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Decreased hyaluronidase 1 expression is associated with early disease recurrence in human endometrial cancer

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HIGHLIGHTS

- Decreased hyaluronidase 1 is associated with invasive properties of endometrial cancer.
- Decreased hyaluronidase 1 is an independent prognostic factor for early disease recurrence.
- Hyaluronidase 2 expression is associated with different phases of endometrial cycle.

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ABSTRACT

Objective. Hyaluronidases (HYAL1 and HYAL2) are key enzymes in the degradation of hyaluronan, and their expression has been altered in various cancer types. We previously showed that hyaluronan accumulation in endometrial carcinomas was correlated with decreased mRNA expression of the *HYAL* genes. In this study, we analyzed HYAL1 and HYAL2 protein expressions in normal and precancerous endometrial tissues and in endometrial carcinomas. We also investigated whether the protein levels were associated with clinicopathological factors, invasion, and disease recurrence.

Methods. A total of 343 tissue specimens from normal, atrophic, hypertrophic, and neoplastic endometria were analyzed immunohistochemically for HYAL1 and HYAL2 expressions. The results were correlated with clinicopathological factors, the expression of the epithelial–mesenchymal transition marker, E-cadherin, and disease recurrence.

Results. Reduced HYAL1 expression was associated with the progression of endometrial carcinomas towards higher grades and also with large tumor sizes, lymph node metastasis, and lymphovascular invasion. Reduced expression of both HYAL1 and HYAL2 was associated with deep myometrial invasion. HYAL2 expression was primarily constant in neoplastic tissues, but its expression was altered in different phases of the endometrial cycle. In addition, a reduction in HYAL1 expression was associated with the depletion of E-cadherin. In a multivariate analysis, reduced HYAL1 expression was an independent prognostic factor for early disease recurrence (HR 5.13, 95% CI: 1.131–23.270, $p = 0.034$).

Conclusions. This study showed that reduced HYAL1 expression was associated with endometrial carcinoma aggressiveness, which further supported the role of hyaluronan degradation in cancer progression.

Background

Endometrial carcinoma is the sixth most common cancer in women worldwide. In addition, endometrial cancer is the most common gynecologic malignancy, with an annual incidence of 15–25 per 100,000 women. Most cases are diagnosed at an early stage of the disease and have a good prognosis, as indicated by the overall 5-year survival rate

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of 80% [1,2]. There are two major subdivisions in endometrial cancer, each with a different histological and genetic profile. The most common lesions (type I) are estrogen-dependent, endometrioid carcinomas with low histological grades. Type II tumors comprise only about 10% of the endometrial cancers. These tumors occur mainly in older women, are not dependent on estrogen, and are mostly associated with endometrial atrophy. These tumors are more aggressive, mainly high grade serous and clear cell carcinomas (non-endometrioid cancers), or poorly differentiated endometrioid adenocarcinomas [3,4].

In many human malignancies, hyaluronan, a high molecular mass glycosaminoglycan of the extracellular matrix, accumulates in the tumor stroma, and its abundance predicts a poor outcome of the disease [5]. Hyaluronan and its receptor, CD44, are both involved in the development and progression of endometrial carcinoma [6,7]. Hyaluronan is synthesized by three hyaluronan synthases (HAS 1–3), and overexpression of these genes increases hyaluronan levels and promotes tumor growth, angiogenesis, and metastasis [8]. Hyaluronan is predominately degraded by the two major hyaluronidases, HYAL1 and HYAL2 [9]. HYAL1 degrades high molecular weight hyaluronan to tetra-hexasaccharides in lysosomes. HYAL2 is a glycosyl-phosphatidylinositol-anchored, lipid raft-associated hyaluronidase that is active under acidic conditions at the cell surface [10]. It has been suggested that HYAL2 initiates the degradation of hyaluronan by processing it to approximately 20 kDa products (50 disaccharide units), and in lysosomes, HYAL1 continues the degradation to smaller oligosaccharides [11].

HYAL1 can function as either a tumor promoter or suppressor [12]. Increased HYAL1 expression has been reported in adenocarcinomas of the prostate [13], bladder [14], colon [15], and breast [16], and in squamous cell carcinomas of the head and neck areas [17]. Among epithelial ovarian carcinomas, the subtype-specific overexpression of HYAL1 was associated with tumors that displayed mucinous or clear cell histology [18]. In contrast, HYAL1 expression was decreased in carcinomas of the lung [19,20] and kidney [20,21]. A recent study indicated that weak expression of HYAL1 was associated with poor survival in pancreatic ductal adenocarcinoma [22]. We previously showed that *HYAL1* mRNA levels were reduced in serous-type ovarian cystadenocarcinoma and in endometrioid endometrial carcinoma, and this reduced expression was associated with stromal hyaluronan accumulation [23,24].

