様式第１９号

学位論文審査の結果の要旨

審査区分 (課)・論	第654号	氏名	朱　若菲
審査委員会委員		主査氏名	花田俊勝
		副査氏名	久保田敏昭
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論文題目

Chemokine expression profiles of ovarian endometriotic stromal cells in 3-dimentional culture

(子宮内膜症におけるコラーゲンゲル三次元培養を用いた遺伝子発現の変化)

論文掲載雑誌名

Journal of Reproductive Immunology

論文要旨

Endometriosis is a chronic inflammatory disease related to abnormal expression of chemokines. For early endometriosis, we established an *in vitro* model of human endometriotic cyst stromal cells (ECSCs) cultured in three-dimensional collagen gel. According to the microarray analysis, we evaluated the gene expression profile of 3D-cultured ECSC and identified six abnormally expressed chemokines. The expression level of these proteins in ECSC was significantly higher in 3D-culture than in 2D-culture. It is suggested that the 3D-culture model of ECSC is more suitable for the study of endometriosis in vitro. Whereas, the present microarray data may provide a new platform to identify the candidate genes involved in the pathogenesis of endometriosis and are masked in conventional 2D-culture.

The gene expression microarray revealed that six chemokine mRNAs were upregulated in 3D-cultured ECSCs compared to 2D-cultured ECSCs. The relative mRNA levels of CXCL1, CXCL2, CXCL3, CXCL8, and CCL20 in the 3D-cultured ECSCs were significantly higher than those in the 2D-cultured ECSCs. The relative protein levels of CXCL1, CXCL2, CXCL8, and CCL20 in the supernatant of 3D-cultured ECSCs were significantly higher than those in the 2D-cultured ECSCs.

Endometriotic lesions are characterized by chronic inflammation. The inflammatory cells and soluble factors, such as proinflammatory cytokines and chemokines, in the diseased tissues are considered to contribute to the development and progression of endometriosis. The ECSC 3-D culture system we have established is a model of fibrosis formation in endometriosis. In this experimental model, ECSCs cultured in floating collagen gels induce the reorganization and compaction of collagen fibers, resulting in the contraction of collagen lattices, which is comparable to the tissue contraction in the early-stage fibrosis formation in endometriosis. Research on endometriotic stromal cell biology in 3D-culture may provide new opportunities to better understand endometriosis-associated fibrosis's pathogenesis.

本研究は、コラーゲンゲル三次元培養法を用いた子宮内膜症の間葉系細胞培養を行い、遺伝子発現解析を試みた。その結果、ケモカイン分子群の高発現を見出し、本疾患における新たな病態機構の一端を明らかにした。このため、審査委員の合議により本論文は学位論文に値するものと判定した。

様式第２０号

~~最終試験~~
の結果の要旨
学力の確認

審査区分 ㊀・論	第６５４号	氏　名	朱　若菲
審査委員会委員		主査氏名	花田俊勝
		副査氏名	久保田敏昭
		副査氏名	黒川竜紀

学位申請者は本論文の公開発表を行い，各審査委員から研究の目的，方法，結果，考察について以下の質問を受けた。

1. What kind of cells is included in the ECSCs?
2. What is the mechanism of the Rho-Rock pathway in endometriosis-associated fibrosis?
3. You emphasized early-stage fibrosis formation in endometriosis in this study. Is chemokines expression increased in endometriotic tissues, especially in its early stage?
4. In this experimental model, 3D-cultured ECSCs induced the contraction of floating collagen gels. Could you explain why the contraction of floating collagen gels occur?
5. The microarray can analyze the expression levels of large numbers of genes. Why did you focus on only chemokines?
6. What kind of markers did you use to confirm ECSC and NESC cell types?
7. About the method of microarray analysis. What is the difference with previous studies (Okamoto 2015 or Aoyagi 2017)?
8. Ovarian endometriotic tissue was obtained from the patients with ovarian endometriotic cysts. You mentioned it in the discussion section. However, you skipped the detailed explanation. Why is it served as a cell culture model of endometriosis?
9. Have you done each experiment at least three times from 8 cases of endometriotic cysts and 10 cases of eutopic normal controls? Did one researcher perform all experiments?
10. The primer sets for CXCL6 mRNA did not work well. Is there any reason why the primer sets did not work well?
11. The ELISA kit for CXCL3 did not work. Have you tried the other kit from other companies?
12. About the result of the microarray analysis. Did you analyze other genes except for chemokines?
13. You mentioned that protein levels of CXCL3 and CXCL6 were below the detection levels. Did you do the western blotting analysis?
14. The expression of some chemokines is attenuated in 2D-cultured ECSCs. In 3D-culture, these chemokines' expressions were restored. Why are the expression of the chemokines restored in 3D- cultured ECSCs?
15. Genome-wide association study is performed to evaluate the candidate genes involved in the pathogenesis of the multifactorial disorders. The last sentence, "The present microarray data may provide a new platform to identify candidate genes involved in the pathogenesis of endometriosis." seems to be too exaggerated. How is your opinion?
16. You found out the expression of CXCL1, CLCL2, CXCL8, and CCL20 in 3-D cultured ECSCs. Previous studies have shown that CXCL1, CXCL8, and CCL20 are produced by endometriotic stromal cells. Is there any report about CXCL2? Can you explain the function of CXCL2?
17. What kind of experiments is further needed to confirm the importance of chemokine in the pathogenesis of endometriosis?

