	検体	症例数
子宮頸癌	組織	7
子宮体癌	組織	5
卵巣癌	組織	20
	腹水	9
	腫瘍内容液	4
合計		45

図1 感受性試験施行例

mer)を培養用基材に用いて再発婦人科癌，特に再発卵巣癌を中心に抗癌剤感受性試験を行い[2]，その結果に基づき化学療法の個別化を図っている．今回はその治療効果を解析し，有用性について評価したので報告する．

対象および方法

2004年2月から2007年12月において，主に婦人科悪性腫瘍再発症例45例に対して検討を行った．十分なインフォームドコンセントのもと，腫瘍の生検，体腔液の採取あるいは摘出手術を施行し，得られた材料を用いて抗癌剤感受性試験を行ない，その結果に基づいて抗癌剤治療を計画・実施した．対象症例の内訳を図1に示す．

まず我々が抗癌剤感受性試験に用いている培養基材のTGPは，転移温度22℃を境にゾル状態，ゲル状態が可逆的に変化する高分子化合物である．TGPを用いた培養の特徴として，

①細胞の包埋・回収が酵素処理を必要とせず，温度変化のみで可能であり，細胞や組織に障害を与えない．

②形態変化を位相差顕微鏡で観察できる．

③培養日数を必要とする時間依存性薬剤でも感受性試験が可能である．

④ゲル内で線維芽細胞の増殖は認めない．

などの特徴がある．

本感受性試験の方法は既に報告している[2]が，以下にその内容を簡単に示す（図2）．細胞または組織を処理後，TGPゲル内に包埋し，各種抗癌剤を添加し4日間培養ののちMTT assayにて吸光度を測定し，各濃度における生存率をプロットし濃

組織の培養法
癌組織を採取（5mm角） Hanks液に保存
↓
Hanks液で反復洗浄，癌組織を細切（厚さ0.5mm）
↓
TGP（4℃）溶解液を各well 300μl注入，ゲル化
↓
ゲル内に組織片を包埋，培養液を各well 650μl添加
↓
培養を開始（37℃，5% CO_2大気条件下）
↓
培養開始24時間後各種抗癌剤を各well 50μl添加，4日間培養
（CDDP，mitomycin C，5-fluorouracil，paclitaxel，CPT-11）
↓
MTT Assay（WST-8＊）
50μl/wellのWST-8を添加
24時間後にOD測定（測定波長450nm，参照波長620nm）
↓
組織蛋白量を測定，単位組織蛋白量当たりのODを求める

＊WST-8：2-(2-methoxyl-4-nitrophenyl)-3-(4-nitrophenyl)-(2,4-disulfophenyl)-2H-tetrazoium, monosodium salt

図2 抗癌剤感受性試験法

度依存曲線を求め各薬剤のIC_{50}値を算出した．得られたIC_{50}値と最高血中濃度（Peak Plasma Concentration；PPC）を比較し，IC_{50}値＜PPCの場合，感受性ありと判定した．

感受性試験の結果を元に，患者の全身状態，再発部位を考慮し，感受性が認められた抗癌剤を中心にレジメンを決定し，個別的な化学療法（点滴静脈投与あるいは動注療法）を施行した．効果判定は婦人科癌化学療法の直接効果判定基準に沿って評価した．

結 果

組織検体を用いた子宮頸癌7例と子宮体癌5例では全例で，また卵巣癌については組織では100%，腹水検体では9例中4例，腫瘍内容液検体では4例中3例において抗癌剤感受性の判定が可能であり，卵巣癌全体の判定可能率は87.8%で，検討した45例全体では41例（91.1%）において判定可能であった．

疾患別の薬剤感受性率の結果を図3に示した．CDDPが各癌種において感受性が最も高く，全体で65.9%と比較的高い感受性を示した．以下感受

	子宮頸癌	子宮体癌	卵巣癌	合計
CDDP	71.4[a)]	40.0	69.0	65.9
MMC	57.1	40.0	55.2	53.7
5FU	57.1	40.0	56.8	56.1
PTX	28.6	20.0	44.8	39.0
CPT-11	0	0	6.90	4.90

a)感受性を認めた症例/判定可能症例（%）
CDDP：cisplatin，MMC：mitomycin C，5FU：5-fluorouracil
PTX：paclitaxel，CPT-11：irinotecan

図3　疾患別の薬剤感受性率

	子宮頸癌	子宮体癌	卵巣癌	合計
CR	2	1	3	6
PR	2	1	8	11
SD	2	1	10	13
PD	0	1	4	5
合計	6	4	25	35
奏効率[a)]	66.7	50.0	42.3	48.6

a)奏効率：(CR + PR)/症例数（%）

図4　評価可能病変を有した症例の疾患別臨床効果

	点滴静注	動注療法
CR	2	1
PR	5	3
SD	5	5
PD	1	3
合計	13	12
奏効率[a)]	53.8	33.3

a)奏効率：(CR + PR)/症例数（%）

図5　再発卵巣癌の治療効果に対する投与径路の比較

抗癌剤	*In vitro* drug response	
	positive	negative
CDDP	18（72%）	7*
PTX	12（48%）	13
CDDP および PTX	9（36%）	5（20%）

*$p < 0.05$, in CDDP treatment, positive vs. negative.

