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ORIGINAL PAPER

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Abstract Metastin/kisspeptin is encoded by *KISS1* and functions as an endogenous ligand of GPR54. Interaction of metastin with GPR54 suppresses metastasis and also regulates release of gonadotropin-releasing hormone, which promotes secretion of estradiol (E2) and progesterone (P4). We have previously demonstrated epigenetic regulation of *GPR54* in endometrial cancer and the potent role of metastin peptides in inhibiting metastasis in endometrial cancer. However, little is known about how the metastin–GPR54 axis is regulated in the endometrium, the precursor tissue of endometrial cancer. Endometrial stromal cells (ESCs) and endometrial glandular cells (EGCs) within the endometrium show morphological changes when exposed to E2 and P4. In this study, we show that metastin expression is induced in ESCs through decidualization, but is repressed in glandular components of atypical endometrial hyperplasia (AEH) and endometrial cancer relative to EGCs. The promoter of *GPR54* is unmethylated in normal endometrium and in AEH. These results indicate metastin may function in decidualized endometrium to prepare for adequate placentation but this autocrine secretion of metastin is deregulated during oncogenesis to enable tumor cells to spread.

Keywords Metastin · GPR54 · Decidualization · Endometrium · Endometrial cancer

Introduction

Uterine endometrial cancer is the leading cause of malignant gynecological disease, and invasion from the endometrium into the deep myometrium and lymphovascular space is a critical life-threatening risk factor that is frequently followed by metastasis. In clinical settings, the rate of nodal metastases approaches 26 % in cases with deep myometrial invasion, and the 5-year survival rate for these cases is 30 % less than those without nodal metastasis. It is crucial to establish novel therapeutic approaches to prevent tumor invasion and metastasis in order to improve the prognosis of patients with endometrial cancer. Various molecules associated with tumor invasion have been intensively investigated as potential therapeutic targets. However, none of these efforts to establish metastasis-suppressing therapies have been successfully translated to clinical practice, even though novel metastasis-regulating molecules or pathways have been identified.

KISS1 was originally described as a metastasis suppressor in melanoma, and has since been identified as a candidate metastasis suppressor in several cancers [1, 2]. The *KISS1* gene encodes a number of metastin/kisspeptin peptides via alternative splicing that function as

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endogenous ligands for the G protein-coupled receptor, GPR54. One of the splice variants, metastin-10, can be chemically synthesized and has been proved safe for administration in vivo. We have previously described that decreased expression of GPR54 is frequently observed in histologically high-grade endometrial cancers and is a poor prognostic factor relevant to both invasive and metastatic capacity for such subtypes [1]. We also showed that metastin was ubiquitously expressed in cancer stromal cells and that administration of metastin-10 could suppress extrauterine tumor metastasis via the metastin–GPR54 axis. However, little is known about how the metastin–GPR54 axis is regulated in the human endometrium, the source of endometrial cancer.

The human endometrium exhibits dynamic changes in the shape and function of both endometrial stromal cells (ESCs) and endometrial glandular cells (EGCs) under ovarian hormonal controls. Previous reports revealed that in ESCs and EGCs there were significant changes in expression observed through the menstrual cycle for several proteins associated with cellular migration, including E-cadherin, Slug, Snail, and S100. There is cross-talk in neuroendocrine cells between the expression of metastin and GPR54 and the release of gonadotropin-releasing hormone (GnRH) to control the timing of puberty and the secretion of estradiol (E2) and progesterone (P4). ESCs demonstrate decidualization and secrete prolactin in late-secretory phase in response to long E2 and P4 exposure, and this phenomenon evokes the notion to consider the endometrium as an endocrine organ. There are two reports describing the expression of metastin and GPR54 in the endometrium [3, 4], but their conclusions are different and these reports did not investigate expression of these proteins specifically in ESCs and EGCs. In this study, the expression of metastin and GPR54 in ESCs and EGCs was investigated to clarify the regulation of the metastin–GPR54 axis in the endometrium and to determine feasibility of metastin-10 use as adjuvant therapy to prevent metastasis and preserve fecundity in young endometrial cancer patients.

Materials and methods

Tissue collection

Human endometrial tissues were obtained following written consent for the use of surgical specimens from patients who underwent hysteroscopy or hysterectomy under protocols approved by the Kyoto University Institutional Review Board. As the source of normal endometrium for immunohistochemical staining, formalin-fixed paraffin-embedded specimens containing the endometrial part of the uterus were used which had been excised from patients with regular menstrual cycles and no hormonal administration but who had a cervical intraepithelial neoplasm or myoma uteri ($n = 12$, age: 32–46 years old). Each endometrial specimen was examined histologically and dated according to published criteria [5]. Non-fixed endometrial specimens for isolated cell culture were obtained by scraping part of the endometrium from patients without endometrial disease or hormonal administration (Table 1) at the time of hysterectomy or endometrial curettage as previously reported [6].

