Acknowledgments

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Figure legends

Figure 1. Expression of C5aR in human primary cancers. ***A***, Human cancer tissues were immunohistochemically stained with an anti-C5aR antibody or control IgG (inset), and representative examples are shown. Colon cancer (top left) and normal colon epithelium (top right); esophagus normal epithelium (bottom, encircled) and cancer (bottom, not encircled). Scale bars = 50 μm. ***B***, Cancer cell C5aR-positive case ratios (positive cases/total cases examined) (•) in primary organs. The vertical scale bar shows the 95% confidence interval that is estimated from the binominal proportion based on the beta distribution. EP, esophagus (n = 20); ST, stomach (n = 20); Col, colon (n = 40); Liv, liver (n = 20); BD, bile ducts (n = 46); Pan, pancreas (n = 10); Kid, kidney (n = 11); UB, urinary bladder (n = 45); Lun, lung (n = 12); PT, prostate (n = 25); Mam, mammary gland (n = 21).

Figure 2. Expression of C5aR on cancer cell lines including HuCCT1/C5aR and HuCCT1/mock. ***A***, Expression of C5aR mRNA in several cancer cell lines shown by RT-PCR. ***B***, Expression of C5aR protein in bile duct and colon cancer cell lines, shown

by immunoblotting using an anti-C5aR antibody. β-actin mRNA and protein were used as controls. ***C***, Expression of C5aR on the cell membrane in MEC and HuCCT1/C5aR, shown by flow cytometry using FITC-conjugated anti-C5aR antibody (gray line) or control antibody (black line).

Figure 3. C5a elicits C5aR expressing cancer cells by inducing cytoskeletal reorganization and changes in cellular morphology. ***A***, HuCCT1/C5aR and HuCCT1/mock cells were incubated with C5a (100 nM) and fixed at indicated time points. F-actin was visualized by immunofluorescence staining with Alexa 488-conjugated Phalloidin. Scale bars: 20 μm. Arrow head and an arrow indicate filopodia and membrane ruffling, respectively. ***B***, Time Lapse analysis of cell motility. Cell images taken at 0, 3, 6 and 9 h are shown. Broken circles indicate the initial cell position shown at 0 h. ***C***, Cell migration distance was measured by tracing a cell. $^{*}P < 0.01$ ($n = 6$).

Figure 4. Enhancement of C5aR-expressing cancer cell invasion by C5a *in vitro*.

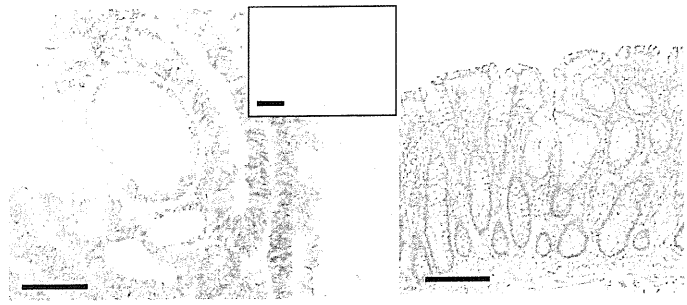
Cancer cell invasion was measured by the Matrigel invasion chamber assay. HuCCT1-derived (***A***) or MEC cells (***B***) were placed in the upper chamber and medium supplemented with various concentrations of C5a was placed in the lower chamber. HuCCT1-derived (***C***) or MEC cells (***D***) were pretreated with various concentrations of C5a for 12 h and 24 h respectively. These cells were washed and were placed in the upper chamber, and medium was placed in the lower chamber. Incubation time for assay: 24 h (***A***, ***C***) and 36 h (***B***, ***D***). (□), HuCCT1/mock (***A***, ***C***); (■), HuCCT1/C5aR (***A***, ***C***); crosshatched and closed bars (***B***, ***D***) indicate MEC cells treated with anti-C5aR antibody or nonspecific IgG, respectively. Gray bars (***A***, ***B***) indicates cells incubated in the upper chamber in the presence of GM6001 (5 μM). ***E***. Checkerboard analysis for C5a cancer cell invasion activity. Closed, crosshatched and open bars indicate C5a concentration 0, 10 or 100 nM, respectively, in the lower chamber.*, $p < 0.01$; n.s., not significant.

