Generation of monoclonal antibodies against n-3 fatty acid desaturase

Dear Editor:

Omega-3 polyunsaturated fatty acids (n-3 PUFAs) are essential fatty acids for normal cellular functions and have been used for prevention and treatment of many diseases, including coronary heart disease, diabetes, and cancers.\(^{[1-3]}\) n-3 PUFAs and n-6 PUFAs have been shown to decrease and increase the severity of several human diseases, respectively.\(^{[4]}\) Unfortunately, mammals have no enzymes to synthesize n-6 and n-3 fatty acids. Therefore, they must rely on a dietary supply. Today, the ratio of n-6 to n-3 PUFAs has reached to an unhealthy 20-30:1 due to people taking n-6 rich diet and eating less sea fish. *Caenorhabditis (C.) elegans* fat-1 gene encodes an n-3 desaturase that introduces a double bond at n-3 position of n-6 fatty acids to form n-3 fatty acids.\(^{[5]}\) Several *fat-1* transgenic mammals (including mouse, pig and cow) have been produced in which n-3 PUFAs were increased and n-6 PUFAs were reduced, resulting in a significant reduction of n-6/n-3 ratio in those transgenic animals.\(^{[6-8]}\)

However, due to lack of commercially available FAT-1 antibody, FAT-1 expression is indirectly determined by measurement of FAT-1 activity including increase of n-3 PUFAs and decrease of n-6/n-3 ratio in animals. In this study, we used recombinant FAT-1 to immunize mice and to generate FAT-1 specific monoclonal antibody. Then, FAT-1 monoclonal antibodies were used to detect FAT-1 protein in tissue samples of the liver, lung, kidney, heart, brain, and spleen of *fat-1* transgenic mice by Western blotting and immunohistochemical staining assays.

*Escherichia (E.) coli* expression system allows rapid and economical production of recombinant FAT-1 protein. To express *C. elegans fat-1* gene in *E. coli* efficiently, we optimized the codons of the full-length *C. elegans fat-1* gene sequence using GeneArt® Gene Synthesis (GeneArt, Regensburg, Germany) followed by subcloning into the expression vector pCold II. The recombinant FAT-1 protein was purified by HisTrap FF affinity chromatography col-

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**Fig. 1 Preparation of FAT-1 monoclonal antibodies.** A: The rFAT-1 expression in transfected bacteria. SDS-PAGE pattern after Coomassie Brilliant Blue G-250 staining of the transfected bacterial cell pellets (lane 1), cell lysate supernatant (lane 2) and the purified rFAT-1 protein (lane 3). The purification of FAT-1 mAb. SDS-PAGE pattern after Coomassie Brilliant Blue G-250 staining of the purified 3A11 mAb concentrations displayed two protein species, the upper band was heavy chain and the lower band was light chain. The titers of the FAT-1 mAb. The titers of the FAT-1 mAb 3A11 was tested using ELISA reactivity and pepck mAb was used as a negative control. Data represent the average absorbance at 450 nm detected by rFAT-1 protein at 0.05, 0.20, 0.5, 5.0, 20.0, 50.0, 100.0 μg/mL, the sensitivity of the mAb was 0.05 μg/mL.
umn (GE Healthcare Life Sciences, Piscataway, NJ, USA) and analyzed by SDS-PAGE and Coomassie brilliant blue staining (Fig. 1A), which showed a protein band of approximately 46 kDa in size, corresponding to the known molecular weight of FAT-1\(^5\). The identity of the FAT-1 recombinant protein was further confirmed by searching sequence databases using mass spectrometry data. Recombinant FAT-1 was used to generate FAT-1 monoclonal antibodies and after three rounds of subcloning, the subclone 3A11 showed the best titer. Coomassie brilliant blue staining revealed the presence of two protein bands 55 kDa and 26 kDa in size, respectively (Fig. 1B). Enzyme-linked immunosorbent assay (ELISA) further showed that the FAT-1 monoclonal antibodies (3A11) between 0.05 and 100 \(\mu\)g/mL exhibited a linear increase in OD\(_{450}\) (Fig. 1C). Further analysis revealed that the antibodies were IgG2a.

Immunoblotting assays using FAT-1 monoclonal antibodies (3A11) demonstrated a protein band of **Fig. 2** Function of FAT-1 monoclonal antibodies. A: Detection of the specificity of FAT-1 mAb by Western blotting. Western blotting was used to analyze binding of FAT-1 mAb 3A11 to denatured FAT-1 in tissues at reducing conditions. Note that the mAb clone binds with two protein species with molecular masses of about 46 kD and 26 kD, the full-length FAT-1 protein and its degradation products, respectively. B-O: Determination of FAT-1 mAb’s effectiveness by Immunohistochemical stains (Magnification×100). Immunohistochemical stains shows the protein of FAT-1 detected in liver tissue (B), alveolus cells (D), glomeruli (F), renal tubulointerstitial cells (H), myocardial cell (J), neurogliocyte, neuron (L) and, splenocyte (N). The normal C57BL/6 tissues yielded some weakly positive staining, especially in the liver tissue (C), alveolus cells (E) and myocardial cell (K).
approximately 46 kDa in size from the homogenates of tissue specimens of the liver, lung, kidney, heart, brain, and spleen of mfat-1 transgenic mice, but not from those of normal C57BL/6 mice (Fig. 2A). A protein band approximately 26 kDa in size was also detected, which may be FAT-1 degradation products. FAT-1 is rich in cysteine and has three successive cysteines at positions 241-243. As the two disulfide bonds can be broken under denaturing conditions, the stability of peptide bond may be affected subsequently. Immunohistochemical staining of tissue specimens of transgenic and normal C57BL/6 mice further demonstrated that FAT-1 protein was present in many tissues of the transgenic mice, but was not found in the wild-type control C57BL/6 tissues (Fig. 2B–O).

The expression of fat-1 gene or FAT-1 in transgenic tissues or cells can be examined previously only by reverse transcription-polymerase chain reaction or by gas chromatography to measure the increase of n-3 PUFAs and the decrease of n-6/n-3 ratios. The generation of FAT-1 monoclonal antibodies provides a new tool to detect FAT-1 protein directly.

This work was supported by grants from the National Natural Science Foundation of China (81202370) and Jiangsu Key Laboratory of Xenotransplantation (BM2012116). Yifan Dai is a Fellow at the Collaborative Innovation Center For Cardiovascular Disease Translational Medicine.

Yours Sincerely,
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