Cell culture

Isolation of endometrial stromal cells (ESCs) and glandular cells (EGCs) was performed as described previously [6, 7]. Endometrial tissues from healthy donors with normal menstrual cycles (Table 1) were minced into small pieces $<1\ mm^3$ and incubated at 37 °C in RPMI1640 (Nikken, Kyoto, Japan) supplemented with 10 % fetal bovine serum (FBS: Funakoshi, Tokyo, Japan), 0.5 % collagenase I (Wako Pure Chemicals, Osaka, Japan) and 0.05 % deoxyribonuclease I (DNAase I; Sigma-Aldrich, St. Louis, MO). After the enzymatic digestion, ESCs were present as single cells or small aggregates, which were repeatedly purified by differential sedimentation at unit gravity. On the other hand, EGCs remained in larger clumps, and these clumps were re-digested into a single cell suspension and incubated on a collagen type IV-coated plate (IWAKI, Tokyo, Japan). After >90 % purification [6, 7], ESCs and EGCs were independently maintained in the conditioned medium, phenol red-free RPMI1640 (Invitrogen, Carlsbad, CA) supplemented with 5000 U/ml penicillin, 5000 μg/ml streptomycin (Nacalai tesque, Kyoto, Japan), and 2 % charcoal/dextran-treated FBS (Thermo Fischer Scientific Inc., Walthum, MA). Endometrial stromal cells and EGCs were incubated for 12 days in this conditioned medium with 10^{-8} M 17-β estradiol (E2; Nacalai tesque) or 10^{-6} M medroxyprogesterone acetate (MPA; Sigma-Aldrich) alone, or with both reagents for further experimentation as previously reported [8–11]. These experiments were carried out independently three times, each in triplicate. An immortalized human endometrial glandular cell line, hEM cells, and a grade 1 endometrioid adenocarcinoma cell line, Ishikawa cells, were maintained as previously reported [1] and used for analysis after incubation in the conditioned media.

Real-time quantitative PCR analysis

Total RNA was extracted from the cultured cells or incised tissues with TRIzol reagent (Invitrogen, Carlsbad, CA) using the manufacturer's recommended protocol. First-

Table 1 Characteristics of donors with cyclic menstrual cycles who provided their endometrial tissue for the primary culture of ESCs and EGCs

Patient	Age	Cycle date	Procedure	Surgical indication	ESCs	EGCs
1	47	9	TAH	CIN3	+	−
2	43	17	TAH	Myoma uteri	+	−
3	34	20	TAH + RSO + pOM	Right ovarian tumor	+	+
4	33	10	RH + BSO + PeN	Cervical cancer stage Ib1	+	−
5	34	15	Hysteroscopy + curettage	Endometrial thickness	+	+
6	45	23	TAH	Myoma uteri	−	+
7	45	22	Hysteroscopy + curettage	Endometrial thickness	−	+

TAH total abdominal hysterectomy, *RSO* right salpingo-oophorectomy, *pOM* partial omentectomy, *RH* radical hysterectomy, *BSO* bilateral salpingo-oophorectomy, *PeN* pelvic lymphadenectomy, *CIN3* cervical intraepithelial neoplasia grade 3, *ESCs* endometrial stromal cells, *EGCs* endometrial glandular cells

strand cDNA was synthesized using the transcriptor high-fidelity cDNA synthesis kit (Roche, Basel, Switzerland). To monitor gene expression, quantitative reverse transcriptase (RT)-PCR amplification of human *IGFBP-1*, *Metastin*, *GPR54* and *GAPDH* mRNAs was performed using the LightCycler 480 II system (Roche). Primers were designed using Universal Probe Library Assay Design Center (https://qpcr.probefinder.com/organism.jsp) and their sequences are as follows: IGFBP-1, 5′-CCA TGT CAC CAA CAT CAA AAA-3′ (forward), 5′-CCT TGG CTA AAC TCT CTA CGA CTC-3′ (reverse); Metastin, 5′-GGT GGT CTC GTC ACC TCA G-3′ (forward), 5′-CTA GAA GTG CCT TGA GGC TTG-3′ (reverse); GPR54, 5′-TTC ATG TGC AAG TTC GTC AAC-3′ (forward), 5′-CAC ACT CAT GGC GGT CAG-3′ (reverse); GAPDH, 5′-AGC CAC ATC GCT CAG ACA C-3′ (forward), 5′-GCC CAA TAC GAC CAA ATC C-3′ (reverse). Cycling parameters were 95 °C for 10 min and 45 cycles of 95 °C for 10 s, 60 °C for 30 s and 72 °C for 1 s, followed by a cooling cycle of 40 °C for 30 s. The expression values for human *IGFBP-1*, *Metastin*, and *GPR54* mRNAs were estimated by dividing their threshold cycle (Ct) values by the GAPDH Ct values. The methylation status of the *GPR54* promoter in endometrial tissues (5 normal and 2 atypical endometrial hyperplasia; AEH) was examined by MS-PCR as previously described [1].