Figure 5. Enhanced invasiveness of C5aR-expressing cancer cells by C5a *in vivo*. HuCCT1/C5aR and HuCCT1/mock cells were incubated separately in the presence or

absence of 100 nM C5a (C5a(+) or C5a(-), respectively) for 12 h. After washing, cells were labeled, mixed, and injected together into nude mouse skin. ***A***. Distribution of HuCCT1/C5aR (*orange*) and HuCCT1/mock (*green*) cells in nude mouse skin two days after implantation. Right panels are corresponding sections stained with hematoxylin-eosin. Bars = 300 μm. ***B***. Cell distribution square ratio (HuCCT1/C5aR cells versus HuCCT1/mock cells). Cells were incubated in the presence (■) or absence (□) of C5a. *, $p < 0.01$ ($n = 5$).

Figure 1

A



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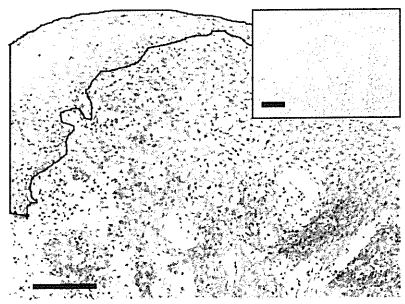

esophagus

B

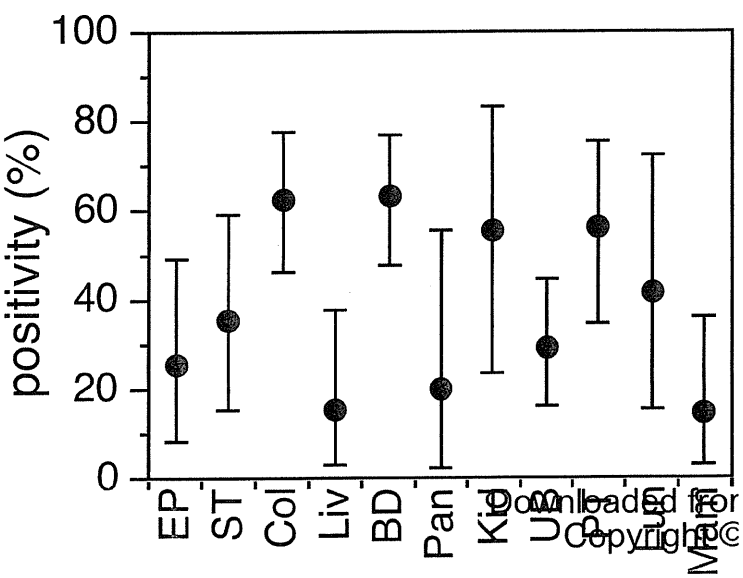

Figure 2

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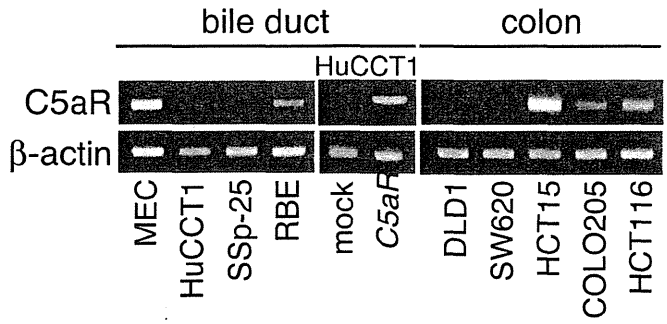

