Prospective study of cesarean scar pregnancy associated with increased MCP-1 and cholesterol synthesis

QIONGWEI WU*, JIAO FAN*, QINGJING SHENG and XIAOYING HE

Shanghai Key Laboratory of Maternal Fetal Medicine, Shanghai Institute of Maternal Fetal Medicine and Gynecologic Oncology, Shanghai First Maternity and Infant Hospital, School of Medicine, Tongji University, Shanghai 200120, P.R. China

Received January 17, 2025; Accepted May 13, 2025

DOI: 10.3892/br.2025.2026

Abstract. In the present study, it was investigated whether gestational trophoblast localization at the cesarean scar was associated with increased monocyte chemoattractant protein-1 (MCP-1) and cholesterol synthesis in women's blood and decidual stromal cells. Patients who underwent abortion at 6-10 weeks of gestation were divided into three groups: cesarean scar pregnancy (CSP), normal intrauterine pregnancy without history of cesarean section (NP), and normal intrauterine pregnancy after cesarean section (NPACS). Blood and decidual tissue at the localization of the gestational sacs were collected from the patients, and RNA sequencing, PCR and ELISA were used to detect differences in the expression of thrombin receptor protease-activated receptor-1 (PAR-1), MCP-1, CCR2, chemokine (C-C motif) receptor 2 (CCR2) and cholesterol synthesis-related proteins sterol regulatory element-binding protein 2 (SREBP2), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) and hydroxy-methyl-glutaryl Coenzyme A synthase (HMGCS). The expression of PAR-1, MCP-1, CCR2, SREBP2, HMGCR and HMGCS in the blood and the decidual stromal cells at the localization of the gestational sac was significantly higher in women with CSP than in the other two groups of patients, whereas the expression of these molecules between NP group and NPACS were not significantly different, and the total blood cholesterol levels of women with cesarean scar pregnancies were significantly higher than those of the other two groups. The development of CSP was associated with a high cholesterol environment and increased MCP-1 expression in women.

Correspondence to: Professor Xiaoying He, Shanghai Key Laboratory of Maternal Fetal Medicine, Shanghai Institute of Maternal Fetal Medicine and Gynecologic Oncology, Shanghai First Maternity and Infant Hospital, School of Medicine, Tongji University, 2699 West Gaoke Road, Pudong New Area, Shanghai 200120, P.R. China
E-mail: hexiaoying@51mch.com

*Contributed equally

Key words: cesarean scar pregnancy, monocyte chemoattractant protein-1, cholesterol synthesis

Introduction

Cesarean scar pregnancy (CSP) is an ectopic pregnancy characterized by the conceptus implanting in the cesarean scar after cesarean section. CSP is a chronic complication of cesarean section, and women in early pregnancy usually present with vaginal bleeding (with or without lower abdominal pain) or no clinically significant symptoms (1). CSP was once considered a rare ectopic pregnancy, occurring in 0.15% of women with previous cesarean deliveries (2). However, the incidence of CSP has risen markedly in the past decade, especially in China, which may be related to the increase in cesarean section rate and the full liberalization of fertility policies (3). Inadequate treatment in early CSP can lead to persistent vaginal bleeding, hemorrhagic shock and even uterine rupture (4), and these events can seriously threaten a woman's reproductive function, health and life. However, the mechanism of cesarean section scar pregnancies was unclear.

The biological processes of trophoblast localization, adhesion and implantation were very analogous to those of tumor cell migration and invasion (5). Tumor cells also have a tendency to metastasize to specific organs; for example, colon cancer was most likely to metastasize to the liver, and breast and choriocarcinoma were especially likely to metastasize to the lungs (6). It remains unclear why do early embryos choose the cesarean scar; this question was worth pondering. Does the embryo choose to localize at the cesarean section scar possessing some similarity with the mechanism of cancer tropism metastasis?

Monocyte chemoattractant protein-1 (MCP-1) and tumor cell cholesterol metabolism possess an important role in the selective metastasis of tumor organs. Cholesterol anabolism is activated during tumor cell metastasis. Lung fibroblasts secrete MCP-1 to stimulate increased cholesterol synthesis in lung breast cancer cells (7). Inhibition of cholesterol synthesis in tumor cells that metastasize from colon cancer to the liver reduce metastasis of cancer cells (8).

MCP-1 is expressed in a variety of cells, including tethered cells, endothelial cells, monocytes and early gestational and full-term decidual cells; and the expression of MCP-1 in these cells was found to be regulated by thrombin (9,10). The expression and secretion of MCP-1 was increased in the placenta. MCP-1 may perform an important role in the normal development of the placenta and the successful maintenance of

pregnancy. Inappropriate MCP-1 secretion during pregnancy can lead to abnormalities in chorionic trophoblast implantation affecting normal placental development, angiogenesis and spiral arterial transformation (11,12). In early pregnancy, the embryo is unable to synthesize its own cholesterol, and the cholesterol needed can only come from the mother (13). Blood cholesterol and triglyceride levels are strongly associated with the development of complications during pregnancy (14).

It was hypothesized that the occurrence of CSP was related to increased thrombin receptor: Protease-activated receptor-1 (PAR-1) in the decidual stromal cells, resulting in the increase of cholesterol synthesis and the increase of MCP-1 expression in the decidual stromal cells, which induced gestational trophoblasts to localize to the cesarean scar. In the present study, it was investigated whether the aforementioned molecules and cholesterol synthesis-related proteins could be reflected in the blood of women with cesarean section pregnancies. A clear understanding of the mechanisms of CSP will facilitate the prevention and management of CSP and reduce the risk of CSP and subsequent associated risks in women after cesarean delivery.