Immunohistochemistry

Immunohistochemical staining was done using the streptavidin–biotin peroxidase complex method as previously reported [12]. An endogenous peroxidase block was followed by nonspecific background blocking and incubation with a 1:100 dilution of anti-human KISS1 monoclonal antibody H00003814-M05 (Abnova, Taipei, Taiwan) or a 1:100 dilution of anti-human GPR54 polyclonal antibody AKR-001 (Alomone Labs, Islael). The primary antibody was omitted for negative controls.

Microarray analysis and statistical analysis

GSE12446 and GSE29981, two independent endometrial tissue gene expression microarray datasets, were obtained from the gene expression omnibus website (http://www.ncbi.nlm.nih.gov/geo).

For statistical analysis, Mann–Whitney *U* test or Fisher's exact test was performed using GraphPad Prism 5 software. Probability values below 0.05 were considered significant.

Results

Expression of metastin and GPR54 in endometrial tissues

The expression of metastin and GPR54 was assessed by immunohistochemistry of proliferative phase ($n = 5$), early secretory phase ($n = 3$), late-secretory phase ($n = 4$) and menopausal ($n = 2$) endometrial tissues. Metastin exhibited weak expression in epithelial glands and was nearly absent in ESCs during the proliferative phase (Fig. 1a). This expression pattern was similar in the early secretory phase, but metastin expression in ESCs was prominent in the decidualized portion at late-secretory phase endometrial tissues (Fig. 1a). After menopause, the endometrium does not exhibit expression of metastin or GPR54, both in epithelial glands and stroma (Fig. 1a).

We previously reported on GPR54 expression in low-grade endometrioid adenocarcinoma [1]. To compare the intensity of expression, we examined staining of endometrium and adjacent grade 1 endometrioid adenocarcinoma on the same slide. The patient was 43 years old, and the non-cancerous endometrium was thick, exhibiting a mid-secretory appearance. GPR54 expression was weaker in the epithelial glands than in the adenocarcinoma lesion,