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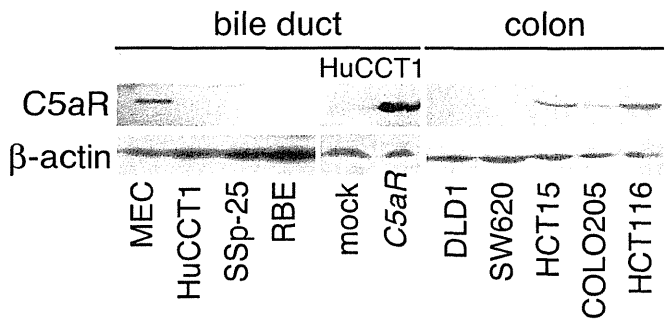

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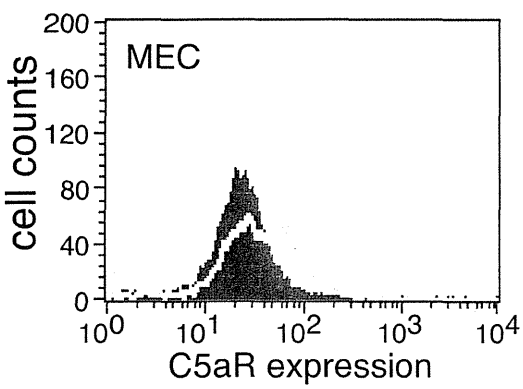

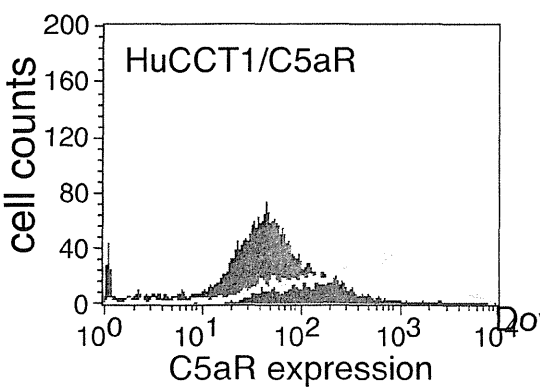

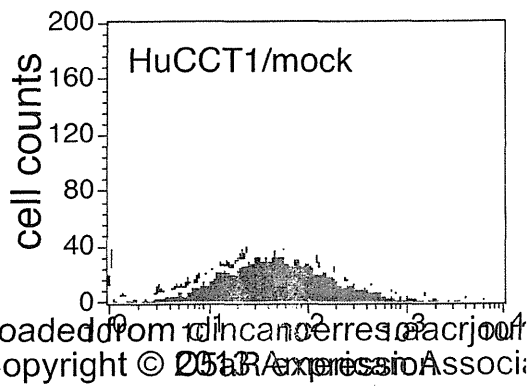

Figure 3

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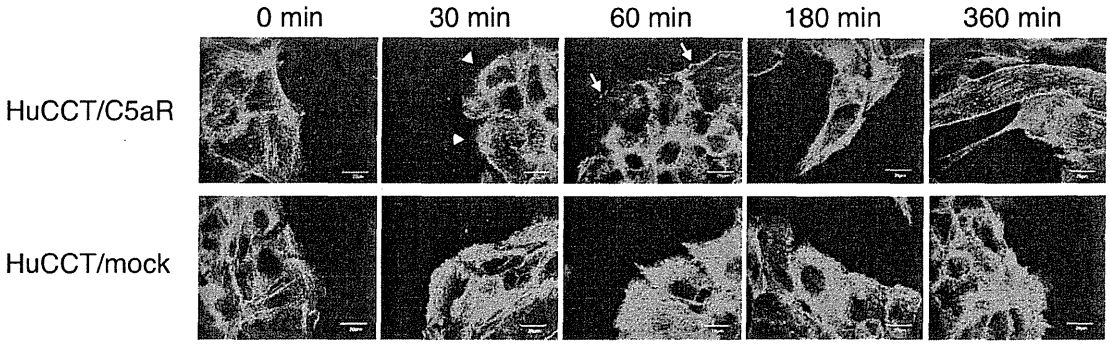

