

miRNA levels are associated with body mass index in endometrial cancer and may have implications for therapy.Ravegnini G, Gorini F, Coada CA, et al. *Cancer Sci.* 2024 Jan 9. doi: 10.1111/cas.15977.**要旨**

子宮内膜癌は高所得国に多い婦人科癌であり、肥満などのリスク因子増加により発症率が増加する。著者らは 29 例の肥満関連内膜癌組織 (BMI \geq 30) と 55 例の非肥満関連類内膜癌組織 (BMI $<$ 30)、9 例の非癌肥満女性の内膜の FFPE から RNA を抽出し、TaqMan Low density array Advanced miRNA array (target miR; 384) を用いて各群の microRNA (miR) の発現状態を比較し、候補 miR を同定した後に、qRT-PCR で validation を行った。また、TCGA コホート中の BMI \geq 30 の内膜癌 235 例と BMI $<$ 30 の内膜癌 149 例を用いて validation を行った。

miR array を用いた肥満関連内膜癌症例群と非癌肥満群の比較により、PCA 分析で両群が異なる事が示され (Fig. 1A)、両群間で 62 miR が有意に発現変動 (up-regulation 51, down-regulation 11) していた (Fig. 1B)。肥満関連内膜癌群と非肥満関連類内膜癌群の比較では、PCA 分析で両群の重複が見られ、miR プロファイルの類似性があることが示された (Fig. 2A)。両群間で多重検定補正した場合、有意な miR は存在しなかったが、未補正 p 値で有意であった 11 miR はいずれも up-regulation していた (Table 1, Fig. 2B)。発現変動を示した miR のうち miR-199a-5p, miR-449a, miR-2110 の 3 miR を qRT-PCR で validation したところ、miR-449a のみが有意な発現変動を示した。TCGA コホートでも miR-199a-5p, miR-449a, miR-2110 の 3 miR は、両群間で有意な発現変動を示していた (Fig. 3)。TargetScan を用いて miR-199a-5p, miR-449a の標的 mRNA を予測し、さらに TCGA コホートの RNA-Seq データを用いて miR と mRNA の逆相関関係に着目し、さらに候補標的 mRNA を絞り込み、潜在的な miR-mRNA ネットワークを予測した (Fig. 4)。miR-199a-5p の標的 mRNA に基づく GO 解析では有意なものはみられなかったが、miR-444a の標的 mRNA に基づく GO 解析では肥満調節関連する GO が call された (Table 2)。TCGA コホートを用いて臨床病理学的事項との関係を検討すると、miR-199a-5p, miR-449a は低異型度、類内膜癌で有意に高値であり (SupFig. 5A-B)、miR-449b はリンパ節陰性例で有意に高値であった (SupFig. 5C)。また、miR-449a 高発現群は OS を延長した (SupFig. 5D)。

Take Home Message

抄読会 令和6年2月9日

肥満がリスク因子となる低異型度類内膜癌は、miR-449a の高発現による肥満関連 mRNA の脱制御環境下にある可能性がある。

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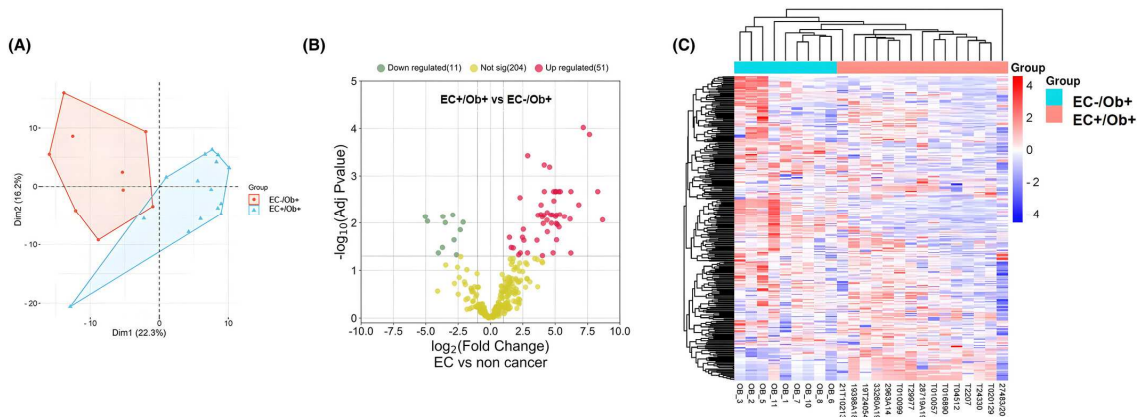