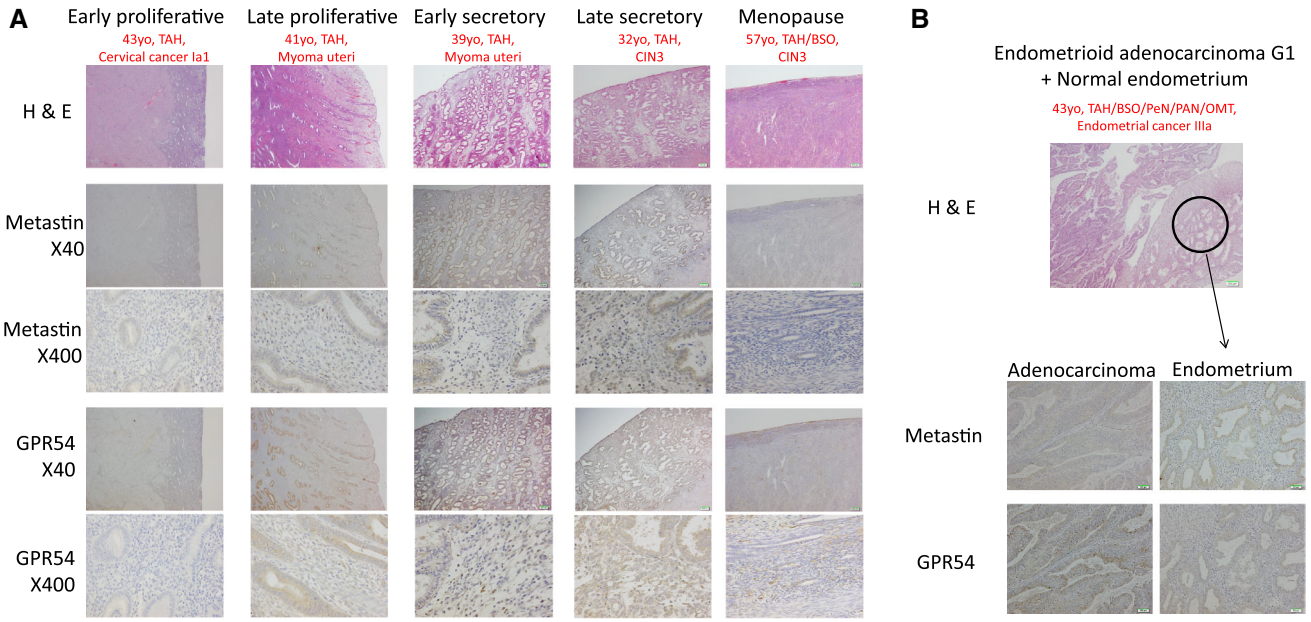

Fig. 1 Immunohistological analysis of metastin and GPR54 in human endometrial tissues. **a** Representative micrographs of metastin and GPR54 expressions in each menstrual phase. Metastin staining is weak in the epithelial glands but nearly absent in ESCs during the proliferative and early secretory phases, while metastin expression in ESCs is prominent in the decidualized component during late-secretory phase. No expression of metastin or GPR54 is observed both in epithelial glands and stroma during the menopausal phase. **b** Comparison of metastin and GPR54 expressions in co-localized endometrial tissue and endometrial cancer grade 1. GPR54 is weakly expressed in the epithelial glands relative to the adenocarcinoma lesion, whereas metastin expression is weak in both components. *H&E* hematoxylin and eosin staining, *yo* years old, *CIN3* cervical intraepithelial neoplasm grade 3, *TAH* total abdominal hysterectomy, *BSO* bilateral salpingo-oophorectomy, *PeN* pelvic lymphadenectomy, *PAN* paraaortic lymphadenectomy, *OMT* omentectomy

whereas metastin expression was weak in both regions (Fig. 1b).

Primary culture of endometrial cells and analysis of metastin and GPR54 expressions in ESCs

Primary cultures of endometrial tissues were established for seven donors listed in Table 1. None of these donors had a menstrual disorder or endometrial malignancy. Viable ESCs and EGCs were successfully isolated from five donors whose endometrium was in secretary phase, four in proliferative phase and four in early secretary phase.

Isolated ESCs were treated with hormone-conditioned medium for 12 days, and mRNA expression was examined by quantitative RT-PCR. *IGFBP1* is induced in ESCs through decidualization [13]. IGFBP1 expression was significantly augmented with E2 plus MPA treatment ($p = 0.0264$, Fig. 2a), while there was no significant induction with E2 or MPA alone ($p = 0.3929$). Mimicking decidualization, metastin expression in cultured ESCs was induced through treatment with E2 plus MPA ($p = 0.0179$, Fig. 2b). In contrast, GPR54 expression in ESCs was lower than that in an endometrioid adenocarcinoma grade 1 cell line, Ishikawa cells, and this was not augmented with treatment.

Metastin and GPR54 expressions in EGCs

We were unable to isolate EGCs from proliferative phase endometrium probably owing to limited glandular components. Metastin expression in endometrial glands appeared relatively higher during late-secretory phase than proliferative phase (Fig. 1a), and that in primarily cultured EGCs derived from secretory phase endometrium (sec-EGCs) was almost equivalent to that in primarily cultured ESCs (Supplementary Fig. 1A). In contrast with the result from ESCs, metastin expression in sec-EGCs was not augmented by treatment with E2 and MPA (Supplementary Fig. 1B). To consider the possibility that sec-EGCs were already differentiated, hEM cells were used as an alternative. However, metastin expression was not induced by E2 with or without MPA in hEM cells (Fig. 2b).

GPR54 expression in sec-EGCs appeared higher than that in ESCs, but was much lower than that in Ishikawa cells (Supplementary Fig. 1A). GPR54 expression in sec-EGCs or hEM cells was also not significantly augmented by E2 with or without MPA (Fig. 2b and Supplementary Fig. 1B). In a microarray dataset of normal endometrial tissues (GSE29981), GPR54 expression in micro-dissected endometrial glands gradually increased through the proliferative phase, and high GPR54 expression was more