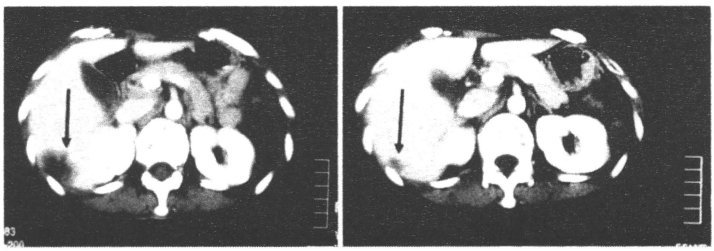
図6　初回治療においてプラチナ＋タキサンを施行後に再発した症例における CDDP，PTX の感受性

性が高かった抗癌剤は5FU，MMC であった．CPT-11 に関しては子宮頸癌，子宮体癌では感受性陽性の症例は認められなかった．

TGP 法で判定可能であった41例について，実際に感受性試験で得られた抗癌剤を使用し化学療法を施行した．その症例のうち評価可能病変を有する症例は35例であった．子宮頸癌では CR 2例・PR 2例，子宮体癌では CR 1例，卵巣癌では CR 3例・PR 8例の効果が得られ，疾患別の奏効率は子宮頸癌 66.7%，子宮体癌 50%，卵巣癌 42.3%，全体の奏効率は 48.6% であった（図4）．

今回の検討において最も多い症例数が得られた卵巣癌について，抗癌剤の投与経路の違いによる治療効果について比較検討したところ，卵巣癌患者33例のうち，実際に化学療法を施行した患者は26例であり，奏効率は点滴静注で 53.8%，動注療法で 33.3% と，点滴静注の方が奏効率が高い傾向が認められた（図5）．初回治療においてプラチナ系化合物とタキサン系化合物の併用化学療法を施行している再発卵巣癌例は25例で，前治療から再発までの期間は1カ月～7年，平均1年2カ月であった．その中で CDDP に感受性を認めたのは18例（72.0%），PTX に感受性を認めたのは12例（48.0%），CDDP および PTX 両方に感受性を認めたのは9例（36.0%）であった．両者に耐性であったのは5例（20%）であり，この5例は MMC に感受性を認めた（図6）．今回感受性試験に基づいて化学療法を施行した評価可能病変を有する症例における平均奏効期間は，CR 5例では11～42カ月で平均 14.4 カ月，また PR 症例 10 例では3～43カ月で平均 13.0 カ月と，ほとんどが再発症例であることを考慮すると比較的良好な成績といえる．

実際に感受性試験の結果に基づいて抗癌剤治療を行なった症例を提示する．症例は55歳，卵巣癌 stage IIIb 未分化癌の患者で，術後標準的治療を行なった後3年2カ月で肝転移，鼡径リンパ節転移および骨盤内再発を認めた．転移鼡径リンパ節を生検し病理組織学的に転移の診断を得たが，イ



治療前　　治療後

縮小率 90%

図7 CT

ンフォームドコンセントの元，その一部を抗癌剤感受性試験に供したところ，MMC に感受性を認めたため肝動脈および内腸骨動脈から MMC による動注化学療法を施行した．4 コース終了後，3,120U/ml と上昇していた CA125 も 17U/ml まで低下し，肝転移も 90% の縮小を認め PR と判定した（図 7）．

考　察

近年癌治療の進歩に伴い，手術，化学療法，放射線療法などを組み合わせた集学的治療により，進行癌症例に対しても寛解導入が可能な症例が多くなっている．その一方で，再発症例に対する治療方針に関してはまだエビデンスが乏しく，婦人科癌領域においても再発症例に対する化学療法は癌種を問わず未だ確立されていないのが現状である．子宮頚癌については IVb 期あるいは進行再発症例に対し，GOG204 において cisplatin と paclitaxel，vinorelbine，gemcitabine，topotecan との併用療法の比較が行なわれたが，各群間で有意差は得られなかったとして study は終了している[3]．子宮体癌に対しては cisplatin，doxorubicin and paclitaxel が有用であると考えられているが，これら薬剤の combination では overall survival に寄与しなかったと報告されている．唯一これら 3 剤の併用療法である TAP 療法で高い奏効率が得られたものの神経毒性が強いため新たなレジメンが検討されている[4]．上皮性卵巣癌では，platinum-taxane による初回化学療法の高い奏効率にも関わらず，多くの症例が再発する．second-line の単剤での化学療法の奏効率はプラチナ感受性症例では 20～35%，プラチナ耐性症例で 8～15% と言われており，その奏効率は満足いくものではない[5]．このように婦人科領域では再発症例に対する化学療法として，より有効なかつ副作用の少ないレジメンの開発が望まれている．

