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SDD17 desaturase can convert arachidonic acid to eicosapentaenoic acid in mammalian cells

Yonggang Chen, Min Zhang, Kemian Gou *

State Key Laboratory for Agrobiotechnology, College of Biological Sciences, China Agricultural University, Beijing 100193, PR China

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ABSTRACT

The possibility of elevating the omega-3 fatty acid contents in mammalian cells using the *sdd17* gene from *Saprolegnia diclina* was investigated in the current study. The nucleotide sequence of the *sdd17* gene was optimized and the pSDD17-IRES-GFP plasmid was introduced into murine 3T3 fibroblast cells by electroporation, following which its heterologous expression was evaluated by fatty acid analysis. Evaluation of GFP co-expression and RT-PCR analysis indicated that *sdd17* could be expressed at very high levels in mammalian cells. Total cellular lipid analysis of transformed cells fed with arachidonic acid (20:4 $n-6$) as a substrate showed that the *sdd17* expression resulted in an 82–155% ($p < 0.05$) increase in eicosapentaenoic acid (20:5 $n-3$) compared with the control. This expression also reduced the arachidonic acid/(eicosapentaenoic + docosapentaenoic + docosahexaenoic acid) ratio from approximately 4:1 in control cells to 1.5:1 in *sdd17*-transformed cells ($p < 0.05$). This study demonstrated that the foreign *sdd17* gene from EPA-rich fungus was expressed at a high efficiency and caused the omega-3 fatty acid contents in mammalian cells to be elevated. It also provided a basis for potential applications of this gene in animal transgenesis.

1. Introduction

Polyunsaturated fatty acids (PUFAs) play critical roles in mammalian infant growth, neural development, and immune function, but mammalian cells are unable to introduce double bonds at the $\omega-3$ ($n-3$) or $\omega-6$ ($n-6$) positions of the fatty acids for lack of the key desaturase enzymes. Therefore, the $n-3$ and $n-6$ classes must be provided in the diet. In general, n – 6 and $n-3$ series fatty acids are not interconvertible in the mammalian body and tend to have opposite biological effects. For the $n-3$ class, the basic form is α-linolenic acid (18:3 $n-3$, ALA), which can be inverted into a physiologically active forms containing at least 20 carbons, including eicosapentaenoic acid (20:5 $n-3$, EPA), docosapentaenoic acid (22:5 $n-3$, DPA), and docosahexaenoic acid (22:6 $n-3$, DHA). These forms are important in mammalian physiology as components of cell membrane phospholipids and precursors to the eicosanoid family of metabolites [1,2]. Deficiency of $n-3$ content in humans usually results in several neuronal specific defects or growth retardation [3,4].

Recently, a few transgenic animal models [5–9] that can synthesize endogenous PUFAs were successfully generated to allow for the study of *de novo* PUFAs metabolism and its effect on mammalian health. Among them, the *fat*-1 transgenic animals had been used widely and were emerging as a new tool for studying the molecular mechanisms of PUFAs action on diseases [10–18] and the benefits of $n-3$ fatty acids [5,9,19,20]. FAT-1 desaturase could efficiently convert $n-6$ to $n-3$ PUFAs in these animals, and the ratio of $n-6/n-3$ in various tissues dropped from 50–20 to almost 1 in transgenic mice [5] or from 21–10 to 11–1 in transgenic pigs [9]. It was demonstrated that the FAT-1 desaturase from *Caenorhabditis elegans* recognized a range of 18- and 20-carbon $n-6$ substrates by heterologous expression in *Arabidopsis* [21], mice [5], and pigs [9]. On the other hand, a novel gene from an EPA-rich fungus (*Saprolegnia diclina*), namely *sdd17*, had been identified. This gene encodes an omega-3 desaturase that is mainly involved in EPA production from its 20-carbon $n-6$ substrate (ARA), specifically by heterologous expression in *Saccharomyces cerevisiae* and somatic soya bean embryos [22]. Here we successful introduced the *sdd17* gene in mammalian cells and demonstrated that SDD17 harbored an $n-3$ desaturase activity for the conversion of the 20-carbon ARA substrate to EPA. Heterologous expression of SDD17 can be used to investigate the ability of the $n-3$ desaturases to recognize the 18- or 20-carbon $n-6$ substrates, and to specifically compare this ability with FAT-1 $n-3$ desaturases in the future. This work can also provide a basis for potential applications of this gene in animal transgenesis experiments or in models of human diseases caused by a lack of ARA.