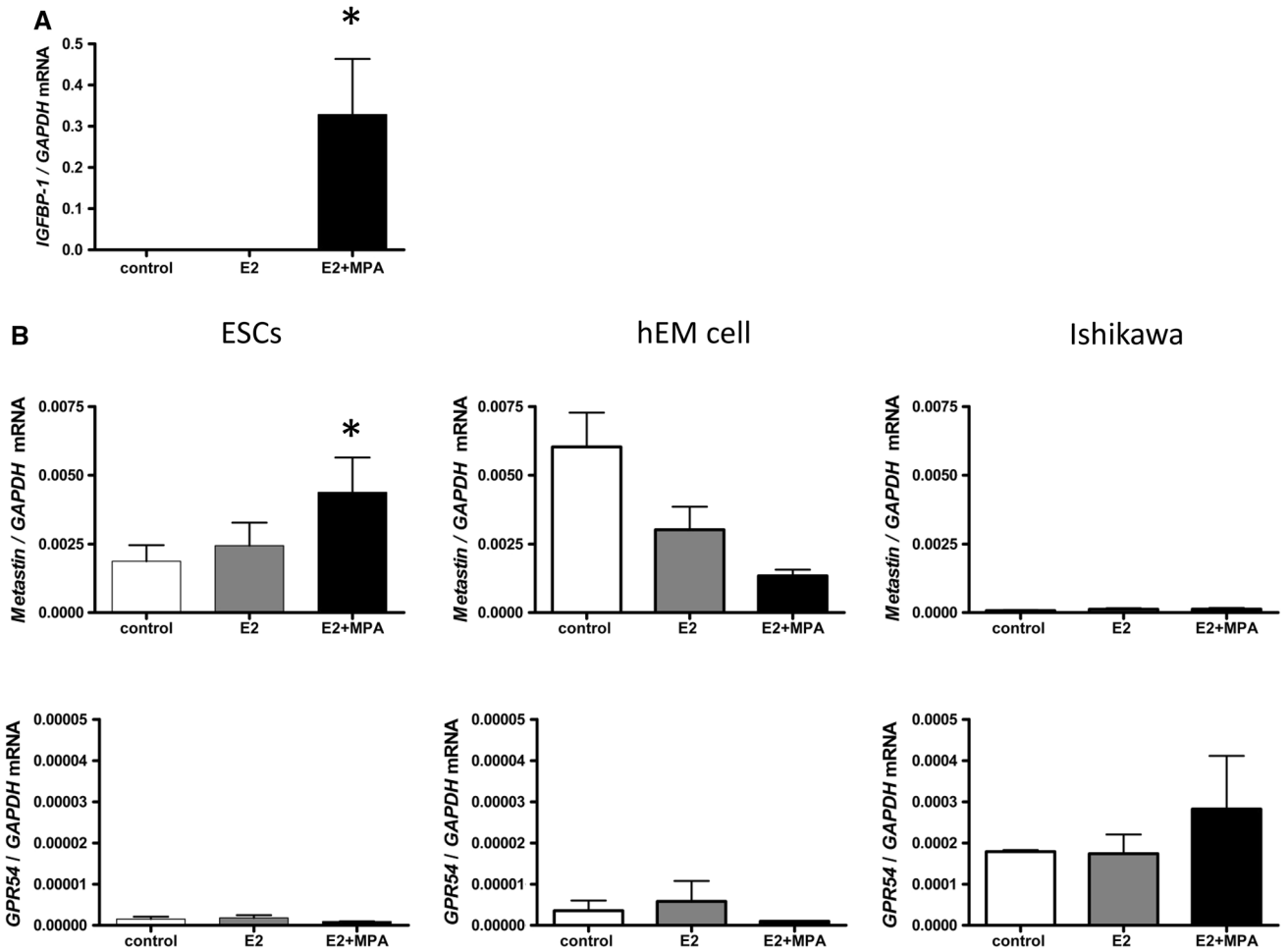

Fig. 2 mRNA expression in ESCs through 12 days of E2 and MPA treatment. **a** IGFBP1 expression in ESCs is significantly augmented following treatment with E2 plus MPA (*: $p = 0.0264$), while no induction is observed with E2 alone. **b** Metastin and GPR54 expressions in cultured ESCs (*left panels*), hEM cells (*middle panels*), and Ishikawa cells (*right panels*). Metastin is induced in ESCs following treatment with E2 plus MPA (*: $p = 0.0179$) but not in other cells. GPR54 expression is lower in ESCs and hEMA cells than in Ishikawa cells, and is not augmented following treatment with E2 alone or with E2 plus MPA

frequently observed from late-proliferative phase to early secretory phase (Supplementary Fig. 2A, $p = 0.01$, Fisher's exact test). In another microarray dataset of whole endometrium treated with E2 and MPA (GSE12446), high metastin expression was more frequently observed (Supplementary Fig. 2B, $p = 0.05$, Fisher's exact test). In GSE12446, *prolactin* expression was significantly higher following E2 and MPA treatment ($p = 0.0001$), indicating that the change in metastin expression occurred with decidualization.