今回の我々の検討では，主に再発婦人科癌に対して行った TGP 法は，91.1% という高い判定可能率であり，腫瘍組織のみならず腹水および腫瘍内容液においても感受性試験が可能であり感受性試験の幅を拡大できた．感受性試験結果に基づいた化学療法の奏効率は，卵巣癌症例においては 42.3% であり，second-line の化学療法としては評価できる数字であった．また卵巣癌の薬剤感受性率の結果より，CDDP，5FU，MMC で比較的高い感受性が認められ，second-，third-line として化学療法を施行する際，これら薬剤が key drug となる可能性が示唆され，特に卵巣癌治療の第一選択となっているパクリタキセル・カルボプラチンの併用化学療法無効症例に MMC が key drug になる可能性が示唆された．再発癌の化学療法は一般的には単剤投与が推奨されているが，プラチナ感受性の再発卵巣癌に対しては多剤併用療法も 1 つのオプションと成りうる[6]．従って感受性試

験法を確立することにより，適切な抗癌剤の選択が可能となり，治療の個別化と奏効率の向上につながると考えられる．

再発腫瘍に関しては，再発部位により薬剤分布が異なる．特に卵巣癌でよく認められる腹膜播種や，子宮頸癌でしばしば認められる骨盤内再発腫瘍は薬剤到達度が低い可能性があり，従って抗癌剤感受性試験の結果感受性ありと判定されても，実際に奏効しない場合があることが推定される．そこで今回は薬剤投与径路を静脈注射と動注療法に分けて検討してみたが，結果は静脈投与の方が奏効率が高かった．今後はよりよい薬剤分布の得られる投与法，例えば腹腔内投与などの検討や，pegylated liposomal doxorubicin などの新しい drug delivery system の開発が望まれる．

結　論

TGP を培養用基材に用いた感受性試験は，採取した組織への障害が少ないため判定率も高く，簡便に行える試験法である．TGP を使用した感受性試験に基づいた抗癌剤治療は，再発した婦人科癌患者の化学療法の個別化を目指す上で有効な手段であると考えられた．

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Heparanase expression and angiogenesis in endometrial cancer: Analyses of RT-PCR and immunohistochemistry

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Abstract

Background: The human heparanase has been shown to function in tumor progression, metastatic spread, and tumor angiogenesis. The aim of the present study was to assess heparanase expression in endometrial cancer in correlation with neovascularization and clinicopathological factors.

Materials and Methods: Fifty-two endometrial cancers were obtained from previously untreated patients (median age, 56 years, range, 35-80 years). The expression of heparanase mRNA was evaluated using a semi-quantitative reverse transcriptase-polymerase chain reaction and immunohistochemical staining (IHC) with anti-heparanase polyclonal antibody. This antibody was raised by immunizing a rabbit

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with a peptide containing the amino acid residues from 238 to 250 of the Heparanase. Tumor angiogenesis was assessed using microvessel counting. The Mann-Whitney U test, one factor ANOVA test, and Spearman's test were used to determine the relationship between heparanase expression, microvessel density, and clinicopathological parameters.

Results: The expression of heparanase mRNA was detected in 26 of 52 (50%) endometrial cancers, and was significantly correlated with FIGO stage IIIc (p=0.0075), the presence of lymph-vascular space involvement (LVSI) (p=0.0041), lymph node metastasis (LNM) (p=0.0049), and histological tumor grade (p=0.003). IHC showed that the heparanase was expressed in 23 of 52 (44.2%) endometrial cancers, which was significantly related to LVSI (p=0.0028), depth of myometrial invasion (p=0.0026), and histological tumor grade (p=0.0135). Microvessel density was also associated with FIGO stage IIIc (p=0.027), LVSI (p=0.001), LNM (p=0.038), ovarian metastasis (p=0.03) and histological tumor grade (p=0.003). Moreover, we found a strong positive correlation between heparanase expression and microvessel density (r^2=0.475, p=0.0001).

Conclusion: These results suggest that the expression of heparanase can promote tumor angiogenesis and develop metastasis in endometrial cancer.

Introduction

The human heparanase gene, an endo-beta-glucuronidase that cleaves heparan sulfate (HS) at specific intrachain sites, has recently been cloned and has been shown to function in tumor progression and metastatic spread. Degradation of heparan sulfate proteoglycans by heparanase appears to play an important role in the invasiveness of tumor cells through the basement membrane and into the extracellular matrix. Recent cloning of the heparanase gene and generation of monoclonal antibodies against the enzyme allow us to examine the tumor cell expression of the enzyme [1, 2]. Heparanase expression is well correlated with the metastatic cancer potential of several different types of cancer. It has been reported that heparanase is strongly expressed in highly metastatic cells, such as melanoma [3], gastric cancer [4], bladder cancer [5], pancreas cancer [6, 7], hematogeneous [8], oral carcinoma [9], hepatocellular carcinoma [10], and colon cancer [11].