* Corresponding author. Fax: +86 10 62731332.
E-mail address: goukm@cau.edu.cn (K. Gou).

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2. Materials and methods

2.1. Gene constructs

According to the nucleotide sequence of *S. diclina* (GenBank Accession No.: AY373823), the DNA sequence of *sdd17* was first optimized for efficient expression with high frequency codons in mouse, using the OptimumGene™ algorithm software (GenScript, USA). In addition, the Kozak consensus sequence was also added immediately upstream of the start codon to improve translational performance. The synthesized *sdd17* gene was then inserted into the pIRES-GFP shuttle vector (Clontech, USA) with an EcoRI digestion to generate the pSDD17-IRES-GFP vector, in which both *sdd17* and an enhanced green-fluorescent-protein (GFP) gene were combined with the internal ribosome entry site (IRES) sequence, all under the control of the cytomegalovirus (CMV) promoter. The shuttle vector contains a neomycin resistance gene to undergo a G418 selection. The construct was confirmed by enzymatic digestion and DNA sequencing.

2.2. Cell culture and transfection

NIH 3T3 cells were cultured in Dulbecco's modified Eagle's medium (Gibco, USA) containing 10% (v/v) new born calf serum (Gibco) at 37 °C under 5% CO_2 in air according to the standard procedure. The cell lines were routinely passaged using an enzymatic solution consisting of 0.05% (w/v) trypsin and 0.04% (w/v) EDTA for 2 min at 37 °C. Plasmids of the pSDD17-IRES-GFP or pIRES-GFP shutter vector as a control were introduced into the cells by electroporation, using a 4-mm Cuvette P/N 640 (BTX Co. Ltd., USA) according to the manufacturer's on-line protocol (No. PR0193). In brief, a total of 5×10^6 well-washed growing cells were resuspended in 400 μl electroporation buffer consisting of 150 mM sucrose and 27 mM Na_2HPO_4 (pH 7.5) followed by thorough mixing with 10 μg plasmid DNA. The cell transfections were then induced by four DC pulses of 1.20 kV/cm for 1.0 ms each (ECM2001, BTX). After the electric stimulus, the transformed cells were carefully transferred to the wells of a 24-well plate (Nunc) for further culture. After incubation for 24 h, the cells were selected by adding 0.6 mg/ml G418 (geneticin, GIBCO) in the culture media for 14 days. The EGFP-positive colonies were then isolated from the G418-resistant cells. Following withdrawal of G418, stable cell lines were routinely passaged and used for analysis of gene expression or fatty acid composition.

2.3. RT-PCR

Total RNAs were extracted from stably transformed cell lines transfected with pSDD17-IRES-GFP or pIRES-GFP vectors, using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. Prior to RT-PCR, the RNA was treated with DNase (TaKaRa). The purified RNA was used for first-strand cDNA synthesis and reverse transcription was performed using M-MLV reverse transcriptase with oligo-dT primers, according to the manufacturer's instructions (Promega, Madison, USA). Reactions in the absence of reverse transcriptase were also performed for each RNA sample tested, in order to check for genomic contamination. The resulting cDNA was used for PCR amplification with the primer pairs (anti-sense: 5′-tctcatcagtcgctcttgg-3′; sense: 5′-tgctgcctacg cttatct-3′) specific for the *sdd17* gene, that produced a 468-bp fragment. As a control, a 352-bp fragment of the *hprt* gene (sense: 5′-cctgctggattacattaaagcactg-3′; anti-sense: 5′-gtcaagggcatatccaacaa caaac-3′), was amplified at the same conditions. PCR amplification was performed as follows: 95 °C for 5 min; 30 cycles of 94 °C for 40 s, 60 °C for 40 s, 72 °C for 60 s, and a final extension at 72 °C for 8 min.

