Loss of monoacylglycerol O-acyltransferase 2 can be compensated for by diacylglycerol O-acyltransferases 1 and 2 resulting in a negligible influence on mammary cancer development found in a mouse model and verified in human tissues

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Abstract – Background: Dietary fat absorption involves the re-esterification of digested triacylglycerol in the enterocytes, it is a biological process catalyzed by monoacylglycerol O-acyltransferase 2 (MOGAT2, aka MGAT2), which is highly expressed in the small intestine. A previous study showed that the loss of the Mogat2 gene can prevent high-fat diet-induced obesity in mice. Obesity is associated with an increased risk of several types of cancer including a postmenopausal mammary tumor. Methods: We collected 147 patients with triple-negative breast adenocarcinoma to explore the relationship between MOGAT2 expression and overall patient survival. The TCGA data were also retrieved for analyzing the prognostic values of MOGAT2 mRNA level as well as the relationships between MOGAT2 and DGAT1/2 mRNA levels. We also used a Mogat2-deficient mouse mammary tumor model by crossing Mogat2-deficient mice with MMTV-PyMT mice to examine the effect of MOGAT2 on mammary tumor development. Results: In human triple-negative breast adenocarcinoma, elevated expression of MOGAT2 correlated with a poorer patient prognosis. Obesity could be induced by a relatively high-fat diet (37% of calories from fat) in the mice with or without Mogat2 knockout. Mammary tumor development was deteriorated by a relatively high-fat diet regardless of Mogat2 deficiency. As a compensation mechanism, upregulation of diacylglycerol O-acyltransferases 1 and 2 (Dgat1 and Dgat2) in the Mogat2 deficient mice was found. Consistently, in human normal tissues adjacent to breast cancer, an inverse correlation between MOGAT2 mRNA level and DGAT1/2 mRNA levels was also found. Conclusions: Elevated expression of MOGAT2 in triple-negative breast adenocarcinoma predicts poorer patient overall survival. With the compensation of Dgat1 and Dgat2, Mogat2 deficiency alone cannot prevent fat diet-induced obesity, nor prevent mammary tumor development in a mouse model.

Key words: Mogat2 depletion, MMTV-PyMT, Mammary tumor, Dgat1, Dgat2.

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Methods

**Background**

A mammary tumor is the most commonly diagnosed cancer and the second most common cause of cancer-induced death in women worldwide [1]. Over 600,000 premenopausal and 1.4 million postmenopausal mammary tumor cases were diagnosed around the world in 2018 [2]. Relatedly, obesity poses an increased risk of the development of a mammary tumor, particularly in postmenopausal women [1]. Equally, sustained weight loss can significantly reduce mammary tumor risk [3]. However, the impact of genetically preventing obesity on mammary tumor risk is unclear.

Triacylglycerols, the bulk of dietary fat, are absorbed in the intestine and readily stored as body fat [4]. The hydrolysis of triacylglycerols to 2-monocacylglycerol and fatty acids is primarily catalyzed by pancreatic lipase in the intestinal lumen, where these hydrolysis products are taken up by enterocytes and resynthesized into triacylglycerols via the monoacylglycerol pathway [5]. The monoacylglycerol pathway is a process catalyzed by the acyl-CoA: monoacylglycerol acyltransferase (MOGAT) family which includes MOGAT1, MOGAT2, and MOGAT3, and accounts for 70–80% of triglyceride re-synthesis in the intestines [6, 7]. Another pathway involved in triglyceride synthesis is the glycerol 3-phosphate pathway [8]. Among the three MOGAT enzymes, only MOGAT2 (also known as MGAT2) is highly expressed in the intestine of both mice and humans [9–12].

It has been reported that a deficiency in MOGAT2 protects mice from obesity and associated metabolic disorders induced by a high-fat diet [13, 14]. MOGAT2 deficiency also protects genetically obese Agouti mice from excess weight gain when on a chow diet [4]. Diacylglycerol (DAG) is usually acylated by the acyl-coenzyme A: diacylglycerol acyltransferase (DGAT) to re-synthesize triacylglycerol (TAG). Importantly, the enzymes DGAT1 [15] and DGAT2 have been shown to acylate MAG to form DAG, and MOGAT2 and MOGAT3 can acylate DAG to form TAG [16, 17].