Patients and methods

Patient and specimen acquisition. The present study was a case-control type; participants were rigorously screened according to predetermined inclusion and exclusion criteria. A total of 60 patients who underwent early abortion from March 2024 to August 2024 in the family planning department of Shanghai First Maternity and Infant Hospital (Shanghai, China) were included in the present study. All patients were between 20 and 40 years of age, with singleton pregnancy confirmed by ultrasound at 6-10 weeks of gestational age. The patients who underwent abortion were categorized into the following three groups according to the current pregnancy: Caesarean scar pregnancy (CSP), normal intrauterine pregnancy after cesarean section (NPACS), and normal intrauterine pregnancy without a history of caesarean section (NP). A total of 20 women were included in each group. The following criteria were used for the diagnosis of CSP in the patients included in the present study: i) Absence of gestational sac in the uterine cavity and cervix on ultrasound and closure of the cervix; ii) embeddedness of the gestational sac in the uterine incisional scar; iii) thickness of uterine myometrium of 3 mm between the gestational sac and the bladder; iv) visible blood flow around the chorionic villus sac; v) visible fetal heartbeat in the gestational sac. Patients with acute inflammatory diseases of the reproductive system, chronic diseases of thyroid gland, heart, liver and kidney, history of diabetes mellitus, hyperlipidemia and smoking or drinking were excluded. Patients who met the criteria were admitted to the hospital for pre-abortion blood testing; 5 ml of blood was retained from each patient, centrifuged (2,000 x g for 10 min at room temperature), and the supernatant was frozen in a -80 refrigerator. The test was performed uniformly after the collection of blood from all patients was completed. On the day of abortion in all patients, the decidual tissue was obtained at the localization of the gestational sac, and the decidual tissue was immediately transferred to a sterile culture solution for the extraction of decidual tissue stromal cells after obtaining. All patients signed an informed consent form, and the study was approved (approval no. KS23330) by the Ethics Committee of Shanghai First Maternity and Infant Hospital (Shanghai, China) and registered with the China Clinical Trial Registry (CCTR) under the registration number: ChiCTR2400079711.

Sample size calculation. The sample size was calculated based on the results of the pre-experiment. As a result of the pre-test, the concentration of MCP-1 in the blood of women with CSP in early pregnancy was 350±150 pg/ml, that of women with NPACS was 200±150 pg/ml, and that of women with NP was 250±150 pg/ml; with α taking the value of 0.05, β taking the value of 0.1, and the degree of certainty of 1-β: 0.9. The sample size required for unilateral testing of these three groups was 18 cases each. For the three groups, a total sample size of 54 cases was required. Eventually 60 people were included in the present study.

Decidual stromal cell extraction. The extraction of decidual stromal cells was performed as previously described (15). Briefly, the obtained decidual was washed in sterile phosphate-buffered saline and chopped into 1-2-mm pieces. Tissues were digested with 0.4% (g/ml) type IV collagenase (Beijing Solarbio Science & Technology Co., Ltd.) in sterile centrifuge tubes for 60 min at 37˚C in a thermostat. The cell suspension was screened for decidual stromal cells by passing through 70- and 40-μm sterile cell sieves sequentially. The filtered cell suspension was centrifuged at 134 x g for 10 min at room temperature. after discarding the supernatant, the cell pellet was resuspended in RPMI-1640 (Gibco; Thermo Fisher Scientific, Inc.) containing 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.), 100 IU/ml penicillin and 100 mg/ml streptomycin, and incubated for 2 h at 37˚C, 5% CO_2. Suspended cells such as leukocytes and erythrocytes were aspirated.

RNA sequencing. After the inclusion of 60 patients, 18 from 60 patients were selected, 6 in each group. RNA sequencing of decidual stromal cell from 6 patients was performed in each group respectively. The selection of patients for RNA sequencing was obtained using a randomization method. Computer-generated random numbers were used for patient selection from three groups. Total RNA was extracted from decidual stromal cell with the Universal RNA Extraction CZ Kit (cat. no. RNC643; ONREW; www.onrew.com) according to the manufacturer's instructions. RNA quantity was analyzed using Qubit 4.0 (Invitrogen; Thermo Fisher Scientific, Inc.) and quality examined by electrophoresis on a denaturing agarose gel. Electrophoresis was performed using a 1% agarose gel with ethidium bromide added to the gel. After electrophoresis, the RNA bands were observed by UV imaging system. RNA libraries were prepared using the VVAHTS® Universal V8 RNA-seq Library Prep Kit for Illumina (cat. no. NR605-0, Vazyme Biotech Co., Ltd.), followed by sequencing using the Illumina NovaSeq 6000 platform (Illumina, Inc.) with the 150 paired-end sequencing strategy. Enrichment of mRNA, library construction, sequencing and data analysis were performed by Shanghai Xu Ran Biotechnology Co., Ltd. (http://www.xurangene.com). Data quality was checked by FastQC v0.11.2 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/).

The read length was 2x150 bp. Differential gene expression was analyzed by DESeq2 (v1.16.1) (https://bioconductor.org/packages/release/bioc/html/DESeq2.html). The thresholds for determining DEGs are P<0.05 and absolute fold change ≥2.

ELISA. ELISA kits were used for the detection of molecules in blood of 60 patients. Human 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) ELISA kit (cat. no. abx250429; Abbexa; www.abbexa.com), human sterol regulatory element-binding protein 2 ELISA kit (cat. no. abx250666; Abbexa), human PAR1/thrombin receptor ELISA kit (cat. no. ab283544; Abcam), human CCR2 ELISA kit (cat. no. NBP2-75114; Novus Biologicals, LLC), hydroxy-methyl-glutaryl Coenzyme A synthase (HMGCS) ELISA kit (cat. no. MBS2019446; MyBioSource, Inc.) and cholesterol assay kit (cat. no. ARG81630; Arigobio; www.arigobio.cn) were used. All operations were performed in strict accordance with the kit instructions. The kits provided the highest concentration of the standard as a positive control, and the kits buffer without antibody or samples as a negative control. A standard curve was made according to the concentration of the standard and the corresponding OD value, and the experiment was repeated three times.