2.4. Fatty acid analysis

Prior to fatty acid analysis, each cell type at an initial concentration of 3×10^5/ml was cultured in a Nunc 100-mm dish and supplemented with 10 μM ARA (Sigma). After incubation for 48 h, the confluent cells were collected in a glass methylation tube for fatty acid analysis. Fatty acid composition of total cellular lipids was analyzed using gas chromatography as previously described by Kang and Wang [23]. Fatty acid methyl esters were quantified using a fully automated 6890 Network GC System (Agilent Technologies, USA) with an Agilent J&W fused-silica capillary column (DB-23). The peaks were identified by comparison with internal fatty acid standards (Sigma), and area percentage for all resolved peaks was analyzed using GC ChemStation software (Agilent Technologies).

2.5. Statistical analysis

All experiments were repeated at least four times and statistical analyses were performed using the SPSS software (version 13.0 for Windows). All values are presented as mean ± SD. Statistical significance in fatty acid composition was assessed by a Student's *t*-test. Differences of $p < 0.05$ were considered to be significant.

3. Results

3.1. Expression of the sdd17 gene in transformed 3T3 cells

To easily assess *sdd17* expression, the pSDD17-IRES-GFP plasmid was introduced into the murine 3T3 fibroblast cells, and the co-expression of GFP allowed us to identify the cells that expressed the *sdd17* gene. After G418 selection, we chose stable cell lines for further GFP examination and found that many cell lines exhibited bright fluorescence, indicating a high expression level of the transgene (Fig. 1). Among them, the expression of the *sdd17* transgene was also determined by RT-PCR analysis. As shown in Fig. 2, the transcription signals were detected successfully in all four examined lines (A–D) that were chosen at random from the GFP positive clones. The mRNA of *sdd17* was not visible in the control cells transfected with the pIRES-GFP plasmid (Fig. 2). These results indicate that *sdd17* can be expressed at a very high level in mammalian cells, which lack the gene naturally.

3.2. Omega-3 desaturase activity

We selected the *sdd17* transformant lines A–D randomly and tested whether the expression of the *sdd17* gene could lead to conversion of $n-6$ fatty acids to $n-3$ fatty acids thereby causing a change in fatty acid composition. ARA-fed cells were used to analyze the total cellular lipids by gas chromatograph analysis (Fig. 3). The results showed that the presence of *sdd17* resulted in an 82–155% increase in EPA among four transformed cell lines, significantly higher than that in control cells ($p < 0.05$, Table 1). On the contrary, the level of ARA substrate in cell line D was reduced by 27.6% ($p < 0.05$), and no significant decrease in ARA was detected in the other three lines. Additionally, there was a substantial reduction of the ARA/(EPA + DPA + DHA) ratio ($p < 0.05$) in all of the *sdd17* lines, compared to control cells (Table 1).

4. Discussion

In the EPA-rich fungus *Saprolegnia*, EPA is the sole $n-3$ PUFAs detected out of all the lipids [24,25], and the novel *sdd17* gene encoding the $n-3$ desaturase only recognizes a 20-carbon substrate. This gene can also convert exogenous ARA to EPA when