To elucidate the role of MOGAT2 in mammary tumor progression, especially under obese conditions, we evaluated the prognostic value of MOGAT2 expression in triple-negative breast adenocarcinoma and generated a MOGAT2-deficient mouse mammary tumor model by crossing MOGAT2-deficient mice with MMTV-PyMT mice.

**Abbreviations**

MOGAT2 monoacylglycerol O-acyltransferase 2;
Dgat1 diacylglycerol O-acyltransferases 1;
Dgat2 diacylglycerol O-acyltransferases 2;
MOGAT monoacylglycerol acyltransferase;
DAG diacylglycerol;
DGAT diacylglycerol acyltransferase;
TAG re-synthesize triacylglycerol.

**Methods**

**Mice and animal care**

FVB/N-Tg (MMTV-PyMT) 634Mul mice and B6.129S4-Mogat2<sup>tm1Far/J</sup> (MOGAT2<sup>–/–</sup>) were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). Mice were housed under specific pathogen-free conditions in the Animal Center of Guangdong Pharmaceutical University. All animal experiments were performed in accordance with institutional guidelines and were approved by Sun Yat-sen University Cancer Center’s Institutional Animal Care and Usage Committee, with the ethical approval number: L102012017000G. The control group was fed a normal diet with 12% kcal from fat. The experimental group was fed a relatively high-fat diet with 37% kcal from fat. The normal diet and the relatively high-fat diet were purchased from the Experimental Medicine Animals Center (Foshan, China).

MMTV-PyMT (PyMT) male animals were bred with Mogat2<sup>–/–</sup> females to produce Mogat2<sup>+/–</sup>PyMT male and Mogat2<sup>+/+</sup> female F1 hybrids. F1 Mogat2<sup>+/+</sup>PyMT male mice were then mated with Mogat2<sup>+/–</sup> female mice to produce F2 Mogat2<sup>+/+</sup>PyMT and Mogat2<sup>–/–</sup>PyMT female mice (Supplementary Fig. 1b, 1c).

The forward and the reverse primers used for mouse genotyping are as follows: Mogat2<sup>+/+</sup> (Forward common 5′–CCGT TTA GCC TGG TCT AGG CAG AG–3′. Reverse wildtype 5′–CAG CAA AGC CCC CTC CTG AAT CTC TC–3′) and Reverse mut 5′–CGT TGA CTC TAG AGG ATC CGA C–3′. MMTV-PyMT (5′–GGA AGC AAC TAC TTC ACA AGG G–3′ and 5′–GGA AAG TCA CTA GGA GCA GGA GGC–3′).

**Histological examination of murine tissues**

Whole-mount carmine alum staining was performed according to the standard protocol [18]. The inguinal mammary glands from 5-week-old F2 Mogat2<sup>+/+</sup>PyMT and Mogat2<sup>–/–</sup>PyMT female mice were fixed overnight in Carnoy’s solution (75% absolute ethanol [EtOH] and 25% glacial acetic acid [Sigma-Aldrich]). Following fixation, the glands were washed with 70% ethanol for 30 min gradually rinsed in water, and stained with carmine alum (Sigma-Aldrich) for at least two days. To examine the lung metastasis foci, tissues were fixed in Bouin’s solution (Sigma-Aldrich) for at least two days, and the number of metastatic lesions larger than 0.5 mm in diameter was counted macroscopically.

**RT-PCR**

At 98 days old, the F2 Mogat2<sup>+/+</sup>PyMT and Mogat2<sup>–/–</sup>PyMT female mice were euthanized for tissue harvesting. Mammary tumors, stomach, small intestine, and colorectal tissues were collected and stored in RNAstore reagent (TIANGEN) for RNA isolation.

Total RNA was extracted from mice tissues using Trizol reagent (Invitrogen, CA, USA) and subjected to reverse transcription using a PrimeScript™ II 1st Strand cDNA Synthesis Kit (Takara), followed by reactions using the Roche Light Cycler 480 System (Roche). Beta-actin served as the normalization genes for these experiments. The relative expression levels of the target genes were calculated using the 2–△△Ct method. The qPCR primers were obtained from PrimerBank [19].