Reverse transcription-quantitative PCR. TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) was used for the extraction of total RNA from decidual stromal cells, and total RNA was quantified by nanodrop spectrophotometer (Thermo Fisher Scientific, Inc.). The reverse transcription kit (Tiangen Biotech Co., Ltd.) was used to synthesize cDNA according to the manufacturer's instructions. The abundance of mRNA was evaluated by quantitative PCR using SYBR Premix Ex Taq (Takara Bio, Inc.). Initial denaturation at 95˚C for 30 sec, followed by 40 cycles of 95˚C for 5 sec (denaturation) and 60˚C for 30 sec (annealing/extension with fluorescence acquisition). The relative expression of molecular RNA was calculated using the $2^{-\Delta\Delta Cq}$ method (16) with GAPDH as an internal reference and ddH_2O was substituted for the template as a negative control. The experiment was repeated four times. The primer sequences are shown in Table I.

Statistical analysis. The SPSS version 20.0 (IBM Corp.) statistical software package was used for the statistical analysis. Data are expressed as the mean ± standard deviation (SD). T-test, paired t-test (with Welch's correction if the variance was not equal), ANOVA, chi-squared test and Fisher's exact test were used as appropriate. Two-by-two comparisons among the three groups were performed using Bonferroni-corrected ANOVA. P<0.05 was considered to indicate a statistically significant difference.

Results

Clinical information of the three groups of patients. A total of 20 women with CSP, NP and NPACS were included as aforementioned. There were no significant differences in age, body mass index (BMI), number of pregnancies, weeks of gestation, or number of miscarriages among the three groups, and the number of cesarean sections between women with CSP and women with NPACS did not differ significantly.

Table I. Sequences of primers used for reverse transcription-quantitative PCR.

Gene name	Primer sequence (5'-3')
MCP-1	F: ACCAGCAGCAAGTGTCCCAAAG
	R: TTTGCTTGTCCAGGTGGTCCATG
CCR2	F: CCAACGAGAGCGGTGAAGAAGTC
	R: CGAGTAGAGCGGAGGCAGGAG
PAR-1	F: TGCCTACCTCCTCTGTGTCTGTG
	R: TGCTGGGATCGGAACTTTCTTTGC
SREBP2	F: CTCACCTTCCTGTGCCTCTC
	R: AGGCATCATCCAGTCAAACC
HMGCR	F: GTCATTCCAGCCAAGGTTGT
	R: CATGGCAGAGCCCACTAAAT
HMGCS	F: AGCTCTTCCAGGATTCAGGCA
	R: AGCTGGATTCCATCCAGTTGG
GAPDH	F: CAGGAGGCATTGCTGATGAT
	R: GAAGGCTGGGGCTCATTT

F, forward; R, reverse; MCP-1, monocyte chemoattractant protein-1; CCR2, chemokine (C-C motif) receptor 2; PAR-1, protease-activated receptor-1; SREBP2, sterol regulatory element-binding protein 2; HMGCR, hydroxy-3-methylglutaryl-CoA reductase; HMGCS, hydroxy-methyl-glutaryl Coenzyme A synthase.

This suggested that the number of cesarean sections may not be related to CSP (Table II). A representative image of early pregnancy is demonstrated in Fig. 1.

Increased MCP-1 and cholesterol synthesis in decidual stromal cells of women with cesarean section scar pregnancies. Chemokines and chemokine receptors play important roles in both embryo implantation and tumor metastasis (7,17-19). Decidual stromal cells were obtained for chemokine and chemokine receptor assays, and the results of RNA sequencing of decidual stromal cells from three types of patients revealed that the expression of PAR-1, MCP-1 and chemokine (C-C motif) receptor 2 (CCR2) in decidual stromal cells from women with CSP was higher than that of women from the other two groups (Fig. 2A and B) , Subsequently, our PCR experiments verified that the expression of PAR-1, MCP-1, and CCR2 genes in the decidual stromal cells of women with cesarean scar pregnancies was significantly higher than that of the other two groups of patients, whereas no significant differences in the expression of PAR-1, MCP-1 and CCR2 genes were observed between the two groups of NP and NPACS (Fig. 2C). Considering that there was a close relationship between MCP-1 and cholesterol (20,21), it was explored if cholesterol synthesis altered in stromal cells at the locus of the gestational sac in women with CSP. The present PCR experiments demonstrated that cholesterol synthesis of the specific transcription factor SREBP2 and the rate-limiting enzymes HMGCR and HMGCS were significantly increased in the decidual stromal cells at the localization of the gestational sacs in women with CSP, whereas no significant differences in the expression of

Table II. Baseline characteristics among three groups.

Variables	CSP group	NP group	NPACS group	P-value
Age, years	29.75±6.52	29.65±4.84	30.75±4.93	0.78
Body mass index	20.76±1.22	20.82±0.73	20.49±0.98	0.54
Gravidity	2.95±0.99	2.55±0.76	2.70±0.73	0.32
Parity	1.50±0.61	1.10±0.55	1.40±0.60	0.08
Number of abortions	0.45±0.69	0.45±0.68	0.30±0.47	0.68
Number of cesarean sections	1.45±0.60	-	1.40±0.59	0.79
Gestational age at time of abortion, days	53.95±4.87	54.50±5.34	56.20±4.10	0.31

CSP, cesarean scar pregnancy; NP, normal intrauterine pregnancy without history of cesarean section; NPACS, normal intrauterine pregnancy after cesarean section.