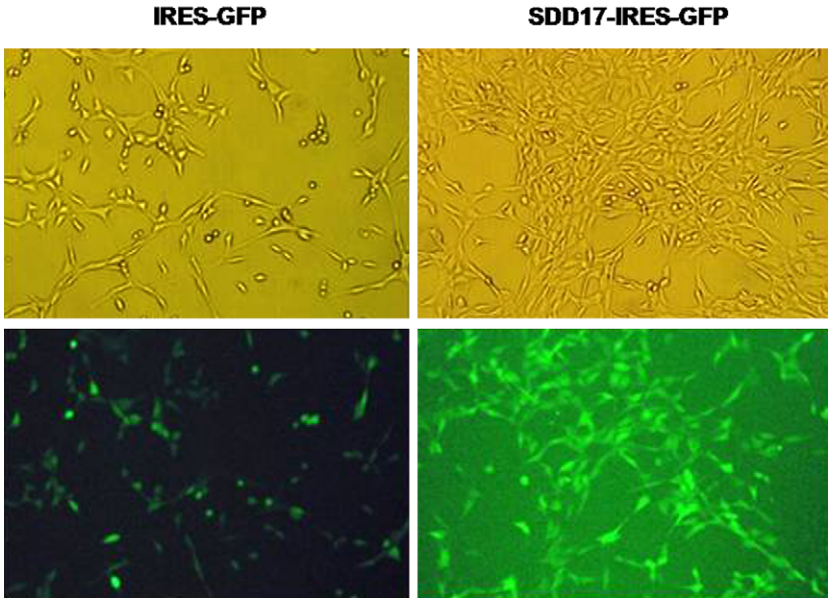

Fig. 1. Photographs showing transformed NIH 3T3 cells expressing GFP protein. Mouse 3T3 cells are transfected with pIRES-GFP (left, control) or pSDD17-IRES-GFP (right, line D). G418-resistant colonies showing green fluorescence at an initial time point (left) or after 48 h of culture (right) are visualized with bright light (upper) and blue light (lower). Visualization of GFP co-expression demonstrates that the *sdd17* gene is being expressed in transformed cells at a high efficiency.

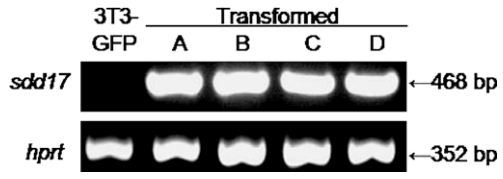

Fig. 2. RT-PCR analysis of *sdd17*-transformed 3T3 cells. Total RNA without DNA contamination from the different lines is used for analysis. The *sdd17*-specific fragments (468 bp) indicate that its transcription is detected in all transformed cell lines tested (A–D), but not in control cells transfected with pIRES-GFP. The *hprt* gene (352 bp) is analyzed concurrently as a control and shows normal expression in all examined samples.

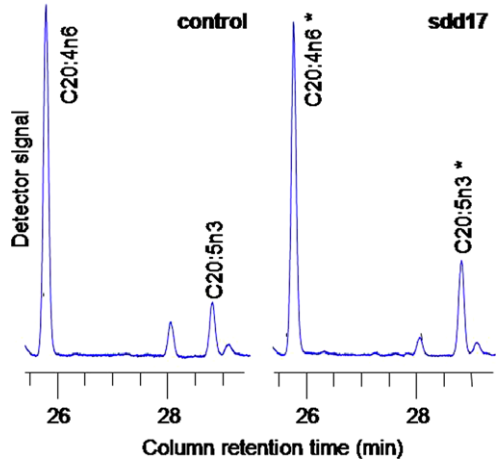

Fig. 3. Partial gas chromatogram traces showing fatty acid profiles of total cellular lipids extracted from *sdd17*-transformed 3T3 cells. Both the control (pIRES-GFP transformed) and *sdd17* line D (pSDD17-IRES-GFP transformed) cells are fed with 10 μM arachidonic acid for 48 h prior to fatty acid analysis. The lipid profiles show that the level of eicosapentaenoic acid (20:5 $n-3$) is markedly increased and the level of arachidonic acid (20:4 $n-6$) is significantly decreased in *sdd17* cells compared with the control cells (asterisks represent $p < 0.05$).

expressed in yeast and soya bean embryos [22]. In the current study, it was demonstrated that the codon-optimized *sdd17* gene could be functionally expressed in mammalian cells, which resulted in an increase in EPA and decrease in ARA in transformed cell lines. This increase led to a balanced ARA/(EPA + DPA + DHA) ratio that went from 4:1 in control cells to 1.5:1 in *sdd17* cells, as the EPA could be converted into DPA and DHA by the action of several specific elongases and desaturases that add additional carbons and double bonds in mammalian cells. On the contrary, no change of ALA (18:3 $n-3$) or its 18-carbon $n-6$ substrate (linoleic acid, LA) compositions were observed between the control and transformed cell lines. This suggested that the $n-3$ desaturase encoded by *sdd17* could also recognize the 20-carbon ARA substrate, and not the 18-carbon LA in mammalian cells. These results were consistent with the heterologous *sdd17* expression in baker's yeast and somatic soya bean embryos, in which the level of ALA did not increase despite the availability of large amounts of LA substrate, whereas approximately 25% and 50% of exogenous ARA was converted into EPA, respectively [22].