**Human tissue specimens**

A total of 147 paraffin-embedded triple-negative primary mammary tumor tissues, diagnosed between 2005 and 2009,
Primary tumor MOGAT2 protein level negatively correlates with poorer mammary tumor patient survival. (a) Eight tissue arrays consisting of 147 triple-negative breast cancer samples were constructed and immunohistochemically stained with a MOGAT2 antibody, followed by an assessment including the percentage of positively stained tumor cells and the intensity of staining on a 13-point scale. (b) Survival analyses showed that in the group with an elevated MOGAT2 protein level in the tumor, breast cancer patients had a poorer overall survival (OS) probability. (c) The analyses of TCGA data showed MOGAT2 mRNA level was reduced along with the triple-negative breast adenocarcinoma progressed from early clinical stages to late stages. (d) Survival analyses of TCGA data showed that in the group with an elevated MOGAT2 RNA level in the tumor, TNBC patients had a poorer OS (optimized cut-off values) and DFS (median value as cut-off value).
were retrieved from the Department of Pathology at Sun Yat-sen University Cancer Center (SYSUCC). All human tissue samples were obtained with patient consent and the approval of the Institutional Clinical Ethics Review Board at SYSUCC. The median follow-up time for all patients was 102 months.

The IHC scores for MOGAT2 (Cat#ab62526, Abcam) in the mammary tumor tissues were calculated by two independent pathologists. For each tumor, two scores were given. One for the percentage of positively stained tumor cells, and another for staining intensity. The percentage of positive cells was categorized as no staining = 0, 1–10% of stained cells = 1, 11–50% = 2, 51–80% = 3, and 81–100% = 4. Cytoplasmic staining intensity was categorized as no staining = 0, weak staining = 1, moderate staining = 2, and strong staining = 3. The proportion and intensity were then multiplied to produce a total score ranging from 0 to 12. A cut-off value of 4 was the optimal balance between sensitivity and specificity with the patients divided into high-level (n = 51) and low-level (n = 96) groups.

**TCGA analysis**

The GEPIA2 (gepia2.cancer-pku.cn) is used to analyze and visualize the TCGA data. Differential expression in different types or stages of breast cancer was evaluated using the “Expression DIY” tool. Survival analyses of the breast cancer patients divided into high- and low-MOGAT2 mRNA groups were visualized in “Survival Analysis” module. The correlation analysis between MOGAT2 and DGAT1/2 were generated by using “Correlation” function in GEPIA2.

**Statistical analysis**

Statistical analyses were performed using GraphPad Prism v7.0 software and the data presented as mean ± SEM. The correlations between MOGAT2 expression and overall survival, mice lacking palpable tumors, and the mice’s age were analyzed with Kaplan–Meier survival curves and the log-rank test. The differences between the groups were compared using the Student’s T-test, and ANOVA for multiple comparisons. P-values were used to denote statistical significance.

**Results**

**Elevated MOGAT2 expression predicts poorer prognosis in mammary tumor patients**

IHC staining showed that MOGAT2 was localized in the cytoplasm of mammary tumor cells (Figure 1a). Survival analysis revealed that the elevated MOGAT2 protein level correlated with poorer overall survival (OS) in patients with triple-negative breast adenocarcinoma (Figure 1b). The TCGA data analyses showed that MOGAT2 mRNA level was decreasing along with the progression of the tumor from stages 1-2 to stages 3-4 (Figure 1c). And MOGAT2 mRNA level correlated with poorer overall survival and disease-free survival (DFS) in patients with triple-negative breast adenocarcinoma (Figure 1d).

**A relatively high-fat diet promotes obesity regardless of MOGAT2 deficiency**

Previous studies [13, 14, 20] reported that MOGAT2 ablation protected mice from high-fat diet-induced obesity (60% of calories from fat). In our study, in the mice fed with a relatively high-fat diet (37% of calories from fat) between 5 and 12 weeks old, obesity was observed regardless of MOGAT2 deficiency (Figure 2).

**MOGAT2 depletion does not inhibit mammary tumorigenesis or metastasis**

To determine whether loss of MOGAT2 in MMTV-PyMT mice affects the development of multifocal dysplastic lesions, we collected the right fourth (inguinal) mammary gland from virgin female Mogat2+/+PyMT and Mogat2−/−PyMT mice at exactly 35 days old, fixed these tissues in Carnoy’s solution, and stained them with carmine dye for at least two days. At 35 days old, Mogat2+/+PyMT and Mogat2−/−PyMT mice both exhibited small hyperplastic focal lesions in the older portions of the mammary ductal tree (Figure 3a). However, there was no significant difference in the frequency or size of these benign lesions in the absence of MOGAT2.