これらの質疑に対して，申請者は概ね適切に回答した。よって審査委員の合議の結果，申請者は学位取得有資格者と認定した。

（注）不要の文字は２本線で抹消すること。

様式第８号　　№　1

学　位　論　文　要　旨

氏名　朱　若菲（Zhu Ruofei）

論　文　題　目

Chemokine expression profiles of ovarian endometriotic stromal cells in 3-dimentional culture

（子宮内膜症におけるコラーゲンゲル三次元培養を用いた遺伝子発現の変化）

要　　旨

【Abstract】Endometriosis is a chronic inflammatory disease related to abnormal expression of chemokines. For early endometriosis, we established an in vitro model of human endometriotic cyst stromal cells (ECSCs) cultured in three-dimensional collagen gel. According to the microarray analysis, we evaluated the gene expression profile of 3D cultured ECSC and identified six abnormally expressed chemokines. The expression level of these proteins in ECSC was significantly higher in 3D culture than in 2D culture. It is suggested that the 3D culture model of ECSC is more suitable for the study of endometriosis in vitro. Whereas, the present microarray data may provide a new platform to identify the candidate genes that are involved in the pathogenesis of endometriosis and are masked in conventionall 2D culture.

【Materials and Methods】Ovarian endometriotic tissues were obtained from patients with regular menstrual cycles at the surgical treatment of ovarian endometriotic cysts. Eutopic endometrial tissues were obtained from premenopausal patients who had undergone hysterectomies for subserousal or intramural leiomyoma and had no evidence of endometriosis. All of the specimens were confirmed as being in the mid-to-late proliferative phases according to pathological observation and/or menstrual cycles. Total RNA from 3D-cultured and 2D-cultured ECSCs were extracted with a miRNeasy Mini kit and subjected to gene expression microarray analyses with a

commercially available human mRNA microarray. CXCL1, CXCL2, CXCL3, CXCL6, CXCL8, and CCL20 were identified by the gene expression microarray analysis. The expressions levels of chemokines in 3D-cultured and 2D-cultured ECSCs and 3D-cultured and 2D-cultured NESCs were evaluated by quantitative RT-PCR and ELISAs.

【Result】The gene expression microarray revealed that 6 chemokine mRNAs were upregulated in 3D-cultured ECSCs compared to 2D-cultured ECSCs. The relative mRNA levels of CXCL1, CXCL2, CXCL3, CXCL8, and CCL20 in the 3D-cultured ECSCs were significantly higher than those in the 2D-cultured ECSCs. The relative protein levels of CXCL1, CXCL2, CXCL8, and CCL20 in the supernatant of 3D-cultured ECSCs were significantly higher than those in the 2D-cultured ECSCs.

【Discussion】Endometriotic lesions are characterized by chronic inflammation. The inflammatory cells and soluble factors, such as proinflammatory cytokines and chemokines, in the diseased tissues is considered to contribute to the development and progression of endometriosis. We have established a three-dimensional collagen gel culture of ECSCs as a model of fi brosis formation in endometriosis. In this experimental model, ECSCs cultured in fl oating collagen gels induce the reorganization and compaction of collagen fibers, resulting in the contraction of collagen lattices, which is comparable to the tissue contraction in the early-stage fi brosis formation in endometriosis. Research on endometriotic stromal cell biology in 3D culture may provide new opportunities for a better understanding of the pathogenesis of endometriosis-associated fibrosis.