The mechanism of substrate specificity and regioselectivity of $n-3$ desaturases has not yet been characterized. Evidently, the SDD17 enzyme has special characteristics that differentiate it from the known $n-3$ desaturases of any plant [26–29] or microbe [30,31]. In these organisms, the $n-3$ desaturases exclusively desaturate the 18-carbon $n-6$ LA substrate to generate ALA fatty acid. The SDD17 enzyme is also different from the known $n-3$ desaturases of any fungi [32,33] or nematode [21], in which the $n-3$ desaturases recognize 18- to 20-carbon $n-6$ fatty acids and convert them to the corresponding $n-3$ PUFAs. Consistent with the results of the conversion of ARA to EPA in *sdd17* cells, the previous studies indicated that the characteristics of substrate specificity and regioselectivity of $n-3$ desaturases was not changed when they were heterologously expressed in mammalian cells. For instance, the *fad3* gene from scarlet flax mainly increased the level of ALA (18:3 $n-3$) in bovine adipocytes [34], and the *fat-1* gene from *C. elegans* resulted in an increase of almost all kinds of $n-3$ PUFAs, including the 18:3 $n-3$, 20:4 $n-3$, 20:5 $n-3$ and 22:5 $n-3$ compositions in rat cardiac myocytes [35] or cortical neurons [36], or the human lung [37] or breast [38] cancer cells.

Table 1
$n-6$ and $n-3$ fatty acid composition in 3T3 cells transfected with pIRES-GFP control and pSDD17-IRES-GFP.

Fatty acids	Control	*sdd17* lines			
		A	B	C	D
18:2 $n-6$	1.52 ± 0.17	1.92 ± 0.14	1.27 ± 0.23	1.77 ± 0.15	2.30 ± 0.40
20:2 $n-6$	1.22 ± 0.53	1.93 ± 0.05	1.11 ± 0.96	2.51 ± 1.05	1.32 ± 0.78
20:4 $n-6$ (ARA)	11.69 ± 1.58	12.96 ± 2.06	10.40 ± 0.45	11.67 ± 0.41	8.46 ± 1.80*
22:4 $n-6$	1.87 ± 0.20	1.67 ± 0.29	1.72 ± 0.21	1.48 ± 0.46	1.22 ± 0.17*
18:3 $n-3$	0.20 ± 0.07	ND	ND	0.16 ± 0.01	0.25 ± 0.07
20:5 $n-3$ (EPA)	1.11 ± 0.09	2.02 ± 0.12**	2.10 ± 0.06**	2.83 ± 0.55*	2.26 ± 0.40*
22:5 $n-3$ (DPA)	1.66 ± 0.58	2.10 ± 0.26	1.91 ± 0.29	1.82 ± 0.13	3.40 ± 1.28
22:6 $n-3$ (DHA)	0.47 ± 0.09	0.61 ± 0.04	0.51 ± 0.08	0.59 ± 0.14	0.47 ± 0.05
ARA/(EPA + DPA + DHA)	4.05 ± 0.33	2.82 ± 0.67*	2.17 ± 0.79*	2.30 ± 0.70**	1.49 ± 0.49**

Fatty acid composition is presented as a percentage of the total cellular lipids from the control (pIRES-GFP transformed) and *sdd17* (pSDD17-IRES-GFP transformed) cell lines A–D. Each value represents the mean ± standard deviation from four independent measurements of each cell line. Statistical significance in fatty acid composition is assessed by Student's *t*-test. $^{*}p < 0.05$, $^{**}p < 0.01$. ND, not detectable.

That is, the mechanism of substrate specificity and regioselectivity of $n-3$ desaturases could be determined by domain swapping using chimeric DNA with *sdd17* and other $n-3$ genes, as described in the previous study [39].