The effect of MOGAT2 ablation on tumor onset was monitored by weekly palpation as shown in Figure 3b. No statistically significant difference was observed between the two groups. All female Mogat2+/+PyMT and Mogat2−/−PyMT mice developed mammary tumors within 77 days.

To assess MOGAT2’s depletion effect on mammary tumor progression, the number of tumor-affected mammary glands and total tumor volume were monitored by weekly palpation. The results showed that there was no statistically significant difference between the two groups (Figures 4a and 4b).
Figure 3. Mammary tumor initiation is not delayed by Mogat2 gene depletion. (a) Whole mount images of the fourth pair of mammary glands from Mogat2^{+/+}PyMT and Mogat2^{−/−}PyMT mice at 5 weeks old. LN = lymph node. (b) Kaplan Meier plots of the tumor latency of Mogat2^{+/+}PyMT (n = 19) and Mogat2^{−/−}PyMT (n = 21) mice. Data were analyzed by the log-rank test. All mice were fed a normal diet (10% of calories from fat).

Figure 4. Mammary tumor progression is not inhibited by Mogat2 gene depletion. (a) The number of mammary glands with palpable tumors for Mogat2^{+/+}PyMT (n = 19) and Mogat2^{−/−}PyMT (n = 19) mice. Data shown are the number of tumor-bearing glands per mouse. Mammary tumors were monitored by weekly palpation. (b) Total tumor weight for Mogat2^{+/+}PyMT (n = 10) and Mogat2^{−/−}PyMT (n = 10) mice. (c–d) Lungs from Mogat2^{+/+}PyMT (n = 20) and Mogat2^{−/−}PyMT (n = 20) mice were stained with Bouin’s solution to identify metastases. Metastatic lesions are indicated by the arrows. (c) The data represents the mean total number of metastases ± SEM from both groups at 15 weeks old (d) Data represents the mean ± SEM. NS = non-significant. All mice were fed a normal diet.
The effect of MOGAT2 ablation on the number of metastases was also determined. Female Mogat2+/+PyMT and Mogat2-/-PyMT mice were euthanized at 16 weeks old, the lungs fixed in Bouin’s solution (Figure 4c), and the metastases quantified (Figure 4d). No statistically significant differences were found between the two groups.

Almost all of the MMTV-PyMT mice developed lung metastases, and all died of lung metastasis within several weeks of development. To observe the effect of MOGAT2 depletion on survival, we collected the survival time of the two groups of mice and found that MOGAT2 depletion did not significantly influence overall survival (P = 0.977) (Figure 5a).

A high-fat diet accelerates MMTV-PyMT mammary tumor incidence and growth regardless of MOGAT2 depletion

Previous studies [13, 14, 20] have reported that MOGAT2 ablation protects mice from high-fat diet (60% of calories from fat) induced obesity. We investigated the effect of MOGAT2’s depletion on mammary tumorigenesis of MMTV-PyMT mice fed with a relatively high-fat diet (37% of calories from fat). Female Mogat2+/+PyMT and Mogat2-/-PyMT mice were produced by mating MMTV-PyMT males with Mogat2-/- females. Both of them were fed with a relatively high-fat diet, commencing at 5 weeks old which continued for 12 weeks. Compared with normal diet-fed Mogat2+/+PyMT mice, a relatively high-fat diet significantly accelerated mammary tumor incidence and growth regardless of MOGAT2 depletion (Figures 5b and 5c).