GPR54 expression differences between normal endometrium and endometrial hyperplasia and promoter methylation status

GPR54 expression was markedly higher in grade 1 endometrioid adenocarcinoma (EmG1) and in Ishikawa cells relative to EGCs (Fig. 1a and Supplementary Fig. 1A). As EGCs are thought to transform into endometrial hyperplasia and EmG1 in a stepwise manner through the oncogenic process, *GPR54* expression in atypical endometrial hyperplasia (AEH) was examined by comparing alongside that in the normal endometrial glandular portion of the same specimens. Among seven AEH cases (age 50.1 ± 3.4 years), two cases exhibited higher relative *GPR54* expression in atypical hyperplastic glands than adjacent EGCs, whereas most cases exhibited similar expression in both. In two peri-menopausal patients, metastin was expressed in EGCs of dilated and elongated endometrial glands although it was scant in AEH (Fig. 3a).

The intensity of *GPR54* expression in endometrial cancer was attenuated by methylation in the promoter region although this region was unmethylated in hEM [1]. We therefore analyzed the methylation status of the *GPR54*

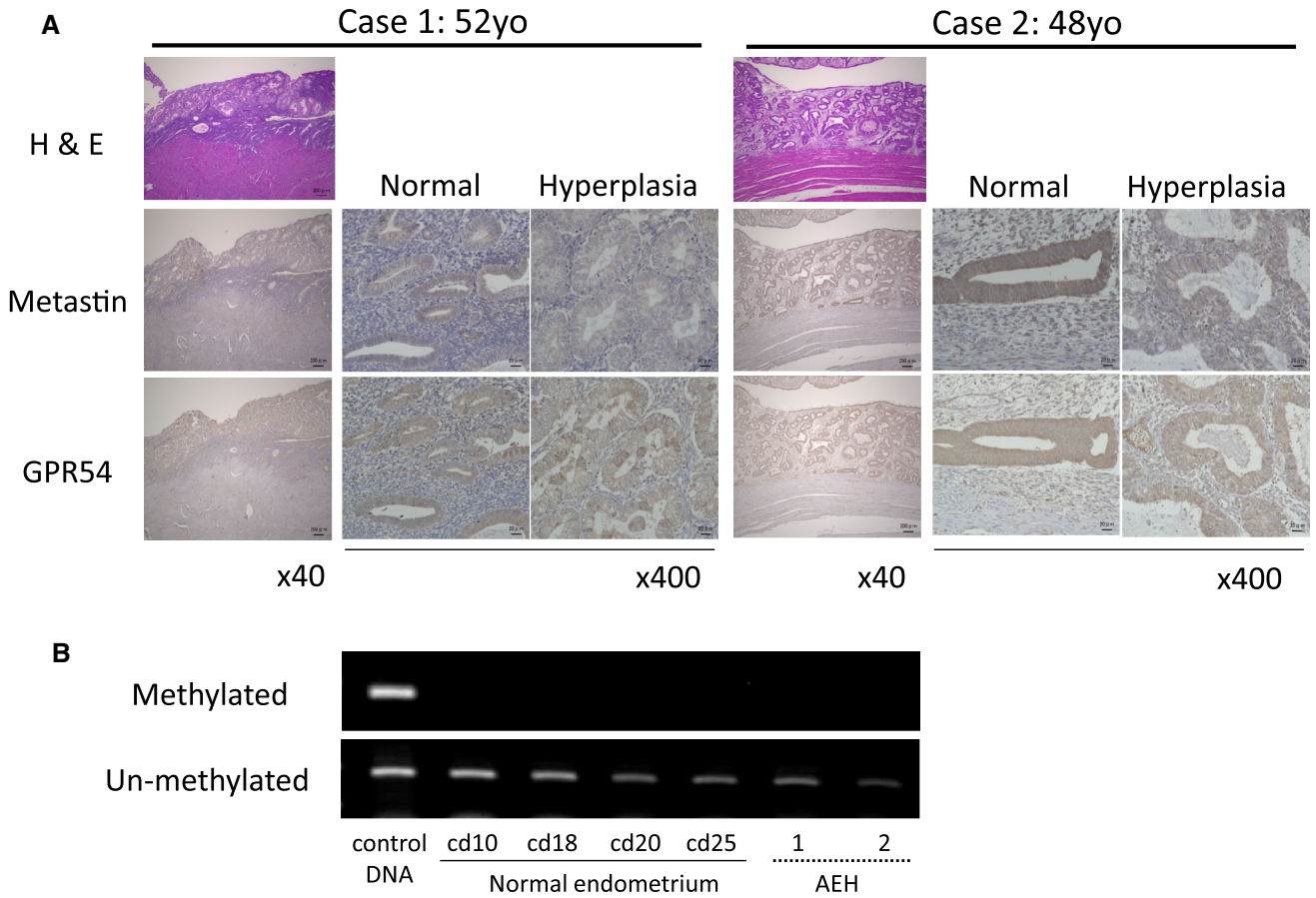

Fig. 3 Metastin and GPR54 expressions in pre-cancerous lesions. **a** Metastin and GPR54 expressions in endometrial hyperplastic lesions adjacent to normal endometrial glandular component from two representative AEH cases. GPR54 expression in the AEH lesion is higher relative to the adjacent EGCs, whereas metastin expression is scant in the AEH portion. **b** Methylation status of the GPR54 promoter region in AEH and in normal endometrium. Methylation is not observed in AEH cases or in normal endometrium specimens. *cd* cycle date

promoter region in AEH cases using MS-PCR. No *GPR54* promoter methylation was observed in AEH cases or in normal endometrium (Fig. 3b).

Discussion

Metastin is a biologically active peptide that was originally described as a metastasis suppressor and was identified from human placental extracts in 2001 [2], but its significance has been more recently described in reproductive research. A couple of studies have shown that sexual maturation does not occur in a pedigree in which there is loss of GPR54, an endogenous receptor of metastin, and *GPR54*-deficient mice do not display any of the characteristic physiological changes associated with sexual maturation of the testes and ovaries [14, 15]. To date, GPR54 is known to be expressed on neurons that secrete gonadotropin-releasing hormones (GnRH), and plays an important role in reproductive function through regulation by its ligand, metastin [16]. Metastin is a neuroendocrine peptide that functions upstream of the hypothalamus–pituitary–gonadal axis to regulate pulsatile secretions of GnRH from the hypothalamus. There are two types of metastin-secreting