Fig. 1. Global microRNA (miRNA) expression analysis. (A) Global views of miRNA expression using principal component analysis of the 24 samples analyzed via the TaqMan Low density array. Each triangle or circle represents the collective expression of all miRNAs in each sample. Each color is indicative of a different group (EC-/Ob+, samples from noncancer patients who were obese; EC+/Ob+, samples from endometrial cancer (EC) patients who were obese). (B) Volcano plot showing the relationship between fold change and statistical significance. Green and red dots represent differentially expressed mRNAs with statistical significance. (C) Heatmap showing differential miRNA expression in the EC group (EC+/Ob+) versus the control group (EC-/Ob+). Each row represents an miRNA and each column represents a sample.

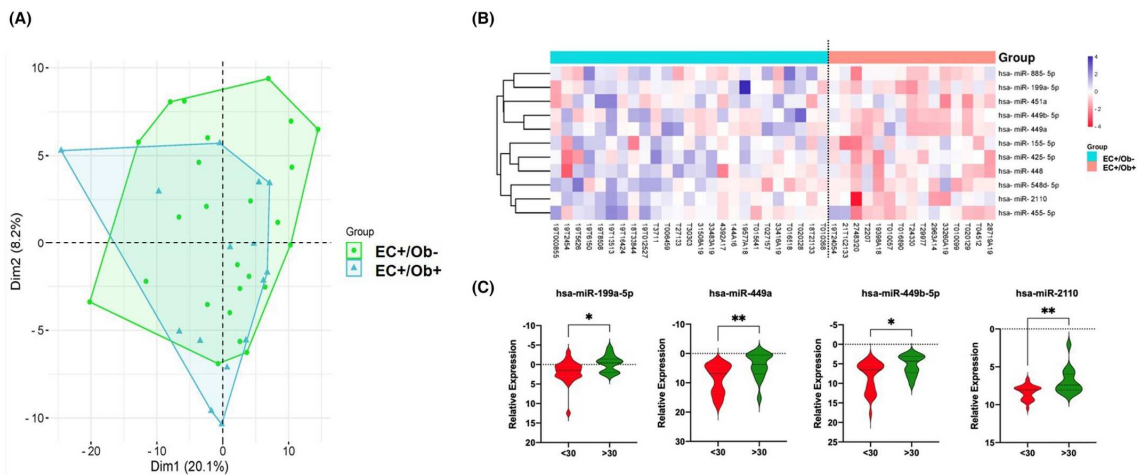


Fig. 2. Global microRNA (miRNA) expression analysis. (A) Principal component analysis of 40 samples analyzed via the TaqMan Low density array. Each triangle represents the collective expression of all miRNAs in one sample. Each color is indicative of a different group (EC+/Ob-, endometrial cancer [EC] samples with BMI < 30; EC+/Ob+, EC samples with BMI \geq 30). (B) Heatmap showing differential miRNA expression in EC patients who were (EC+/Ob+) or were not (EC+/Ob-) obese. Blue indicates lower expression and red represents higher expression. Each row represents a miRNA and each column represents a sample. (C) Expression levels of the four topmost significant miRNAs in EC patients stratified by BMI.