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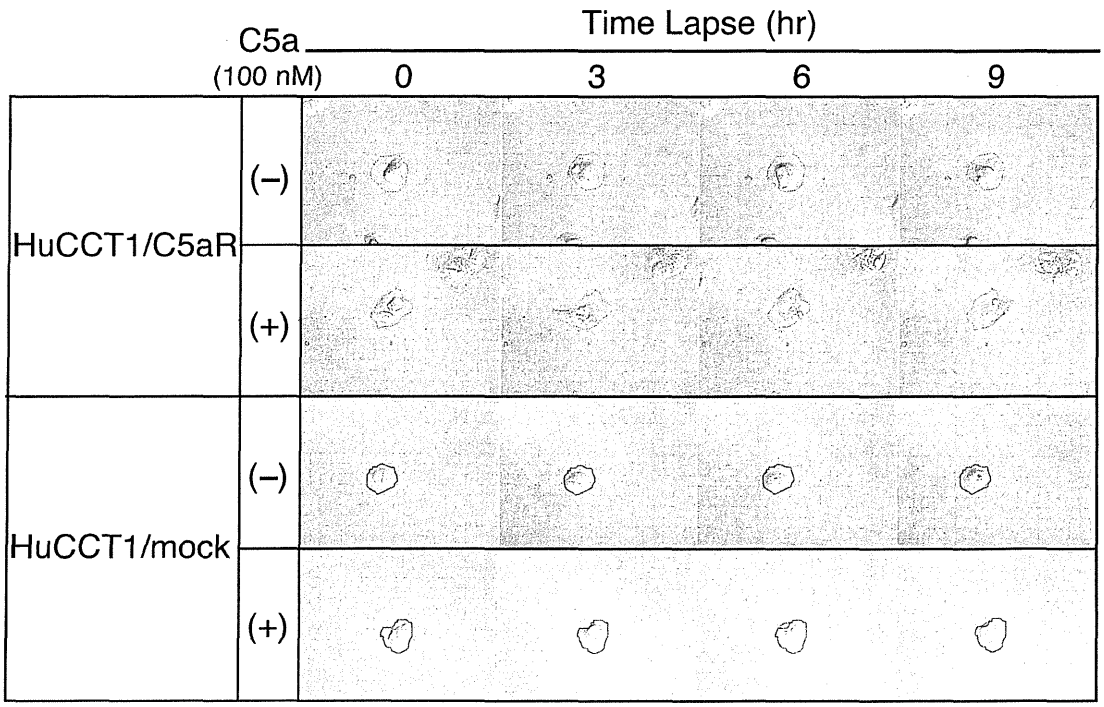

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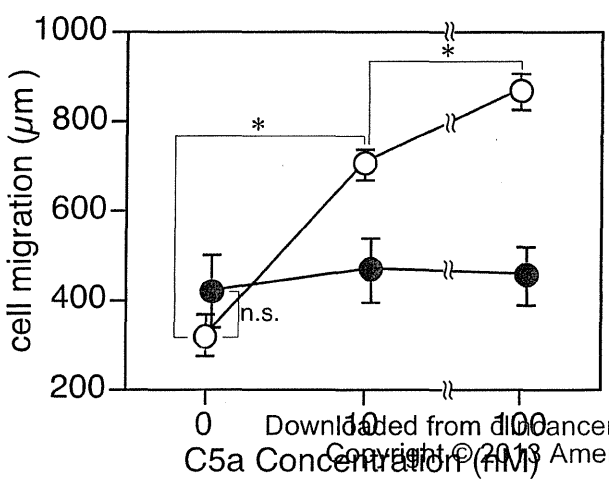