Heparan sulfate proteoglycans also bind and sequester a variety of bioactive proteins, including growth factors, chemokines, cytokines, and enzymes [12, 13]. Heparanase produced by a given tumor may facilitate tumor invasion and angiogenesis through the release of HS-

bound growth factors, such as basic fibroblast growth factor (bFGF) [14, 15], vessel endothelial growth factor (VEGF) [16], hepatocyte growth factor (HGF) [17], and platelet-derived growth factor (PDGF) [18]. Thus, expression of heparanase is thought to play an important role in cancer invasion and metastasis [19-21].

A majority of patients with endometrial cancer are diagnosed as being without clinical evidence of extrauterine spread (the International Federation of Gynecology and Obstetrics (FIGO) stage I and II) and have a 5-year survival rate of approximately 90%. However, 15-25% of patients with a tumor extending outside the uterus but limited to the true pelvis (FIGO stage III) and have an estimated 5-year survival of 40 to 70% [22, 23]. Also, in patients with stage III disease, lymph vascular space involvement, deep myometrial invasion, and lymph node metastasis are reported to be independent prognostic factors [24-26]. It is quite interesting to investigate the relationship between heparanase expression and angiogenesis in association with the prognostic variables in endometrial cancer. Here we review our data on heparanase expression and angiogenesis in endometrial cancer [27-29].

Materials and Methods

Tissue Samples

A total of 52 endometrial cancers were obtained from previously untreated patients (median age, 56 years, range, 35-80). Tissue samples were snap-frozen in liquid nitrogen and maintained at -80°C until use. All cases underwent curative operations for endometrial cancer and were surgically categorized into different stages according to FIGO staging.

RNA extraction and cDNA synthesis

Total cellular RNA was extracted from tissues using Isogen (Nippon Gene) according to the manufacturer's specific protocol. First-strand cDNA was synthesized from total cellular RNA primed by random hexamers. Briefly, 1 μg of total cellular RNA was incubated in 20 μl reactions containing 200 U of Moloney murine leukemia virus reverse transcriptase (BRL), 50 mM Tris-HCl (pH 8.3), 75 mM potassium chloride, 3 mM magnesium chloride, 10 mM DTT, 100 mg/ml BSA, 0.625 mM each of four dNTPs (Pharmacia), 0.4 μg of random hexamers (Pharmacia), and 20 U of RNasin (Promega) for 45 min at 37°C. The reaction was terminated by boiling for 5 min.

Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis

PCR primers were designed from the cDNA sequences of the heparanase [1]. The sequence of the nucleotides used for heparanase was: forward 5'-TTC GAT CCC AAG AAG GAA TCA AC-3' and reverse 5'-GTA GTG ATG CCA TGT AAC TGA ATC-3'. The primers used for β-actin were as follows; forward: 5'-GAT GAT GAT ATC GCC GCG CT-3' and reverse: 5'-TGG GTC ATC TTC TCG CGG TT-3'. Two μl of the cDNA reaction was amplified in a volume of 100 μl containing 50 mM potassium chloride, 10 mM Tris-HCl (pH 9.0), 1.5 mM magnesium chloride, 0.1% Triton X-100, 100 mM each of four dNTPs (Pharmacia), 250 nM of a pair of primers, one unit of Taq polymerase (Takara). The amplification reaction consisted of 33 cycles, including denaturation at 94°C for 1 min, annealing for 1 min at 60°C and extension at 72°C for 1.5 min. At the end of the reaction a polymerization step at 72°C for 7 min was included. PCR products were run on a 1.2% agarose gel and visualized by ethidium bromide (Sigma). Gel images were obtained using FAS-II UV-image analyzer (Toyobo), and the densities of the products were quantified using the Quantity One eversion 3.0 (PDL, Inc.). The relative expression levels were calculated as the density of the product of the respective target genes divided by that of the β-actin from the same cDNA.

Immunohistochemistry (IHC)

Tissue sections were dewaxed with xylene and rehydrated in alcohol. Slides were then heat inactivated in 10 mmol/L sodium citrate (pH 6.0) in a microwave for 3 minutes. Cooled slides were rinsed with PBS and then incubated with 1% H_2O_2 in methanol for 30 minutes at room temperature. Sections were then blocked with 5% normal goat serum in PBS for 30 minutes at room temperature followed by 1 hour incubation with an antiheparanase rabbit serum (1:500 dilution) in PBS. This antibody was raised by immunizing a rabbit with a peptide containing the amino acid residues from 238 to 250 the 50-kDa HPR1 subunit and is able to detect both the 50-kDa and 65-kDa forms of expressed HPR1. Specificity of this antibody was well characterized by Western blot. Slides were washed and then incubated with goat antirabbit IgG-biotin conjugate (PharMingen) diluted at 1:300 in 5% human serum in PBS. Streptavidin-heparanase conjugate (Zymed Laboratories) diluted at 1:200 in PBS with 5% normal human serum was added and incubated for 45 minutes at room temperature. Color was developed by DAB substrate (Sigma) followed by 3,3 =diaminobenzidine enhancer (Vector Laboratories). Slides were counterstained with Mayer's hematoxylin for 2 minutes, dehydrated, and mounted. Heparanase expression was determined by a board-certified pathologist and corroborated