neurons in the arcuate nucleus (Arc) and the anteroventral periventricular nucleus (AVPV) in rodents [17, 18]. These neurons co-express estrogen receptor alpha (ERα) [19], and E2 differentially regulates the expression of *metastin* mRNA through ERα in distinct forebrain nuclei [20]. These metastin-secreting Arc and AVPV neurons are regulated by E2 in an inhibitory and stimulatory manner, respectively. Specifically, E2-mediated negative feedback on gonadotropin secretion in the Arc inhibits pulsatile GnRH/LH secretion but stimulates follicle maturation in the follicular period, whereas E2-mediated positive feedback in the AVPV induces the GnRH/LH surge resulting in ovulation [18, 21]. Thus, it is thought that metastin peptides are produced mainly in hypothalamus in vivo, but metastin is also present in peripheral organs such as the pancreas, which also implies extra-hypothalamic

production of metastin. In addition, serum metastin levels increase dramatically during pregnancy, implying that the metastin source is the placenta [22]. On the other hand, circulating metastin levels increase significantly in adult women compared with prepubertal and pubertal girls. Metastin levels are stable in males throughout puberty and adulthood [23], although metastin-10 stimulates gonadotropin release even in men [24]. These results support the notion that metastin may not only be produced in the hypothalamus but also produced in female-specific organs responsive to ovarian hormones.

The uterus and endometrium are regulated coordinately by the cyclical ovarian hormones, estrogen and progesterone. The endometrium is composed of endometrial epithelial cells (EGCs) and endometrial stromal cells (ESCs) that have various functions that through mutual interaction are geared toward receiving a fertilized egg after conception. That metastin and GPR54 are expressed by the endometrium is still not conclusive [3, 4], nor has the function of the metastin/GPR54 axis in the endometrium been clarified. We have shown that metastin is produced by EGCs and ESCs to a certain extent, and this is promoted when ESCs are decidualized by the coordinated effects of the sex steroid hormones, E2 and MPA. On the other hand, GPR54, an endogenous metastin receptor, is mainly expressed in EGCs, and GPR54 expression appears to not be affected by sex steroid hormones. Although further investigation is required to determine the mechanism, these findings suggest that endogenous metastin production is induced in ESCs through stimulation by sex steroid hormones to act on GPR54 in an autocrine/paracrine-like manner. Furthermore, this mechanism may be involved in embryo implantation and placentation, as the expression of metastin and GPR54 in the chorionic villi is prominent enough to be used as positive control in immunohistochemistry [1].