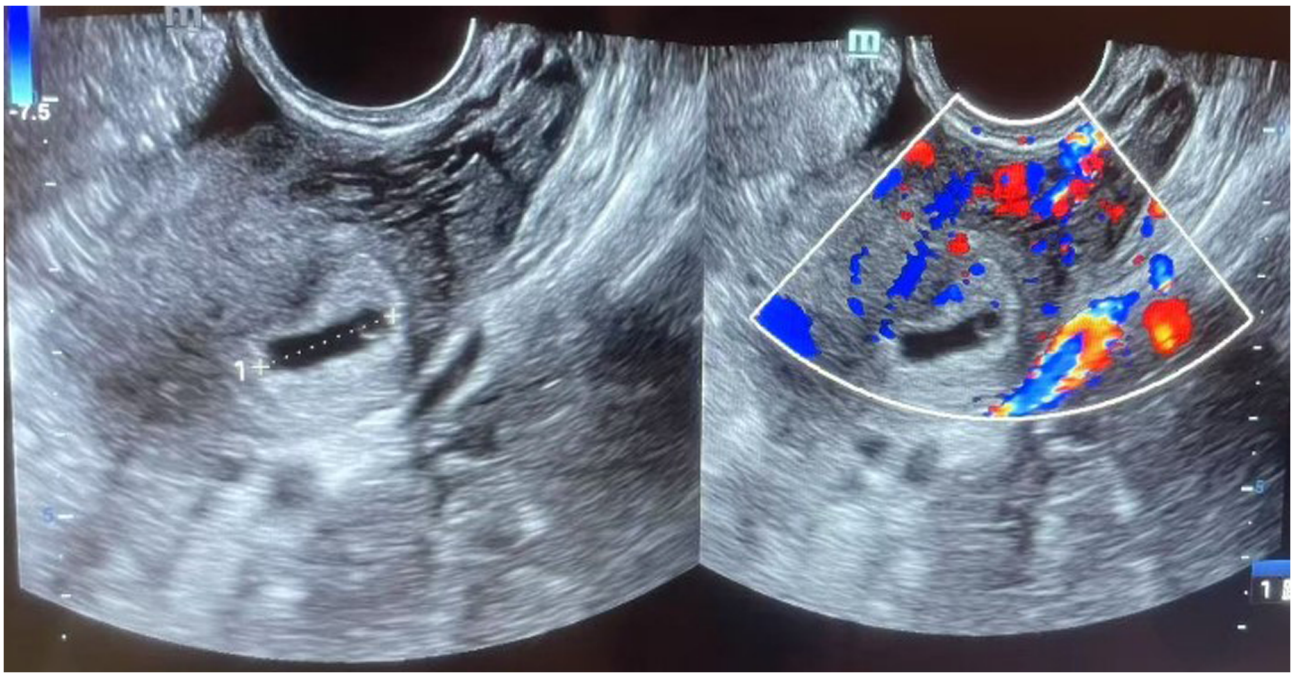

Figure 1. A representative ultrasound of early pregnancy.

cholesterol-synthesis-related proteins were observed between the two groups of NP and NPACS (Fig. 2D).

Increased blood MCP-1 and cholesterol synthesis in women with CSP. It was investigated whether the blood of women with CSP revealed alterations in chemokines and cholesterol synthesis. ELISA proved that the concentrations of PAR-1, MCP-1, CCR2, SREBP2, HMGCR and HMGCS in the blood of women with CSP were significantly higher than that in the two groups of NP and NPACS, and there was no significant difference in the expression of MCP-1 and cholesterol synthesis-related proteins in the blood of women between NP and NPACS (Fig. 3A). Blood tests also identified that the level of cholesterol in the blood of women with CSP was significantly higher than that of the other two groups (Fig. 3B), suggesting that the occurrence of scar pregnancies after cesarean section was associated with a high cholesterol milieu in the body.

Discussion

Active trophoblasts, tolerant endometrium, endometrium and inter-embryonic dialog were essential for successful embryo implantation (22). The mutual communication between the endometrium and the embryo was accomplished by various cytokines affecting cellular metabolism (23,24). Metabolic alterations in endometrial cells inevitably affected the process of embryo localization, adhesion and implantation. The present study found that MCP-1 and cholesterol synthesis of the specific transcription factor SREBP2 and the rate-limiting enzymes HMGCR and HMGCS in stromal cells at the cesarean scar was significantly higher than in control patients, suggesting that the occurrence of CSP was associated with increased cholesterol synthesis and MCP-1 in endometrial stromal cells.

Chemokines exert an important role in tumor cell metastasis (25). The synthesis of MCP-1 by stromal cells in the lungs promotes the localization of breast