independently by three investigators in this study, all blinded to other clinicopathologic information. Heparanase expression was judged as positive by the presence of brown staining, specifically within 20% of the tumor areas. In the majority of specimens, heparanase staining was present in all tumor cells. A graded scoring system was not used because of the variation in intensity of heparanase signal between the experiments conducted at different times.

Evaluation of microvessel density (MVD)

Microvessel counting was used for assessing tumor angiogenesis. Three areas of high vascular density (hot spot) were selected at low power (x40 and x100). Counts were performed with a microscope at x250 magnification, corresponding to 0.384 mm^2 field size, and the average value of these three areas was taken as the microvessel score. Only blood vessels with a well-defined lumen or a linear vessel shape were taken into account.

Statistical analysis

A one-factor ANOVA test, and a Mann-Whitney U test were used to determine the relationship between the heparanase expression, or microvessel density, and clinicopathological factors. Associations between heparanase expression and the clinicopathologic parameters of endometrial cancer specimens were determined by chi-square or Fisher's exact test. The correlation between heparanase mRNA expression and microvessel density was assessed using a Spearman's test. P values less than 0.05 were considered statistically significant for all tests.

Results

Heparanase mRNA expression and clinicopathological factors

Heparanase expression was detected in 26 of 52 (50%) endometrial cancer tumors using RT-PCR. Figure 1 demonstrates the final RT-PCR products of heparanase and β-actin on a 1.2 % agarose gel. The ratios of heparanase/ β-actin ranged from 0 to 0.933 with a mean value of 0.062±0.284. The ratios of heparanase/β-actin in each clnicopathological factors are presented in Table 1. FIGO stage IIIc tumors expressed significantly higher heparanase mRNA compared with stage I (p= 0.0005) or IIIa (p=0.0126) tumors. Grade 2 and 3 tumors showed a higher heparanase expression than those of grade 1 tumors, significantly (0.425±0.334 vs. 0.137±0.213, p=0.0033). In respects to LVSI tumors with positive LVSI had a significantly stronger expression of heparanase than those with negative LVSI (0.361±0.262 vs. 0.157±0.274,

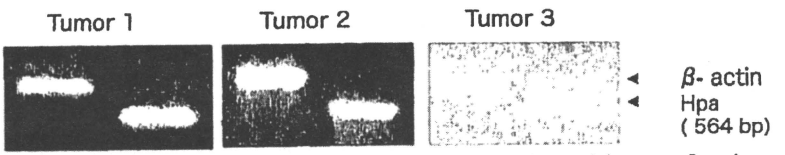

Figure 1: Detection of heparanase mRNA by RT-PCR in endometrial cancer; β-actin was used as a positive control.

Table 1: Correlation between heparanase expression by RT-PCR and clinicopathological factors

Variables	No. of patients	Mean±S.D.[b]	P-value[c]
FIGO stage			p=0.0075
IA	6	0	p=0.0005
IB	27	0.228±0.304	p=0.0097
IC	7	0.116±0.114	p=0.061
II	1	0.237	p=0.0126
IIIa	4	0.193±0.145	
IIIc	7	0.558±0.357	
Tumor grade			
1	40	0.137±0.213	p=0.0033
2+3	12	0.425±0.334	
Myometrial invasion			NS
a+b	36	0.080±0.158	
c	16	0.284±0.360	
LVSI[a]			p=0.0041
Yes	18	0.361±0.262	
No	34	0.157±0.274	
Lymph node metastasis			p=0.0049
Yes	6	0.526±0.336	
No	46	0.159±0.230	
Peritoneal cytology			NS
Yes	2	0.322±0.248	
No	50	0.206±0.289	
Ovarian metastasis			NS
Yes	4	0.131±0.162	
No	48	0.234±0.294	
Cervical invasion			NS
Yes	5	0.343±0.081	
No	47	0.210±0.296	

[a] LVSI, lymph-vasucular space involvement
[b] S.D., standard deviation
[c] one factor ANOVA test, Mann-Whitney U test
NS, not significant

p=0.0041). In six tumors with positive lymph nodes, the heapranase expression was observed as being significantly high compared to tumors with negative lymph nodes (0.526±0.336 vs. 0.159±0.230, p=0.0049). In terms of depth of myometrial invasion, peritoneal cytology, ovarian metastasis, and cervical invasion, we observed no significant difference in the heparanase expression assessed using RT-PCR.