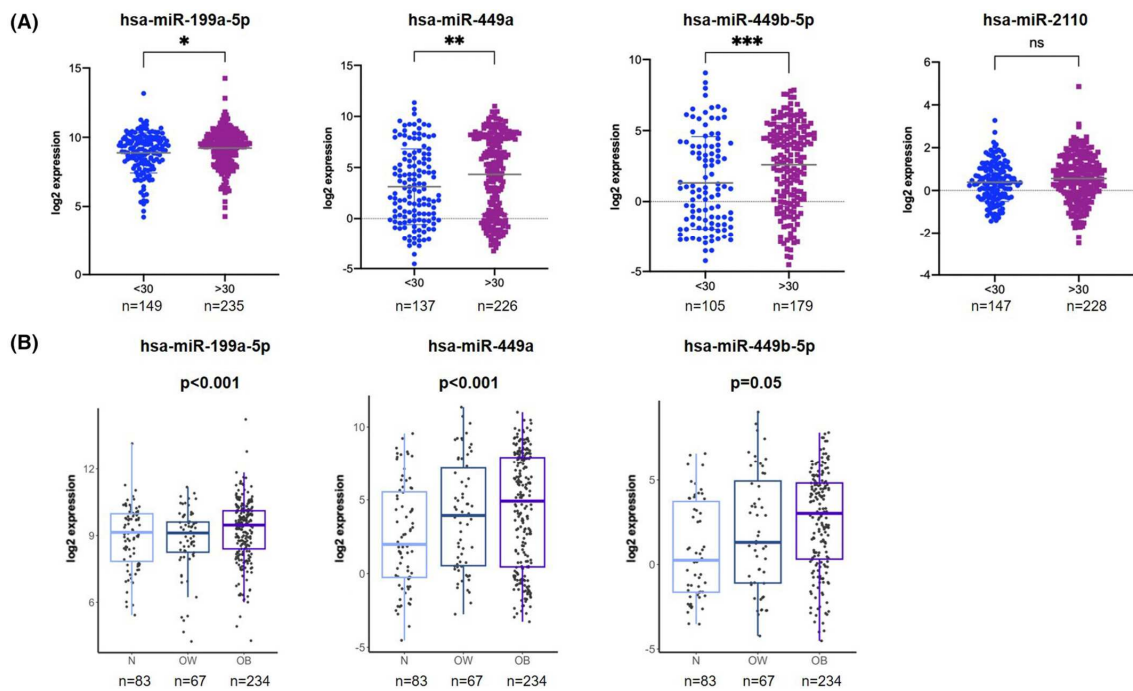


Fig. 3. (A) Analysis of miR-199a-5p, miR-449a, miR-449b-5p, and miR-2110 in TCGA endometrial cancer cohort stratified based on BMI \geq or $<$ 30. miRNA levels are expressed as log₂ expression: *P < 0.05, **P < 0.01, ***P < 0.001. (B) Expression levels of miR-199a, miR-449a, and miR-449b-5p in the TCGA cohort, stratifying the patients as normal weight (N; BMI < 25, n = 83), overweight (OW; 30 > BMI \geq 25, n = 67), and obese (OB; BMI \geq 30, n = 234). miRNA levels are expressed as log₂ expression.