Furthermore, our results also provide a basis for potential applications of this gene in animal transgenesis to produce livestock that can synthesize essential $n-3$ PUFAs, or in animal models to study syndromes caused by lack of ARA.

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References

[1] C.D. Funk, Prostaglandins and leukotrienes: advances in eicosanoid biology, Science 294 (2001) 1871–1875.
[2] D. Hwang, Fatty acids and immune responses—a new perspective in searching for clues to mechanism, Annu. Rev. Nutr. 20 (2000) 431–456.
[3] A.P. Simopoulos, The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases, Exp. Biol. Med. (Maywood) 233 (2008) 674–688.
[4] J.R. Marszalek, H.F. Lodish, Docosahexaenoic acid, fatty acid-interacting proteins, and neuronal function: breast milk and fish are good for you, Annu. Rev. Cell Dev. Biol. 21 (2005) 633–657.
[5] J.X. Kang, J. Wang, L. Wu, Z.B. Kang, Transgenic mice: fat-1 mice convert $n-6$ to $n-3$ fatty acids, Nature 427 (2004) 504.
[6] K. Saeki, K. Matsumoto, M. Kinoshita, I. Suzuki, Y. Tasaka, K. Kano, Y. Taguchi, K. Mikami, M. Hirabayashi, N. Kashiwazaki, Y. Hosoi, N. Murata, A. Iritani, Functional expression of a Delta12 fatty acid desaturase gene from spinach in transgenic pigs, Proc. Natl. Acad. Sci. USA 101 (2004) 6361–6366.
[7] G. Zhu, H. Chen, X. Wu, Y. Zhou, J. Lu, H. Chen, J. Deng, A modified $n-3$ fatty acid desaturase gene from *Caenorhabditis briggsae* produced high proportion of DHA and DPA in transgenic mice, Transgenic Res. 17 (2008) 717–725.
[8] Q. Chen, Q. Liu, Z. Wu, Z. Wang, K. Gou, Generation of fad2 transgenic mice that produce omega-6 fatty acids, Sci. China C: Life Sci. 52 (2009) 1048–1054.
[9] L. Lai, J.X. Kang, R. Li, J. Wang, W.T. Witt, H.Y. Yong, Y. Hao, D.M. Wax, C.N. Murphy, A. Rieke, M. Samuel, M.L. Linville, S.W. Korte, R.W. Evans, T.E. Starzl, R.S. Prather, Y. Dai, Generation of cloned transgenic pigs rich in omega-3 fatty acids, Nat. Biotechnol. 24 (2006) 435–436.
[10] C.A. Hudert, K.H. Weylandt, Y. Lu, J. Wang, S. Hong, A. Dignass, C.N. Serhan, J.X. Kang, Transgenic mice rich in endogenous omega-3 fatty acids are protected from colitis, Proc. Natl. Acad. Sci. USA 103 (2006) 11276–11281.
[11] S. Xia, Y. Lu, J. Wang, C. He, S. Hong, C.N. Serhan, J.X. Kang, Melanoma growth is reduced in fat-1 transgenic mice: impact of omega-6/omega-3 essential fatty acids, Proc. Natl. Acad. Sci. USA 103 (2006) 12499–12504.
[12] A. Bhattacharya, B. Chandrasekar, M.M. Rahman, J. Banu, J.X. Kang, G. Fernandes, Inhibition of inflammatory response in transgenic fat-1 mice on a calorie-restricted diet, Biochem. Biophys. Res. Commun. 349 (2006) 925–930.
[13] C. Schmocker, K.H. Weylandt, L. Kahlke, J. Wang, H. Lobeck, G. Tiegs, T. Berg, J.X. Kang, Omega-3 fatty acids alleviate chemically induced acute hepatitis by suppression of cytokines, Hepatology 45 (2007) 864–869.
[14] J. Nowak, K.H. Weylandt, P. Habbel, J. Wang, A. Dignass, J.N. Glickman, J.X. Kang, Colitis-associated colon tumorigenesis is suppressed in transgenic mice rich in endogenous $n-3$ fatty acids, Carcinogenesis 28 (2007) 1991–1995.
[15] Q. Jia, J.R. Lupton, R. Smith, B.R. Weeks, E. Callaway, L.A. Davidson, W. Kim, Y.Y. Fan, P. Yang, R.A. Newman, J.X. Kang, D.N. McMurray, R.S. Chapkin, Reduced colitis-associated colon cancer in Fat-1 ($n-3$ fatty acid desaturase) transgenic mice, Cancer Res. 68 (2008) 3985–3991.
[16] K.H. Weylandt, A. Nadolny, L. Kahlke, T. Kohnke, C. Schmocker, J. Wang, G.Y. Lauwers, J.N. Glickman, J.X. Kang, Reduction of inflammation and chronic tissue damage by omega-3 fatty acids in fat-1 transgenic mice with pancreatitis, Biochim. Biophys. Acta 1782 (2008) 634–641.