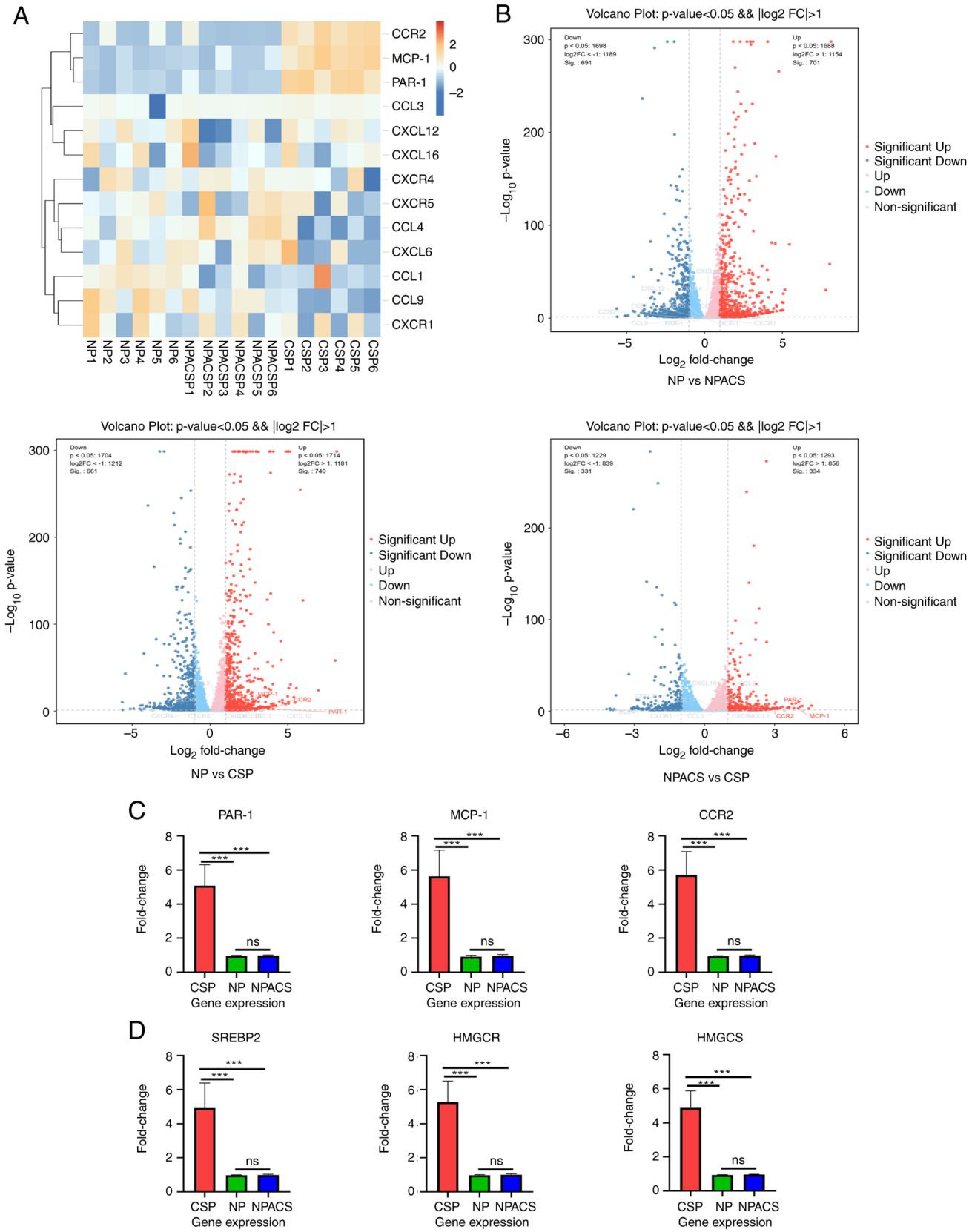

Figure 2. Increased MCP-1 and cholesterol synthesis in decidual stromal cells localized at the gestational sac of women with CSP. (A) Heat map results of decidual stromal cell chemokine and chemokine receptor extracted from the gestational sac localization of the three groups of patients. (B) Volcano map results of two-by-two comparison of chemokine and chemokine receptor of decidual stromal cells extracted from patients' gestational sac localization. (C) Gene expression of PAR-1, MCP-1 and CCR2 in decidual stromal cells extracted from patients' gestational sac localization in the three groups. (D) Gene expression of SREBP2, HMGCR and HMGCS in decidual stromal cells extracted from patients' gestational sac localization in the three groups. Data are expressed as the mean ± standard deviation. ns, P>0.05 and ***P<0.001. MCP-1, monocyte chemoattractant protein-1; CSP, cesarean scar pregnancy; PAR-1, protease-activated receptor-1; CCR2, chemokine (C-C motif) receptor 2; SREBP2, sterol regulatory element-binding protein 2; HMGCR, hydroxy-3-methylglutaryl-CoA reductase; HMGCS, hydroxy-methyl-glutaryl Coenzyme A synthase; NP, normal intrauterine pregnancy without history of cesarean section; NPACS, normal intrauterine pregnancy after cesarean section; ns, not significant (P>0.05).