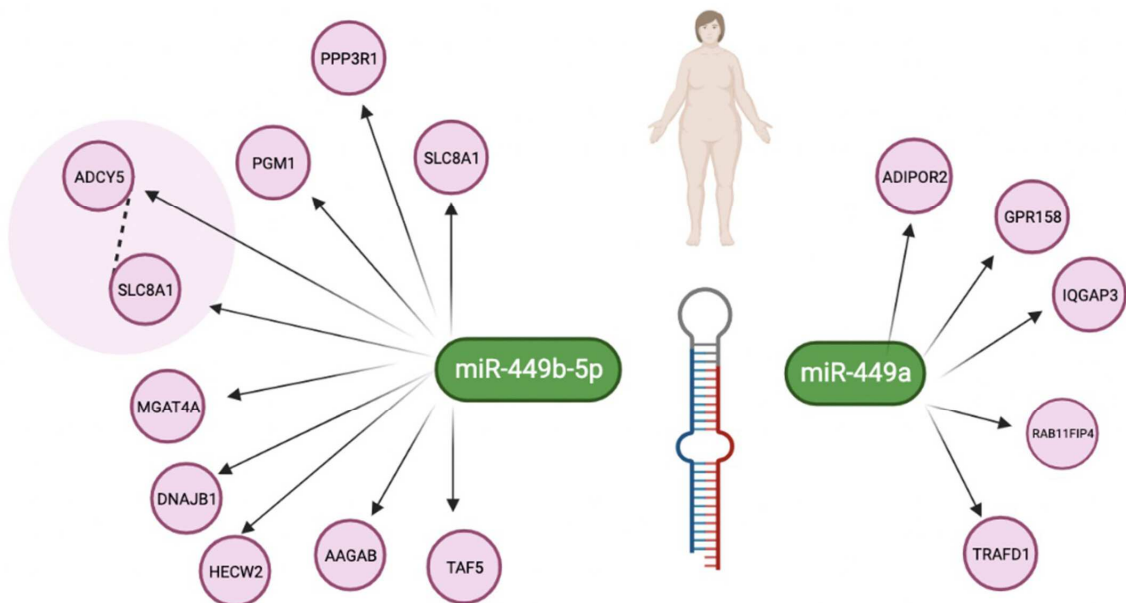
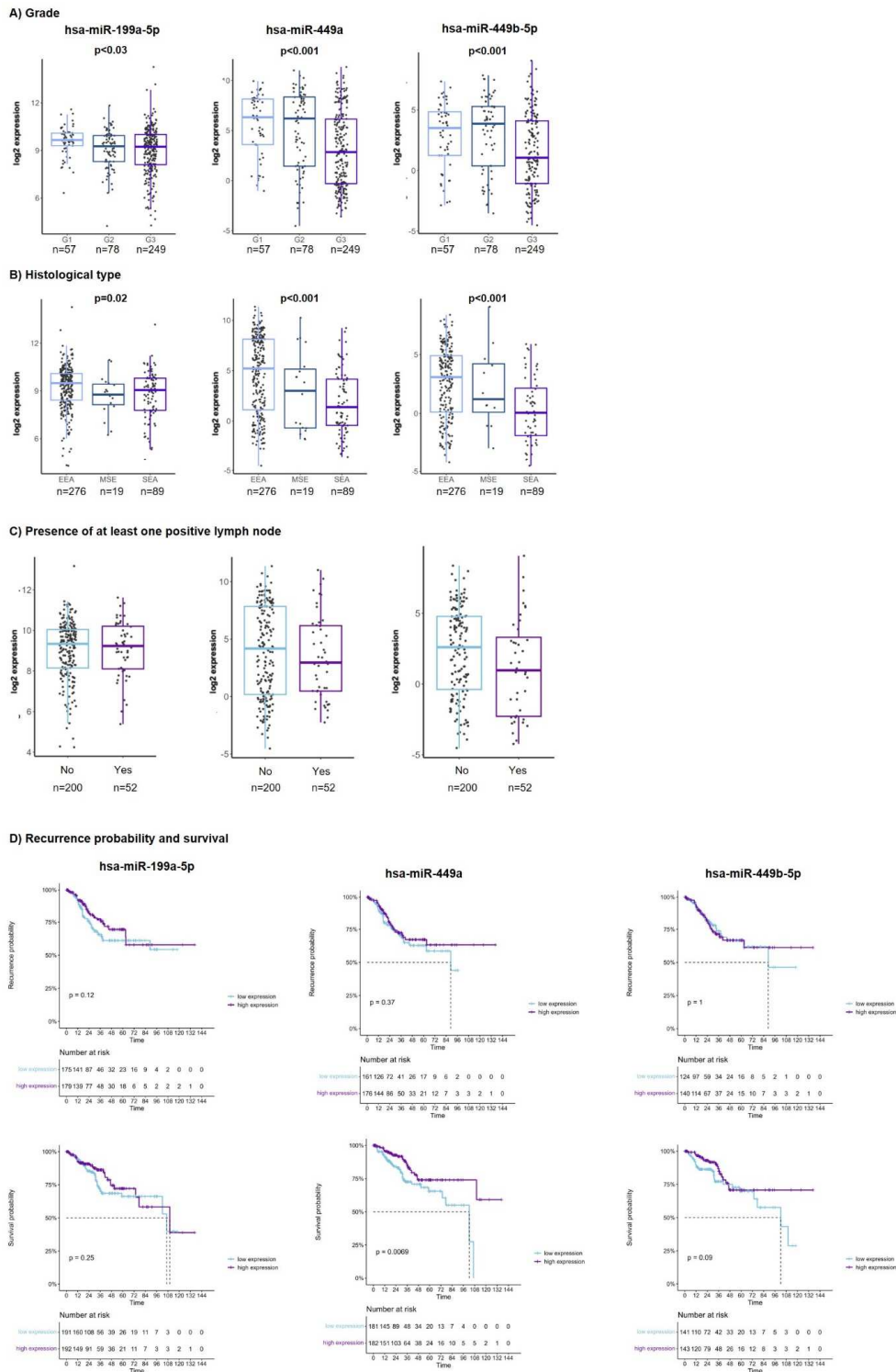