DGAT1 and DGAT2 act as functional substitutes in MOGAT2-/- PyMT mice

To investigate the mechanism of functional cover-up upon MOGAT2 depletion in mice, we collected the mammary tumors, stomach, small intestine, and colorectal tissues of the
Figure 6. Depletion of the Mogat2 gene results in compensational overexpression of Dgat1 and Dgat2 genes. (a) Mogat1 gene mRNA was detected by qPCR in mammary tumors, stomach, small intestine, and colorectal tissues from Mogat2+/+PyMT (n = 6) and Mogat2−/−PyMT (n = 6) mice. As expected, Mogat1 gene expression was unaltered upon Mogat2 depletion. (b) In the same tissues, the Mogat2 expression level was suppressed in all the analyzed organs. (c) To compensate, Dgat1’s mRNA level was upregulated in the gastrointestinal system, especially in the small intestine, which is the major organ for fat absorption. (d) The level of Dgat2 mRNA had a similar overexpression pattern to that of Dgat1. (e, f) By analyzing TCGA data containing XXX cases of normal tissues adjacent to triple-negative breast adenocarcinoma, a significantly inverse correlation between MOGAT2 mRNA level and DGAT1/2 mRNA levels was found. Data represent the mean ± SEM. NS = non-significant, *P < 0.05, **P < 0.01 and ***P < 0.001.
mice with or without MOGAT2 depletion, and examined the RNA levels of MOGAT1, MOGAT2, DGAT1, and DGAT2 by qPCR (Figure 6). Compared with the high-fat diet-fed MOGAT2+/+PyMT mice, a significant reduction in MOGAT2 was evident in the high-fat diet-fed MOGAT2−/−/−PyMT mice, while MOGAT1 expression was unaltered (Figure 6b). However, DGAT1 and DGAT2 were significantly upregulated in the gastrointestinal tract in Mogat2−/−/−PyMT mice (Figures 6c and 6d). The TCGA data analyses showed that the reduction of MOGAT2 mRNA level was negatively correlated with the increase of DGAT1 and DGAT2 mRNA levels in normal tissues adjacent to breast cancer (Figures 6e, 6f). That means whenever the MOGAT2 level was reduced, DGAT1/2 levels would be increased. These findings are consistent with our data from animal experiments showing compensatory increases of Dgat1 and Dgat2 mRNA levels upon Mogat2 depletion. These data suggest that both DGAT1 and DGAT2 may play some roles in the absence of MOGAT2 in a pathological condition.

Discussion

Previous studies [13, 14] have shown that MOGAT2 ablation protects mice from high-fat diet-induced obesity. In our study, a relatively high-fat diet (37% of calories from fat) promoted obesity and subsequent mammary tumor formation and metastasis regardless of Mogat2 depletion. This discrepancy, however, may be the result of a lower amount of fat in the food: 60% versus 37%.

In terms of dietary habits, most countries in Asia have a relatively low total fat intake (< 30% total energy, TE), such as 20% TE for China [21], 22.5% TE for India [22], 21.1% TE for South Korea [23] and 23.3–26.3% TE for Japan [24], whereas, most western countries have a relatively higher total fat intake (> 30% TE), with 34% TE for the USA [25], 37.6% TE for Germany [26] and 38.2% TE for France [27]. A systematic review of data from 40 countries found that the total fat intake ranged from 11.1 to 46.2% TE [25]. Therefore, the relatively high-fat diet (37% of calories from fat) used in our study is more in line with human dietary habits compared with the higher-fat diet (60% of calories from fat) used in other studies [4, 13, 14].

Our findings about the upregulation of Dgat1 and Dgat2 expression upon Mogat2 depletion suggest that the genetic depletion of MOGAT2 alone is insufficient in preventing obesity and mammary tumor formation. DGAT1 and DGAT2 are abundantly expressed in the small intestine [28, 29] and mediate the final step in TAG re-synthesis during dietary fat absorption. Previous studies have shown that DGAT1 [15] and DGAT2 have the capacity to acylate MAG to form DAG [16]. These results imply that DGAT1 and DGAT2 can act as functional substitutes in Mogat2−/−/−PyMT mice (Video 1).

Conclusion

In summary, elevated expression of MOGAT2 in a triple-negative mammary tumor correlates with a poorer patient overall survival and disease-free survival. With the compensation of DGAT1 and DGAT2, MOGAT2 depletion alone, cannot prevent fat-food-induced obesity, and has no effect on mammary tumor initiation, growth, or metastasis. Targeting multiple key enzymes in fat metabolism might generate better outcomes in controlling obesity and probably in inhibiting mammary tumor development as well.

Ethics approval and consent to participate

This animal study was approved by Sun Yat-sen University Cancer Center’s Institutional Animal Care and Usage Committee (L102012017000G). All human tissue samples were obtained with patient consent and the approval of the Institutional Clinical Ethics Review Board at SYSUCC.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors have nothing to disclose.

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Supplementary material

The supplementary material of this article is available at https://vcm.edpsciences.org/10.1051/vcm/2023006/olm

Supplementary Figure 1: Survival analyses of the patients with breast cancer as well as genotyping MMTV-PyMT mice and MOGAT2-deficient mice. (a) Survival analyses of different molecular types of breast cancer patients divided by high- and low-mRNA levels of MOGAT2 using the TCGA database.

(b, c) Diagrams and genotyping of the mice with MMTV-PyMT or deletion of the first exon of Mogat2.

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References