[17] A.Y. Taha, P.S. Huot, S. Reza-Lopez, N.R. Prayitno, J.X. Kang, W.M. Burnham, D.W. Ma, Seizure resistance in fat-1 transgenic mice endogenously synthesizing high levels of omega-3 polyunsaturated fatty acids, J. Neurochem. 105 (2008) 380–388.
[18] K. Mayer, A. Kiessling, J. Ott, M.B. Schaefer, M. Hecker, I. Henneke, R. Schulz, A. Gunther, J. Wang, L. Wu, J. Roth, W. Seeger, J.X. Kang, Acute lung injury is reduced in fat-1 mice endogenously synthesizing $n-3$ fatty acids, Am. J. Respir. Crit. Care Med. 179 (2009) 474–483.
[19] D.W. Ma, V. Ngo, P.S. Huot, J.X. Kang, $N-3$ polyunsaturated fatty acids endogenously synthesized in fat-1 mice are enriched in the mammary gland, Lipids 41 (2006) 35–39.
[20] B.T. Kao, K.A. Lewis, E.J. DePeters, A.L. Van Eenennaam, Endogenous production and elevated levels of long-chain $n-3$ fatty acids in the milk of transgenic mice, J. Dairy Sci. 89 (2006) 3195–3201.
[21] J.P. Spychalla, A.J. Kinney, J. Browse, Identification of an animal omega-3 fatty acid desaturase by heterologous expression in *Arabidopsis*, Proc. Natl. Acad. Sci. USA 94 (1997) 1142–1147.
[22] S.L. Pereira, Y.S. Huang, E.G. Bobik, A.J. Kinney, K.L. Stecca, J.C. Packer, P. Mukerji, A novel omega3-fatty acid desaturase involved in the biosynthesis of eicosapentaenoic acid, Biochem. J. 378 (2004) 665–671.
[23] J.X. Kang, J. Wang, A simplified method for analysis of polyunsaturated fatty acids, BMC Biochem. 6 (2005) 5.
[24] A. Kendrick, C. Ratledge, Lipids of selected molds grown for production of $n-3$ and $n-6$ polyunsaturated fatty acids, Lipids 27 (1992) 15–20.
[25] J.L. Gellerman, H. Schlenk, Methyl-directed desaturation of arachidonic to eicosapentaenoic acid in the fungus, *Saprolegnia parasitica*, Biochim. Biophys. Acta 573 (1979) 23–30.
[26] J. Browse, M. McConn, D. James Jr., M. Miquel, Mutants of *Arabidopsis* deficient in the synthesis of alpha-linolenate. Biochemical and genetic characterization of the endoplasmic reticulum linoleoyl desaturase, J. Biol. Chem. 268 (1993) 16345–16351.
[27] T. Flores, O. Karpova, X. Su, P. Zeng, K. Bilyeu, D.A. Sleper, H.T. Nguyen, Z.J. Zhang, Silencing of GmFAD3 gene by siRNA leads to low alpha-linolenic acids (18:3) of fad3-mutant phenotype in soybean [Glycine max (Merr.)], Transgenic Res. 17 (2008) 839–850.
[28] T. Nishiuchi, M. Nishimura, V. Arondel, K. Iba, Genomic nucleotide sequence of a gene encoding a microsomal [omega]-3 fatty acid desaturase from *Arabidopsis thaliana*, Plant Physiol. 105 (1994) 767–768.
[29] H. Kodama, H. Akagi, K. Kusumi, T. Fujimura, K. Iba, Structure, chromosomal location and expression of a rice gene encoding the microsome omega-3 fatty acid desaturase, Plant Mol. Biol. 33 (1997) 493–502.
[30] T. Sakamoto, D.A. Los, S. Higashi, H. Wada, I. Nishida, M. Ohmori, N. Murata, Cloning of omega 3 desaturase from cyanobacteria and its use in altering the degree of membrane-lipid unsaturation, Plant Mol. Biol. 26 (1994) 249–263.
[31] K. Suga, K. Honjoh, N. Furuya, H. Shimizu, K. Nishi, F. Shinohara, Y. Hirabaru, I. Maruyama, T. Miyamoto, S. Hatano, M. Iio, Two low-temperature-inducible Chlorella genes for delta12 and omega-3 fatty acid desaturase (FAD): isolation of delta12 and omega-3 fad cDNA clones, expression of delta12 fad in *Saccharomyces cerevisiae*, and expression of omega-3 fad in *Nicotiana tabacum*, Biosci. Biotechnol. Biochem. 66 (2002) 1314–1327.
[32] T. Oura, S. Kajiwara, Substrate specificity and regioselectivity of delta12 and omega3 fatty acid desaturases from *Saccharomyces kluyveri*, Biosci. Biotechnol. Biochem. 72 (2008) 3174–3179.