Fig. 4. Potential miRNA-mRNA networks. miR-449a and miR-449b-5p, belonging to the same miRNA family, were upregulated in EC+/Ob+ patients while their mRNA targets were downregulated. Each arrow indicates an upregulated target gene, while the dashed line represents a protein-protein association according to the String database.



Supplementary Figure 5. Association of miR-449a/miR-449b-5p and miR-199a with tumor grade (A), histological type (B) and lymph nodes status in the TCGA cohort C). D) Association of miR-449a/miR-449b-5p and miR-199a with recurrence probability and overall survival in the TCGA cohort. G1: grade 1; G2: grade 2; G3: grade 3; EEA: endometrioid endometrial adenocarcinoma; MSE: mixed serous and endometrioid; SEA: serous endometrial adenocarcinoma.

TABLE 1 Differentially expressed microRNA (miRNA) endometrial cancer (EC) patients who were and were not obese (Ob⁺ or Ob⁻).

miRNA ID	P value	Fold change (EC ⁺ /Ob ⁺ vs. EC ⁺ /Ob ⁻)
hsa-miR-2110	0.0022	1.2
hsa-miR-449a	0.0045	4.5
hsa-miR-449b-5p	0.009	3.0
hsa-miR-199a-5p	0.016	2.2
hsa-miR-455-5p	0.025	0.9
hsa-miR-548d-5p	0.026	0.9
hsa-miR-885-5p	0.031	2.1
hsa-miR-425-5p	0.033	0.8
hsa-miR-155-5p	0.041	0.9
hsa-miR-451a	0.043	1.3
hsa-miR-448	0.045	1.0

TABLE 2 Gene ontology of the miR-499a/499b targets with lower expression in endometrial cancer patients who were and who were not obese.

Category	ID	Name	P value	q value FDR B&H
GO:MF	GO:0005516	Calmodulin binding	0.0005	0.031
GO:MF	GO:0070856	Myosin VI light chain binding	0.0007	0.031
GO:MF	GO:0086038	Calcium: sodium antiporter activity involved in regulation of cardiac muscle cell membrane potential	0.0014	0.031
GO:MF	GO:0099580	Ion antiporter activity involved in regulation of postsynaptic membrane potential	0.0014	0.031
GO:MF	GO:0055100	Adiponectin binding	0.0021	0.031
GO:MF	GO:0008453	Alanine-glyoxylate transaminase activity	0.0021	0.031
GO:MF	GO:0070853	Myosin VI binding	0.0021	0.031
GO:MF	GO:1905060	Calcium: cation antiporter activity involved in regulation of postsynaptic cytosolic calcium ion concentration	0.0021	0.031
GO:MF	GO:0097003	Adipokinetic hormone receptor activity	0.0028	0.031
GO:MF	GO:0008454	Alpha-1,3-mannosylglycoprotein 4-beta-N-acetylglucosaminyltransferase activity	0.0028	0.031
GO:MF	GO:0008597	Calcium-dependent protein serine/threonine phosphatase regulator activity	0.0035	0.031
GO:MF	GO:0008294	Calcium- and calmodulin-responsive adenylate cyclase activity	0.0035	0.031
GO:MF	GO:0004614	Phosphoglucomutase activity	0.0035	0.031
GO:MF	GO:0032027	Myosin light chain binding	0.0055	0.047
GO:CC	GO:0042383	Sarcolemma	0.0002	0.028

Abbreviations: CC, cellular component; FDR B&H, False discovery rate Benjamini and Hochberg; GO, gene ontology; MF, molecular function.