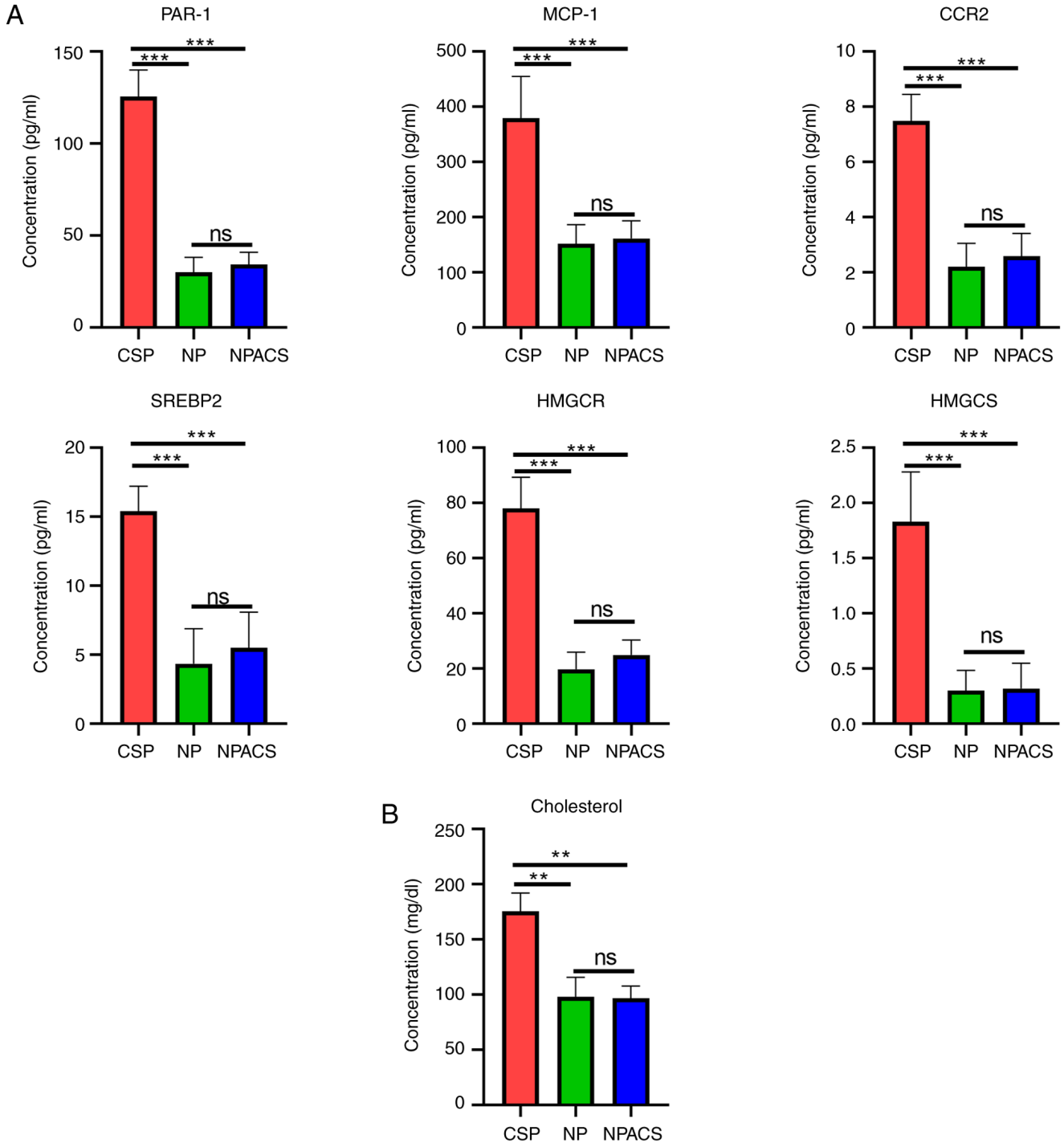

Figure 3. Increased blood MCP-1 and cholesterol synthesis in women with CSP. (A) Detection of PAR-1, MCP-1, CCR2, SREBP2, HMGCR and HMGCS concentrations in the blood of three groups of patients. (B) Comparison of blood cholesterol levels in three groups of patients. Data are expressed as the mean ± standard deviation. **P<0.01 and ***P<0.001. MCP-1, monocyte chemoattractant protein-1; CSP, cesarean scar pregnancy; PAR-1, protease-activated receptor-1; CCR2, chemokine (C-C motif) receptor 2; SREBP2, sterol regulatory element-binding protein 2; HMGCR, hydroxy-3-methylglutaryl-CoA reductase; HMGCS, hydroxy-methyl-glutaryl Coenzyme A synthase; NP, normal intrauterine pregnancy without history of cesarean section; NPACS, normal intrauterine pregnancy after cesarean section; ns, not significant (P>0.05).

cancer cells in the lungs. Elevated MCP-1 in the blood also facilitates metastasis of tumor cells to lymph nodes and liver metastasis of colon cancer cells (26-28). Knockdown of MCP-1 in breast cancer cell lines was found to reduce cancer cell metastasis (29,30). Decreased breast cancer lung metastasis by reducing of MCP-1 was also validated in a mouse model (31,32).

Cholesterol is a cell membrane component and a precursor for the synthesis of bilirubin and steroid hormones (33,34). Cholesterol synthesis is also important in tumor growth and progression (35). Cholesterol anabolism was found to be regulated by the following major transcription factors: SREBP2 and its downstream genes including HMGCR, HMGCS and squalene synthase (36). High cholesterol diet facilitates

breast cancer progression and lung metastasis in mice (37). Statins, a common medication for lowering cholesterolemia, reduce mortality from recurrence of breast cancer (38,39). Mouse models have also demonstrated that statins reduce lung metastases from breast cancer (40,41).

The aforementioned studies illustrated that tumor selection of specific organs for metastasis was associated with MCP-1 and cholesterol synthesis. Considering the similarity of biological behaviors of embryo implantation and tumor metastasis, the present study also found that MCP-1 and the enzymes associated cholesterol synthesis in the blood and in the decidual stromal cells at the localization of the gestational sacs in women with CSP were significantly higher than in control patients. This may be a possible mechanism for the development of CSP.

Thrombin regulates MCP-1 expression and activation of the PAR-1 upregulates MCP-1 expression and secretion in trophoblast cells (9,10). Trophoblasts expressed MCP-1 and CCR2 (17). In the present study, it was identified that the expression of MCP-1 and CCR2 was higher in decidual stromal cells at the localization of the gestational sac in patients with CSP than in control patients, which may be responsible for the induction of trophoblasts to the scar.

The limitation of the present study is that randomization was not used to include patients; participants were screened strictly according to predetermined inclusion and exclusion criteria, resulting in no statistically significant differences in baseline characteristics among the three groups (CSP, NP and NPACS) (as shown in Table II). Although randomization or variable-specific matching were not used, potential confounders were effectively controlled through strict inclusion criteria. The importance of randomization and matching in reducing bias was taken into consideration, but in practice, complete randomization or matching of all variables (for example, age, BMI) is not always feasible due to the limitations of study design (case-control design). Nonetheless, it was confirmed by statistical analysis that the three groups were balanced in terms of baseline characteristics, which reduced the risk of bias to some extent.

Basic research is now conducted by the authors on the regulation of PAR-1 on cholesterol synthesis and MCP-1 expression in decidual stromal cells, the interrelationships between cholesterol synthesis and MCP-1, and whether blocking the expression of MCP-1 alters the localization of trophoblast cells. The results of these studies will help to further understand the mechanism of CSP and may become a means of preventing CSP.