[33] H.G. Damude, H. Zhang, L. Farrall, K.G. Ripp, J.F. Tomb, D. Hollerbach, N.S. Yadav, Identification of bifunctional delta12/omega3 fatty acid desaturases for improving the ratio of omega3 to omega6 fatty acids in microbes and plants, Proc. Natl. Acad. Sci. USA 103 (2006) 9446–9451.
[34] Y. Indo, A. Tatemizo, Y. Abe, I. Suzuki, K. Matsumoto, Y. Hosoi, M. Kinoshita, K. Mikami, N. Murata, A. Iritani, K. Saeki, Functional expression of a humanized gene for an omega-3 fatty acid desaturase from scarlet flax in transfected bovine adipocytes and bovine embryos cloned from the cells, Biochim. Biophys. Acta 1791 (2009) 183–190.
[35] Z.B. Kang, Y. Ge, Z. Chen, J. Cluette-Brown, M. Laposata, A. Leaf, J.X. Kang, Adenoviral gene transfer of *Caenorhabditis elegans* $n-3$ fatty acid desaturase optimizes fatty acid composition in mammalian cells, Proc. Natl. Acad. Sci. USA 98 (2001) 4050–4054.
[36] Y. Ge, X. Wang, Z. Chen, N. Landman, E.H. Lo, J.X. Kang, Gene transfer of the *Caenorhabditis elegans* $n-3$ fatty acid desaturase inhibits neuronal apoptosis, J. Neurochem. 82 (2002) 1360–1366.
[37] S.H. Xia, J. Wang, J.X. Kang, Decreased $n-6/n-3$ fatty acid ratio reduces the invasive potential of human lung cancer cells by downregulation of cell adhesion/invasion-related genes, Carcinogenesis 26 (2005) 779–784.
[38] Y. Ge, Z. Chen, Z.B. Kang, J. Cluette-Brown, M. Laposata, J.X. Kang, Effects of adenoviral gene transfer of *C. elegans* $n-3$ fatty acid desaturase on the lipid profile and growth of human breast cancer cells, Anticancer Res. 22 (2002) 537–543.
[39] R.J. Sasata, D.W. Reed, M.C. Loewen, P.S. Covello, Domain swapping localizes the structural determinants of regioselectivity in membrane-bound fatty acid desaturases of *Caenorhabditis elegans*, J. Biol. Chem. 279 (2004) 39296–39302.