generation of fad2 transgenic mice that produce omega-6 fatty acids

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Fatty acid desaturase-2 (FAD2) introduces a double bond in position Δ12 in oleic acid (18 : 1) to form linoleic acid (18 : 2 n-6) in higher plants and microbes. A new transgenic expression cassette, containing CMV promoter/fad2 cDNA/SV40 polyA, was constructed to produce transgenic mice. Among 63 healthy offspring, 10 founders (15.9%) integrated the cotton fad2 transgene into their genomes, as demonstrated by PCR and Southern blotting analysis. All founder mice were fertile and heterozygous fad2 female and nontransgenic littermates were used for fatty acid analysis using gas chromatography. One fad2 transgenic line showed substantial differences in the fatty acid profiles and the level of linoleic acid was increased 19% (P<0.05) in transgenic muscles compared to their nontransgenic littermates. Moreover, it exhibited an 87% and a 9% increase (P<0.05) in arachidonic acid (20 : 4 n-6) in muscles and liver, compared to their nontransgenic littermates. The results indicate that the plant fad2 gene can be functionally expressed in transgenic mice and may play an active role in conversion of oleic acid into linoleic acid.

Plant fatty acid desaturase-2 (FAD2) introduces a double bond in position Δ12 in oleic acid (18 : 1) to form linoleic acid (18 : 2 n-6, LA). Because mammals lack the FAD2 enzyme and cannot synthesize de novo n-6 fatty acids, they must take it from plant or seafood in their diets. Polyunsaturated fatty acids (PUFAs) including n-6 fatty acids are constituents of cellular membrane phospholipids which affect membrane fluidity and the function of many membrane proteins (enzymes and receptors)[1]. The serve as signaling molecules or precursors of other signaling molecules, and influence the physiological function of cardiovascular, brain and nerve systems[2]. n-6 fatty acids may be associated with several diseases such as hyperinsulinism, arteriosclerosis, and cancer[3]. Therefore fad2 transgenic mice are used to study the mechanism of the endogenous synthesis of polyunsaturated fatty acids. Which are also significant factors in clinical fields and livestock breeding. Saeki and colleagues in 2004 showed functional expression of a spinach (Spinacia oleracea) desaturase gene in transgenic pigs[4]. It was the first study wherein a plant gene was functionally expressed in transgenic animals[5]. In their study, the level of LA in white adipose tissue was increased by 20% in the fad2 transgenic pigs, but the level of arachidonic acid (20 : 4 n-6, AA) did not change[4]. In early 2005, we constructed a cotton (Gossypium hirsutum) fad2 expression vector (pFAD2) driven by alpha-actin promoter and produced the transgenic mice. Unfortunately, the transgenic mouse failed to express the exogenous gene[6]. In this study, the fad2 gene was constructed into the expression vector (pCMV-FAD2) driven by a CMV promoter and successfully produced

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transgenic mice expressing cotton FAD2 desaturase. The transgenic line endogenously synthesize not only LA but also AA in the tested tissues. This transgenic model may have value in the investigation of the mechanism of related diseases.

1 Materials and methods

1.1 Chemicals
All chemicals for manipulating the mouse embryo and PUFA Mix No. 2 (47.015-U) as the lipid analytical standard were purchased from Sigma/Aldrich (St. Louis, MO, USA). Restriction enzymes and Taq polymerase were purchased from Takara Biomedical (Dalian, China). DNase, Klenow enzyme, T4 ligase, oligo-dT primers and MMLV reverse transcriptase were purchased from Promega Incorporation (Madison, USA). DNA markers, TRIzol Reagent and TIANgel Midi Purification Kit were purchased from Tiangen Company (China).

1.2 Animals
Mice C57BL/6 and DBA/2 at 8 weeks of age were obtained from the Beijing Experimental Animal Center. Hybrid B6D2F1 mice were produced by the natural mating of C57BL/6 female and DBA2 males in our animal facility and used to produce the transgenic mice. Transgenic mice were backcrossed with C57BL/6 mice through at least four generations before PUFA analysis. All animals were raised with a diet deficient in omega-3 fatty acids and maintained in a light-controlled room (14L:10D, lights on at 06:00 AM) at a temperature of 22°C. All animal procedures in the present study were approved by the Committee for Experimental Animals of our university.

1.3 Construction of the expression vector
The vector pCMV-FAD2 was constructed by modifying a plasmid pFAD2 (6.3 kb) used in our previous study[6]. Briefly, the pFAD2 was digested by restriction enzymes Nhe I and Hind III to remove the 0.9 kb alpha-actin promoter, and then polished with a Klenow enzyme, ligating the vector using T4 ligase (Figure 1). The new plasmid was named pCMV-FAD2, which carried the fad2 cDNA driven by the CMV promoter and was confirmed by enzyme digestion and DNA sequencing. The 5.4-kb transgene was digested using the restriction enzyme Afl II, isolated by preparative electrophoresis on an agarose gel, purified using a TIANgel Midi Purification Kit, and dissolved in TE buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 8.0) at a final concentration of 5 μg/mL for DNA microinjection.

1.4 Generation of transgenic mice
B6D2F1 female mice, 6—8 weeks old, were i.p. injected with 5.0 IU pregnant mare’s serum gonadotropin (Hua-Fu Hi-tech Biological Company, Tianjin, China) followed by 5.0 IU human chorionic gonadotropin (hCG; Hua-Fu Hi-tech) 48 h later, and mated with B6D2F1 males of proven fertility. Pronuclear-stage embryos were collected from excised oviducts at 24-h post-hCG, freed from the cumulus by treatment with 300 IU/mL hyaluronidase (Sigma) in a modified CZB with 20 mmol/L

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**Figure 1**  Construction of the expression vector pCMV-FAD2. The vector pFAD2 (Left) driven by an alpha-actin promoter is digested with Nhe I and Hind III and re-ligate to construct the pCMV-FAD2 vector (Right).