Microvessel density (MVD) and cilnicopathological factors

The mean values of the microvessel score ranged from 8 to 30 (mean±SD, 17.8±6.2). FIGO stage IIIa (22.7±6.3), and IIIc (20.8±4.7) tumors were found to have a significantly higher microvessel score compared with stage I (Ia; 14.8±5.7, Ib; 15.1±4.3, Ic; 19.2±8.8) tumors. Tumors with a positive LVSI (22.5±5.9), grade 2/3 (21.7±4.8), lymph node metastasis (22.0±5.3), and ovarian metastasis (23.8±5.4) showed a significantly high microvessel score compared to those lacking these factors. The relationship between microvessel score and clinicopathological variables, and p values are shown in Table 2.

Correlation between heparanase mRNA expression and MVD

We used Spearman's correlation coefficient by rank. Figure 2 demonstrates a strong correlation between heparanase expression and MVD (correlation coefficient r^2=0.475, Spearmann's provability p=0.0001).

IHC analysis was performed to analyze heparanase expression

As shown in Figures 3 to 5, heparanase was abundantly and equally expressed in both the cytoplasm and the cell membrane of the cells in endometrial cancer (Figure 3, x40), and no heparanase expression was detected (Figure 4, x40). Strong heparanase -positive staining was also seen at the apex of cancer invasion into myometrium (Figure 5). Of specimens staining positive for heparanase, the majority expressed heparanase uniformly throughout the tumor. The IHC positive for heparanase in each clnicopathological factors are presented in Table 3. Tumors with deep myometrial invasion (depth c) expressed significantly higher heparanase positive rate compared with those in depth a or b tumors (p=0.0026). Grade 2 and 3 tumors showed a higher heparanase expression than those of grade 1 tumor, significantly (p=0.0135). In respects to LVSI, tumors with positive LVSI had a significantly higher expression rate of heparanase than those with negative LVSI (p=0.0028). In six tumors with positive lymph nodes, the heparanase expression was observed as being higher compared to tumors with negative lymph nodes, which

Table 2 : The correlation between microvessel count and clinicopathological factors

Variables	No.of patients	Mean±S.D.[b]	P-value[c]
FIGO stage			
IA	6	14.8±5.7	p=0.005
IB	27	15.1±4.3	
IC	7	19.2±8.7	p=0.027
II	1	16	
IIIa	4	22.7±6.3	
IIIc	7	20.8±4.7	
Tumor grade			*p*=0.003
1	40	16.1±6.0	
2+3	12	21.7±4.8	
Myometrial invasion			NS
a+b	36	16.7±5.4	
c	16	19.8±7.1	
LVSI[a]			*p*=0.001
Yes	18	22.5±5.9	
No	34	15.5±4.9	
Lymph node metastasis			*p*=0.038
Yes	6	22.0±5.3	
No	46	16.9±6.0	
Peritoneal cytology			NS
Yes	2	21.8±6.4	
No	50	17.1±5.9	
Ovarian metastasis			*p*=0.030
Yes	4	23.8±5.4	
No	48	17.1±5.9	
Cervical invasion			NS[d]
Yes	5	21.5±7.5	
No	47	17.3±5.9	

[a] LVSI, lymph-vasucular space involvement
[b] S.D.,standard deviation
[c] one factor ANOVA test, Mann-Whitney U test
[d] NS, not significant

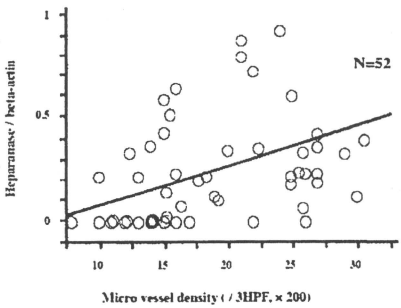

Figure 2: The correlation between ratios of heparanase/β-actin expression and microvesel score in endometial cancer as evaluated using the Spearmann's correlation test. Tumor MVD was correlated with the level of the ratios of heparanase/β-actin expression in endometrial cancer with correlation coefficient r^2=0.475, and Spearmann's provability; p=0.0001.

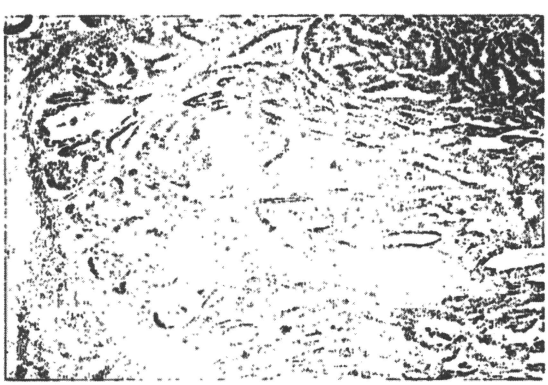

Figure 3: Heparanase was abundantly and equally expressed in both the cytoplasm and the cell membrane of the cells in endometrial cancer (x40). Of specimens staining positive for heparanase, the majority expressed heparanase uniformly throughout the tumor.