Modulation of MCP-1 and cholesterol metabolism has shown promising potential in reducing the occurrence of CSP, but its clinical application still requires caution. For example, MCP-1 inhibitors may suppress immune function and increase the risk of infection. The potential effects of the drug on fetal development need to be evaluated to ensure safety. The safety of long-term use of MCP-1 inhibitors requires further study. Cholesterol metabolism regulating drugs such as statins may affect fetal development and should be used with caution. Statins may cause side effects such as muscle pain. Liver function should be monitored to prevent drug induced liver injury. Future studies should be designed for in-depth investigation of the mechanisms. Based on the clarification of the regulatory network of MCP-1 and cholesterol metabolism, the authors are searching for more upstream and specific targets of intervention to improve therapeutic efficacy and reduce side effects.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The data generated in the present study may be requested from the corresponding author. The data generated in the present study may be found in the BioSample under accession number PRJNA1238512 or at the following URL: https://www.ncbi.nlm.nih.gov/biosample/?term=1238512.

Authors' contributions

JF and QWW were responsible for writing the article, data collection and statistical analysis. QJS was responsible for data collection. XYH was responsible for revising the article and the research concept. XYH and JF confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

All patients signed an informed consent form, and the study was approved (approval no. KS23330) by the Ethics Committee of Shanghai First Maternity and Infant Hospital (Shanghai, China) and registered with the China Clinical Trial Registry (CCTR) under the registration number: ChiCTR2400079711.

Patient consent for publication

All patients provided consents for their information to be published.

Competing interests

The authors declare that they have no competing interests.

References

1. Luo L, Ruan X, Li C, Chen S, Hu Q and Mueck AO: Early clinical features and risk factors for cesarean scar pregnancy: A retrospective case-control study. Gynecol Endocrinol 35: 337-341, 2019.
2. Seow KM, Huang LW, Lin YH, Lin MY, Tsai YL and Hwang JL: Cesarean scar pregnancy: Issues in management. Ultrasound Obstet Gynecol 23: 247-253, 2004.
3. Li HT, Hellerstein S, Zhou YB, Liu JM and Blustein J: Trends in cesarean delivery rates in China, 2008-2018. JAMA 323: 89-91, 2020.
4. Miller R, Timor-Tritsch IE and Gyamfi-Bannerman C: Society for Maternal-fetal medicine (SMFM) Consult series #49: Cesarean scar pregnancy. Am J Obstet Gynecol 222: B2-B14, 2020.
5. Murray MJ and Lessey BA: Embryo implantation and tumor metastasis: Common pathways of invasion and angiogenesis. Semin Reprod Endocrinol 17: 275-290, 1999.

