1. [The extracellular vesicular pseudogene LGMNP1 induces M2-like macrophage polarization by upregulating LGMN and serves as a novel promising predictive biomarker for ovarian **endometriosis** recurrence.](https://pubmed.ncbi.nlm.nih.gov/34893848/)

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## Abstract

**Study question:**How does ectopic endometrial stromal cell (Ecto-ESC)-derived extracellular vesicular Legumain pseudogene 1 (EV-LGMNP1), a newly identified pseudogene of Legumain (LGMN), contribute to M2-phenotype macrophage polarization, and does it predict recurrence in patients with ovarian endometriosis (EMs)?

**Summary answer:**EV-LGMNP1, which is abundant in Ecto-ESCs and serum from ovarian EMs, can direct macrophages towards an M2 phenotype by upregulating LGMN expression and is a promising biomarker for predicting ovarian EMs recurrence.

**What is known already:**Extracellular vesicles (EVs) can mediate cell-to-cell crosstalk to promote disease progression via cargo molecule transport. Recently, LGMNP1, a newly identified pseudogene of LGMN, has been reported to promote cancer progression by upregulating LGMN. LGMN is a well-studied protein that can induce M2-like polarization.

**Study design, size, duration:**An in vitro study was conducted with Ecto-ESCs isolated from ectopic endometrial samples, collected from two patients with ovarian EMs (diagnosed by laparoscopy and histological analysis). A clinical retrospective cohort study of 52 ovarian EMs patients and 21 controls with available preoperative serum samples was carried out (2013-2017). The follow-up period ended either at the time of recurrence or on 31 December 2018.

**Participants/materials, setting, methods:**Ecto-ESC-derived EVs (EV/Ecto-ESCs) were characterized by nanoparticle tracking analysis, transmission electron microscopy and western blotting. EV internalization by THP-1 cells, which are the most widely used primary human macrophages model, was detected by fluorescence labelling. After EV treatment, THP-1 cell polarization was detected by quantitative real-time PCR (qRT-PCR) and western blot analyses of CD86 (M1-related marker) and CD206 (M2-related marker). LGMNP1 mRNA expression level in EVs from both primary ectopic endometrioc stromal cells and serum was examined using qRT-PCR. Additionally, the expression of LGMN, the downstream target gene of LGMNP1, in THP-1 cells was evaluated using qRT-PCR and western blotting. Kaplan-Meier and multivariate Cox regression analyses were applied to evaluate the independent predictive factors of EMs recurrence-free survival. A novel nomogram model based on serum EV-LGMNP1 was then formulated to predict EMs recurrence.

**Main results and the role of chance:**In vitro assays demonstrated that EV/Ecto-ESCs drove macrophages towards an M2-like phenotype. Moreover, LGMNP1 contributed to EV/Ecto-ESC-induced M2 macrophage polarization by upregulating LGMN mRNA expression levels. Clinically, serum EV-LGMNP1 was more highly expressed in recurrent EMs patients than in controls and EMs patients without recurrence. Survival analysis and our novel nomogram reconfirmed that serum EV-LGMNP1 was a novel promising and meaningful non-invasive biomarker for predicting EMs recurrence.

**Large scale data:**N/A.

**Limitations, reasons for caution:**In vitro experiments were only performed on samples from two patients with ovarian endometriosis, and a larger sample size is needed. ESCs isolated from the eutopic endometrium of EMs and non-EMs patients should be studied in the future. Additionally, in vitro experiments should be performed using endometrial epithelium cells and further in vivo experiments, such as using mice endometriotic models to investigate whether EV/Ecto could induce M2 macrophage polarization, should be conducted. Moreover, multicentre, large-sample data are needed to validate our predictive nomogram model.

**Wider implications of the findings:**Our study provides novel insights into the mechanism of M2 polarization involved in ovarian EMs progression mediated by an 'EV-shuttled pseudogene LGMNP1' mode. In addition, serum EV-LGMNP1 may serve as a novel non-invasive biomarker for predicting recurrence, providing a new therapeutic target for ovarian EMs.

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**Keywords:**ectopic microenvironment; extracellular vesicles; legumain pseudogene 1; macrophage polarization; ovarian endometriosis; pseudogene.