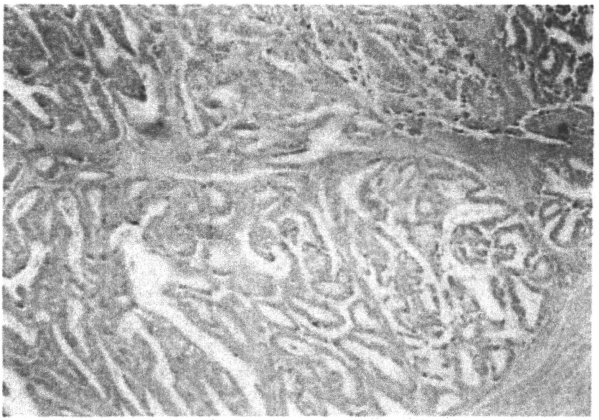

Figure 4: No heparanase expression was detected (x40)

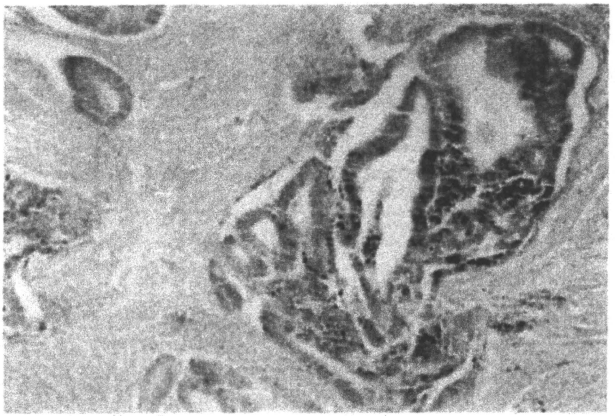

Figure 5: Strong heparanase -positive staining was also seen at the apex of cancer invasion into myometrium (x40).

Table 3: Correlation between heparanase expression by IHC and clinicopathological factors

Variables	No. of patients	Hep positive	P-value[b]
FIGO stage			NS
IA	6	1	
IB	27	9	
IC	7	5	
II	1	1	
IIIa	4	2	
IIIc	7	5	
Tumor grade			0.0135
1	40	14	
2+3	12	9	
Myometrial invasion			0.0026
a+b	36	11	
c	16	12	
LVSI[a]			0.0028
Yes	18	13	
No	34	10	
Lymph node metastasis			NS
Yes	6	4	
No	46	19	
Peritoneal cytology			NS
Yes	2	2	
No	50	21	
Ovarian metastasis			NS
Yes	4	3	
No	48	20	
Cervical invasion			NS
Yes	5	4	
No	47	19	

[a] LVSI, lymph-vascular space involvement

[b] Fisher's exact test

NS, not significant

was not significant (p=0.2349). In terms of peritoneal cytology, ovarian metastasis, and cervical invasion, we observed no significant difference in the heparanase expression assessed by IHC.

Discussion

Tumor spread involves degradation of various components of the extracellular matrix and blood vessel wall. Among these is heparan sulfate proteoglycan, which plays a key role in the self-assembly, insolubility and barrier properties of basement membranes and extracellular matrices [30, 31]. Expression of heparanase, which degrades heparan sulfate correlates with the metastatic potential of tumor cells, and treatment with heparanase inhibitors markedly reduces the incidence of metastasis in experimental animals [1, 32, 33]. Heparanase may thus facilitate both tumor cell invasion and neovascularization, two critical steps in tumor progression. Our results showed that heparanase mRNA was detectable in 50% of the endometrial cancers [27, 28] according to the method of Vlodavsky et al. [1] and that its expression was closely associated with LVSI, lymph node metastasis, and tumor grade. The enzyme may play an important role in metastatic spread of the cancerous cells, as previously reported in several types of cancer, such as melanoma [3], gastric cancer [4], bladder cancer [5], pancreas cancer [6,7], hematogeneous [8], oral carcinoma [9], hepatocellular carcinoma [10], and colon cancer [11].

Heparin-binding angiogenic proteins are stored as a complex with heparan sulfate in the microenvironment of tumors. These proteins are released and can induce new capillary growth when heparan sulfate is degraded by heparanase. Heparanase may influence the bioavailability of different growth factors including FGFs, VEGF, HGF, and PDGF, which are stored in HS and possess HS-binding sequences [17, 18, 34, 35]. It is quite reasonable to assume that the release of such growth factors may influence tumor growth and angiogenesis. In hepatocellular carcinoma, the expression of heparanase enhances growth, invasion, and angiogenesis of the tumor, and bFGF seems to be a potent angiogenic factor [10]. In our study, we tested whether or not heparanase could influence tumor vascularity, and whether there was a direct correlation between heparanase expression and tumor angiogenesis in endometrial cancer [28]. El-Assal et al. [36] have previously demonstrated that different angiographic findings were not directly correlated with the MVD in hepatocellular carcinoma, because the degree of tumor stain is likely to be influenced by different factors, such as arterial supply, specificity of the canulation, lymphatic and venous drainage, and the amount of the contrast materials, besides the