In the chorionic villus during early gestation, cytotrophoblasts adhere to the maternal decidualized endometrium and differentiate into extravillous trophoblasts (EVTs), forming a stratified structure called the cell column in which EVTs acquire the ability to invade the decidualized endometrium. In the floating villus, cytotrophoblasts differentiate into multinucleated syncytiotrophoblasts and take part in exchange of gas and nutrients [25]. Metastin is expressed in the stromal cells of the decidual endometrium and GPR54 was expressed in cytotrophoblasts [1, 22]. The reason that circulating metastin levels increase in pregnancy might result from the outer syncytiotrophoblasts being positioned adjacent to blood vessels, allowing easy passage of metastin into the maternal blood. A recent report described findings that decreased metastin expression in trophoblasts is associated with repeated pregnancy loss [26]. Another study showed that circulating metastin levels in early pregnancy are low in patients who later developed pre-eclampsia [27]. Repeated miscarriage and pre-eclampsia are considered to be a consequence of abnormal implantation of EVTs in early pregnancy. Metastin was previously reported to suppress villous motility [28], but these findings indicate that metastin secreted in decidua may adequately modulate the invasive activity of trophoblasts for ideal implantation and pregnancy maintenance.

Cancer invasion frequently shares invasive characteristics that are inherent to trophoblasts. We previously reported that GPR54 expression was inversely associated with tumor progression and histological grade of endometrial cancer [1]. With regard to GPR54-positive, low-grade cancers that comprise the majority of endometrial cancers, prognosis is fairly good and tumor invasion and metastasis are inhibited by metastin treatment. As low-grade cancers are known to arise through prolonged exposure to unopposed estrogen, it is remarkable that the source of metastin is the endometrium which is susceptible to sex hormones, and that endogenous metastin would play an inhibitory role in tumor progression. However, it has not been clarified whether or not endogenous metastin secretion is suppressed in endometrial cancers. In this study, metastin expression in endometrial cancer cells was lower than that in normal endometrium, while *GPR54* expression in cancer cells was higher than that in normal endometrium. Although it is still unclear why *GPR54* expression is paradoxically exaggerated in low-grade cancers, it is possible that prolonged estrogen exposure without progesterone might attenuate endogenous secretion of metastin in ESCs, which may make it easier for cancer cells to invade into the myometrium and metastasize beyond the uterus. Furthermore, *GPR54* expression in high-grade endometrial cancer is down-regulated through epigenetic or genetic mechanisms, and this down-regulation likely contributes to the more aggressive features of this subtype. Lymph node metastasis is a critical determinant of prognosis for women with endometrial cancers, and as this is fairly common even in low-grade subtypes that highly express GPR54, GPR54-targeted therapy is worthwhile developing for the control of lymph node metastasis.

Due to the recent increasing trends in obesity and later age at marriage, the number of young patients wishing to preserve fertility is increasing. As most of these patients bear low-grade cancers that appear localized within the endometrium by imaging, uterus-preserving therapy is sometimes employed with high-dose progestin administration and cyclic endometrial curettage. Thanks to this therapy, about 80 % of patients achieve a clinical response and more than 35 % subsequently bear children [29]. More than 40 % of patients, however, relapse during the follow-up period after achieving a clinical response. There is no

predictive marker for recurrence or preventive method that can block tumor progression during current uterus-preserving therapy [30]. Recently, several phase I–II endocrinology studies were conducted in which metastin or its analog was administered and reproducibly confirmed its safety and efficacy [31–33]. Utilizing characteristics of low-grade endometrial cancers, such as high GPR54 expression with poor metastin secretion from ESCs, metastin-10 treatment may be effective as an adjuvant therapy to prevent cancer cells from metastasizing during MPA therapy to preserve fertility in young patients.

Grade1 endometrioid adenocarcinoma is thought to arise from hyperplastic EGCs exposed to long-term unopposed estrogen. GPR54 expression was not epigenetically impaired in atypical endometrial hyperplasia (AEH), while metastin expression in AEH was lower than in the normal endometrial glandular component on the same specimen from several AEH patients. Atypical endometrial hyperplasia patients with menstrual disorders usually experience unopposed estrogen, and as such ESCs are expected to produce insufficient metastin. This attenuation of metastin may not only contribute to the pro-oncogenic status of AEH through initiation of the ability to acquire invasiveness but also to the persistent infertile status after mechanical eradication [34]. From this perspective, administration of metastin-10 might provide a means to support embryo implantation as well as to prohibit tumor progression.

In this study, we describe that metastin secretion is induced in ESCs through decidualization with E2 and MPA. Although further studies are needed, this process is expected to play an important role not only to maintain adequate placentation in pregnancy but also for the development of adjuvant therapy to prevent low-grade endometrioid adenocarcinoma from progression during fertility-sparing therapy with MPA.

Conflict of interest The authors declare no conflict of interest.

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