In type I endometrial cancer, myometrial invasion is one of the most important prognostic factors. To acquire the ability to invade, tumor cells must progress through an epithelial to mesenchymal transition (EMT). In this process, epithelial cells lose their polarity and cell–cell contacts, and acquire a migratory phenotype, which results in a mesenchymal-like gene expression program [25]. E-cadherin is the central cell adhesion molecule in normal epithelia, and it plays a critical role in the suppression of tumor invasion and metastasis. Thus, a critical molecular feature of EMT is the downregulation of E-cadherin expression [26]. In endometrial cancer, downregulation of E-cadherin is associated with disease aggressiveness [27,28]. Interestingly, increased hyaluronan content can induce EMT in normal epithelial cells [29].

We have previously shown that hyaluronan accumulation in ovarian and endometrial cancers can result from decreased degradation, due to downregulation of the primary *HYAL* genes. The present study aimed to examine the expression of HYAL1 and HYAL2 proteins in normal and hyperplastic endometria and in endometrial carcinoma, and to determine whether HYAL expression was associated with clinicopathological markers, E-cadherin expression, and disease progression.

Methods

Patients

Endometrial tissue specimens were collected from 343 patients with normal, atrophic, or premalignant (complex atypical hyperplasia) endometria and from endometrial carcinomas (Table 1). All patients were diagnosed and treated in Kuopio University Hospital during 2000–2012.

Table 1
Clinicopathological data of the study population.

		No. of patients
Age at diagnosis (median; range)	65; 32–90	
Histology		343
Pre-menopausal endometria		
Secretory phase		24
Proliferating phase		22
Interphase		6
Post-menopausal endometria		
Atrophy		14
Complex atypical hyperplasia		27
Neoplastic endometria		
Endometrioid adenocarcinoma		234
Serous carcinoma		12
Clear cell carcinoma		4
Grade		
I		107
II		94
III[a]		49
FIGO stage		
IA		132
IB		56
II		21
IIIA		15
IIIB		2
IIIC1		9
IIIC2		5
IVA		0
IVB		10

[a] Serous and clear cell carcinomas included.

The nonmalignant endometrial samples were obtained from hysterectomies (e.g., due to leiomyoma or a uterus prolapse). All patients with endometrial carcinoma underwent surgery. The surgery included peritoneal cytology, total hysterectomy, bilateral salpingo-oophorectomy, and pelvic and para-aortic lymph node sampling, when considered necessary. No patient experienced chemotherapy or radiotherapy before surgery.

Histological typing and grading of the tumors were performed according to WHO classifications [30] and staged according to the international federation of gynecology and obstetrics (FIGO) guidelines [31]. The ethics committee of Kuopio University Hospital approved the study protocol, and all patients provided signed, informed consent.

Tissue samples

All tissue samples were fixed in 10% buffered formalin and were embedded in paraffin. Representative samples of carcinomas and hyperplastic endometria were cut into 3-μm thick sections for immunohistochemical analyses. Samples of the normal endometria that represented different phases of menstrual cycle or atrophic endometrium were evaluated in tissue microarrays. For tissue microarrays, three regions of the endometrium were chosen from each sample, and they were incorporated into microarrays (core diameter, 1.3 mm) with a tissue microarrayer I device (Beecher Instruments, Silver Spring, MD, USA).

HYAL1 and HYAL2 immunohistochemical staining

The deparaffinized sections were incubated in 10 mM citrate buffer, pH 6.0, for 15 min in a pressure cooker at 120 °C, washed with phosphate buffered saline (PBS), and treated for 5 min with 1% H_2O_2 to block endogenous peroxidase activity. Thereafter, the sections were incubated in 1% bovine serum albumin (BSA), 0.05% Tween-20, and 0.1% gelatin (Sigma G-2500, Sigma) in PBS for 30 min to block nonspecific binding. The sections were incubated overnight at 4 °C with polyclonal primary antibodies against HYAL1 and HYAL2, diluted in 1% BSA (HYAL1: HPA002112 Atlas Antibodies, Stockholm, Sweden, dilution 1:100; and HYAL2: Ab68608 Abcam, Cambridge, UK, dilution 1:100).