MVD. Accordingly, we used MVD as a direct index for tumor neovascularization. Our study demonstrated that MVD was closely associated with LVSI, lymph node metastasis, ovarian metastasis, and tumor grade as well as heparanase expression. Furthermore, we found that the presence of positive correlation between heapranase expression and endometrial cancer angiogenesis in the present study. Tumors with strong heparanase expression had a significantly higher MVD compared with weak heparanase expression tumors. We can conclude that heparanase expression plays a pivotal role not only in the tumor growth and invasion but also in the angiogenesis of endometrial cancer [27, 28].

The degree of histologic differentiation of endometrial cancer has long been accepted as one of the most sensitive indicators of prognosis. Also, the grade of tumor correlates with other prognostic factors. As the tumor loses its differentiation, the chances of survival decrease [37]. Interestingly in our study, as the tumor becomes less differentiated, both the heparanase expression and the MVD increase [27-29]. This may be one of the reasons that the histological tumor grade in endometrial cancer is a strong prognostic variable.

In conclusion, our study demonstrated the biological importance of heparanase expression in endometrial cancer. The expression of heparanase was found to influence different malignant behaviors in endometrial cancer, including lymph-vascular space involvement, lymph node metastasis and angiogenesis.

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Endometrial Cancer State of the Science Meeting

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and for the Endometrial Cancer Working Group of the Gynecologic Cancer Intergroup

Abstract: There is a pressing need to improve our understanding of endometrial cancer (EC) and uterine carcinosarcoma and to develop new treatment strategies to improve outcomes. In recognition of this, a State of the Science meeting on EC was held last November 28 and 29, 2006, in Manchester, United Kingdom. The meeting was cosponsored by the National Cancer Research Institute (UK), the National Cancer Institute (US), and the Gynecological Cancer Intergroup.

The objectives of the meeting were as follows:

1. To review current knowledge and understanding of EC and its treatments.
2. To identify key issues for translational research and clinical trials.
3. To identify the most important trials for women with endometrial carcinoma and uterine carcinosarcoma, both those already underway or to be done, for which the Gynecological Cancer Intergroup might facilitate international cooperation.

Key Words: Endometrial cancer, Clinical trials, Translational research

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Endometrial cancer (EC), the second most common gynecologic cancer worldwide, has now become the most common gynecologic cancer in developed countries. Its rising incidence is related to increasing life expectancy, tamoxifen use, and the epidemic of obesity. The last is also responsible for comorbidity, notably adult-onset diabetes and hypertension. Together, comorbidity and obesity present challenges in delivering optimal therapy for many women with EC. The rising incidence of EC has been associated with a rising death rate. Although the prognosis of early disease is good with a survival rate of 80%, those with very high-risk disease and advanced disease at presentation have a survival rate below 50% with very little gain in therapeutic efficacy during the past 30 years. This lack of progress in treatment is, in part, related to our limited understanding of the molecular pathology of EC. There is a pressing need to improve our understanding of EC and to develop new treatment strategies to improve outcomes. In addition, compared with ovarian and cervical cancer, EC and uterine carcinosarcoma (CS) have been studied much less extensively. Fewer trials have been opened for women with these cancers, and accrual to those trials has been slow.

In recognition of this, a State of the Science meeting on EC was held last November 28 and 29, 2006 in Manchester, United Kingdom. The meeting was cosponsored by the National Cancer Research Institute (NCRI, UK), the National Cancer Institute (US), and the Gynecological Cancer Intergroup (GCIG). A multidisciplinary group of 75, drawing on surgeons, gynecologic oncologists, radiation (clinical) oncologists, medical oncologists, pathologists, translational scientists, and patient advocates from 18 countries and representing 14 trial groups attended.

The objectives of the meeting were as follows:

1. To review current knowledge and understanding of EC and its treatments.
2. To identify key issues for translational research and clinical trials.
3. To identify the most important trials for women with endometrial carcinoma and uterine CS, both those already underway or to be done, for which the GCIG might facilitate international cooperation.

The first half of the proceedings was dedicated to a series of presentations, which outlined our current knowledge. The second half of the meeting began with parallel sessions of early disease and advanced/recurrent disease to define staging, treatment, and translational research issues to lead to candidate clinical trials questions. This was followed by plenary discussion of the questions to be addressed in these candidate trials and an attempt to develop an international consensus of the most favored concepts for future development and international collaboration.

This paper reports the content and conclusions arising from this meeting.

CURRENT KNOWLEDGE

Molecular Pathology of EC

Endometrial Hyperplasia

There is broad agreement that type 1 (estrogen-related) EC progresses via a precursor lesion, atypical hyperplasia or endometrial intraepithelial neoplasia.[1] This has been clearly demonstrated

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