6. Obenauf AC and Massagué J: Surviving at a distance: Organ-specific metastasis. Trends Cancer 1: 76-91, 2015.
7. Han B, Alonso-Valenteen F, Wang Z, Deng N, Lee TY, Gao B, Zhang Y, Xu Y, Zhang X, Billet S, *et al*: A chemokine regulatory loop induces cholesterol synthesis in lung-colonizing triple-negative breast cancer cells to fuel metastatic growth. Mol Ther 30: 672-687, 2022.
8. Zhang KL, Zhu WW, Wang SH, Gao C, Pan JJ, Du ZG, Lu L, Jia HL, Dong QZ, Chen JH, *et al*: Organ-specific cholesterol metabolic aberration fuels liver metastasis of colorectal cancer. Theranostics 11: 6560-6572, 2021.
9. Grandaliano G, Valente AJ and Abboud HE: A novel biologic activity of thrombin: Stimulation of monocyte chemotactic protein production. J Exp Med 179: 1737-1741, 1994.
10. Colotta F, Sciacca FL, Sironi M, Luini W, Rabiet MJ and Mantovani A: Expression of monocyte chemotactic protein-1 by monocytes and endothelial cells exposed to thrombin. Am J Pathol 144: 975-985, 1994.
11. Chaiworapongsa T, Yoshimatsu J, Espinoza J, Kim YM, Berman S, Edwin S, Yoon BH and Romero R: Evidence of in vivo generation of thrombin in patients with small-for-gestational-age fetuses and pre-eclampsia. J Matern Fetal Neonatal Med 11: 362-367, 2002.
12. Bagot CN, Leishman E, Onyiaodike CC, Jordan F and Freeman DJ: Normal pregnancy is associated with an increase in thrombin generation from the very early stages of the first trimester. Thromb Res 157: 49-54, 2017.
13. Ghio A, Bertolotto A, Resi V, Volpe L and Di Cianni G: Triglyceride metabolism in pregnancy. Adv Clin Chem 55: 133-153, 2011.
14. Ferriols E, Rueda C, Gamero R, Vidal M, Payá A, Carreras R, Flores-le Roux JA and Pedro-Botet J: Relationship between lipid alterations during pregnancy and adverse pregnancy outcomes. Clin Investig Arterioscler 28: 232-244, 2016 (In Spanish).
15. Xiong M, Wang Q, Zhang X, Wen L and Zhao A: Decidual stromal cells-derived exosomes incurred insufficient migration and invasion of trophoblast by disturbing of β-TrCP-mediated snail ubiquitination and degradation in unexplained recurrent spontaneous abortion. Eur J Med Res 29: 39, 2024.
16. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
17. Shi JW, Yang HL, Fan DX, Yang SL, Qiu XM, Wang Y, Lai ZZ, Ha SY, Ruan LY, Shen HH, *et al*: The role of CXC chemokine ligand 16 in physiological and pathological pregnancies. Am J Reprod Immunol 83: e13223, 2020.
18. Lin Z, Shi JL, Chen M, Zheng ZM, Li MQ and Shao J: CCL2: An important cytokine in normal and pathological pregnancies: A review. Front Immunol 13: 1053457, 2022.
19. Valles CS and Domínguez F: Embryo-endometrial interaction. Chang Gung Med J 29: 9-14, 2006.
20. Sun R, Fang P, Jiang J, Huang C, Wang J, Guo Q, Li H, Wu X, Xie X, Jiang Y, *et al*: Insulin rescued MCP-1-suppressed cholesterol efflux to large HDL2 particles via ABCA1, ABCG1, SR-BI and PI3K/Akt activation in adipocytes. Cardiovasc Drugs Ther 36: 665-678, 2022.
21. Blanco-Colio LM, Martín-Ventura JL, de Teresa E, Farsang C, Gaw A, Gensini G, Leiter LA, Langer A, Martineau P and Egido J; ACTFAST investigators: Elevated ICAM-1 and MCP-1 plasma levels in subjects at high cardiovascular risk are diminished by atorvastatin treatment. Atorvastatin on Inflammatory Markers study: A substudy of Achieve Cholesterol Targets Fast with Atorvastatin Stratified Titration. Am Heart J 153: 881-888, 2007.
22. Damsky CH, Fitzgerald ML and Fisher SJ: Distribution patterns of extracellular matrix components and adhesion receptors are intricately modulated during first trimester cytotrophoblast differentiation along the invasive pathway, in vivo. J Clin Invest 89: 210-222, 1992.
23. Jones RL, Hannan NJ, Kaitu'u TJ, Zhang J and Salamonsen LA: Identification of chemokines important for leukocyte recruitment to the human endometrium at the times of embryo implantation and menstruation. J Clin Endocrinol Metab 89: 6155-6167, 2004.
24. Red-Horse K, Drake PM, Gunn MD and Fisher SJ: Chemokine ligand and receptor expression in the pregnant uterus: Reciprocal patterns in complementary cell subsets suggest functional roles. Am J Pathol 159: 2199-2213, 2001.
25. Roussos ET, Condeelis JS and Patsialou A: Chemotaxis in cancer. Nat Rev Cancer 11: 573-587, 2011.
26. Qian BZ, Li J, Zhang H, Kitamura T, Zhang J, Campion LR, Kaiser EA, Snyder LA and Pollard JW: CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis. Nature 475: 222-225, 2011.
27. Lu Y, Cai Z, Galson DL, Xiao G, Liu Y, George DE, Melhem MF, Yao Z and Zhang J: Monocyte chemotactic protein-1 (MCP-1) acts as a paracrine and autocrine factor for prostate cancer growth and invasion. Prostate 66: 1311-1318, 2006.
28. Yoshidome H, Kohno H, Shida T, Kimura F, Shimizu H, Ohtsuka M, Nakatani Y and Miyazaki M: Significance of monocyte chemoattractant protein-1 in angiogenesis and survival in colorectal liver metastases. Int J Oncol 34: 923-930, 2009.
29. Fang WB, Yao M, Brummer G, Acevedo D, Alhakamy N, Berkland C and Cheng N: Targeted gene silencing of CCL2 inhibits triple negative breast cancer progression by blocking cancer stem cell renewal and M2 macrophage recruitment. Oncotarget 7: 49349-49367, 2016.
30. Nam JS, Kang MJ, Suchar AM, Shimamura T, Kohn EA, Michalowska AM, Jordan VC, Hirohashi S and Wakefield LM: Chemokine (C-C motif) ligand 2 mediates the prometastatic effect of dysadherin in human breast cancer cells. Cancer Res 66: 7176-7184, 2006.
31. Cranford TL, Velázquez KT, Enos RT, Bader JE, Carson MS, Chatzistamou I, Nagarkatti M and Murphy EA: Loss of monocyte chemoattractant protein-1 expression delays mammary tumorigenesis and reduces localized inflammation in the C3(1)/SV40Tag triple negative breast cancer model. Cancer Biol Ther 18: 85-93, 2017.
32. Yan L and Sundaram S: Monocyte chemotactic protein-1 deficiency reduces spontaneous metastasis of Lewis lung carcinoma in mice fed a high-fat diet. Oncotarget 7: 24792-24799, 2016.
33. Simons K and Ikonen E: How cells handle cholesterol. Science 290: 1721-1726, 2000.
34. Russell DW: The enzymes, regulation, and genetics of bile acid synthesis. Annu Rev Biochem 72: 137-174, 2003.
35. Clendening JW, Pandyra A, Boutros PC, El Ghamrasni S, Khosravi F, Trentin GA, Martirosyan A, Hakem A, Hakem R, Jurisica I and Penn LZ: Dysregulation of the mevalonate pathway promotes transformation. Proc Natl Acad Sci USA 107: 15051-15056, 2010.
36. Shimano H: Sterol regulatory element-binding proteins (SREBPs): Transcriptional regulators of lipid synthetic genes. Prog Lipid Res 40: 439-452, 2001.
37. Llaverias G, Danilo C, Mercier I, Daumer K, Capozza F, Williams TM, Sotgia F, Lisanti MP and Frank PG: Role of cholesterol in the development and progression of breast cancer. Am J Pathol 178: 402-412, 2011.
38. Sakellakis M, Akinosoglou K, Kostaki A, Spyropoulou D and Koutras A: Statins and risk of breast cancer recurrence. Breast Cancer (Dove Med Press) 8: 199-205, 2016.
39. Manthravadi S, Shrestha A and Madhusudhana S: Impact of statin use on cancer recurrence and mortality in breast cancer: A systematic review and meta-analysis. Int J Cancer 139: 1281-1288, 2016.
40. Beckwitt CH, Clark AM, Ma B, Whaley D, Oltvai ZN and Wells A: Statins attenuate outgrowth of breast cancer metastases. Br J Cancer 119: 1094-1105, 2018.
41. Baek AE, Yu YA, He S, Wardell SE, Chang CY, Kwon S, Pillai RV, McDowell HB, Thompson JW, Dubois LG, *et al*: The cholesterol metabolite 27 hydroxycholesterol facilitates breast cancer metastasis through its actions on immune cells. Nat Commun 8: 864, 2017.