Figure 4

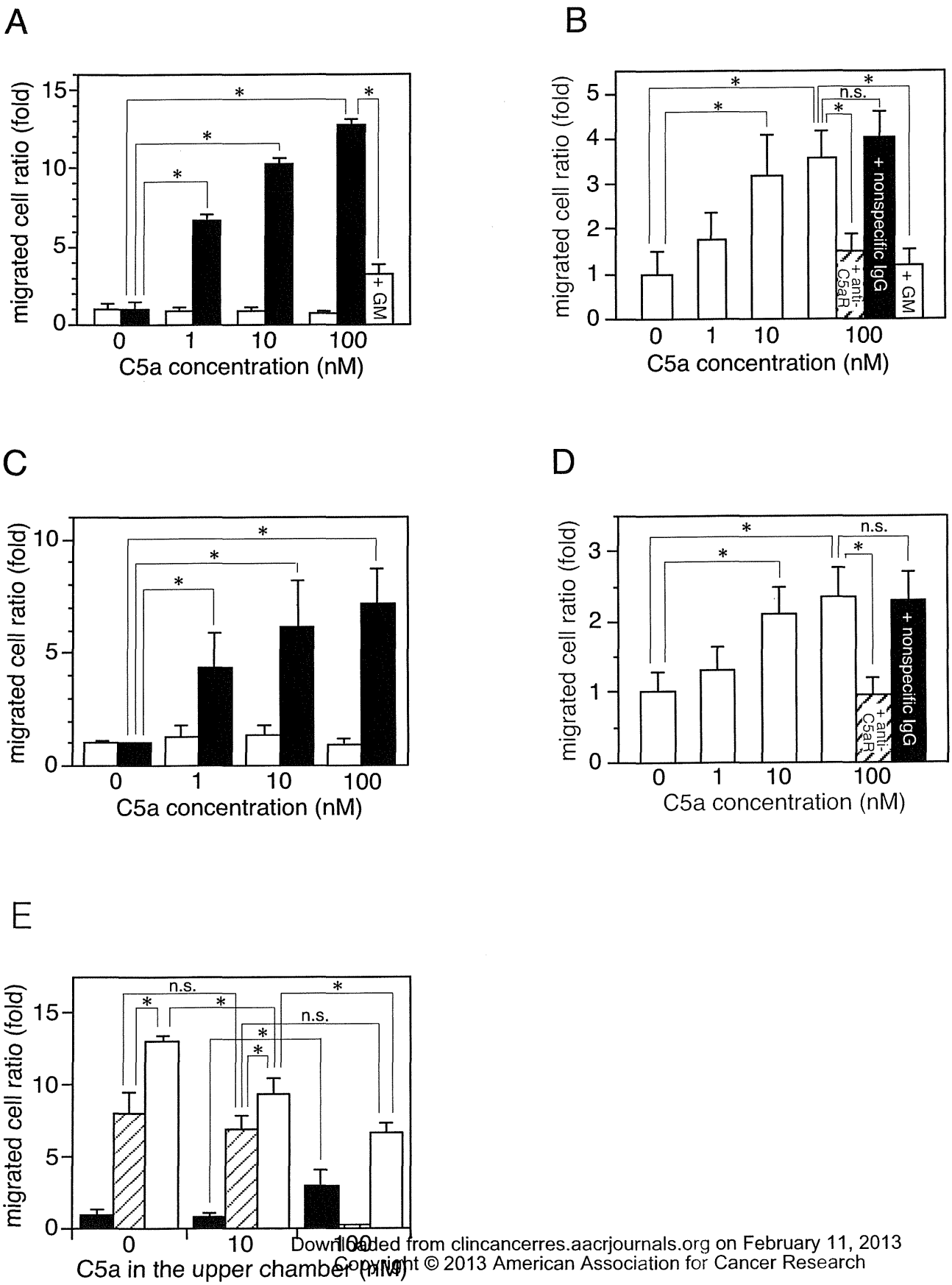
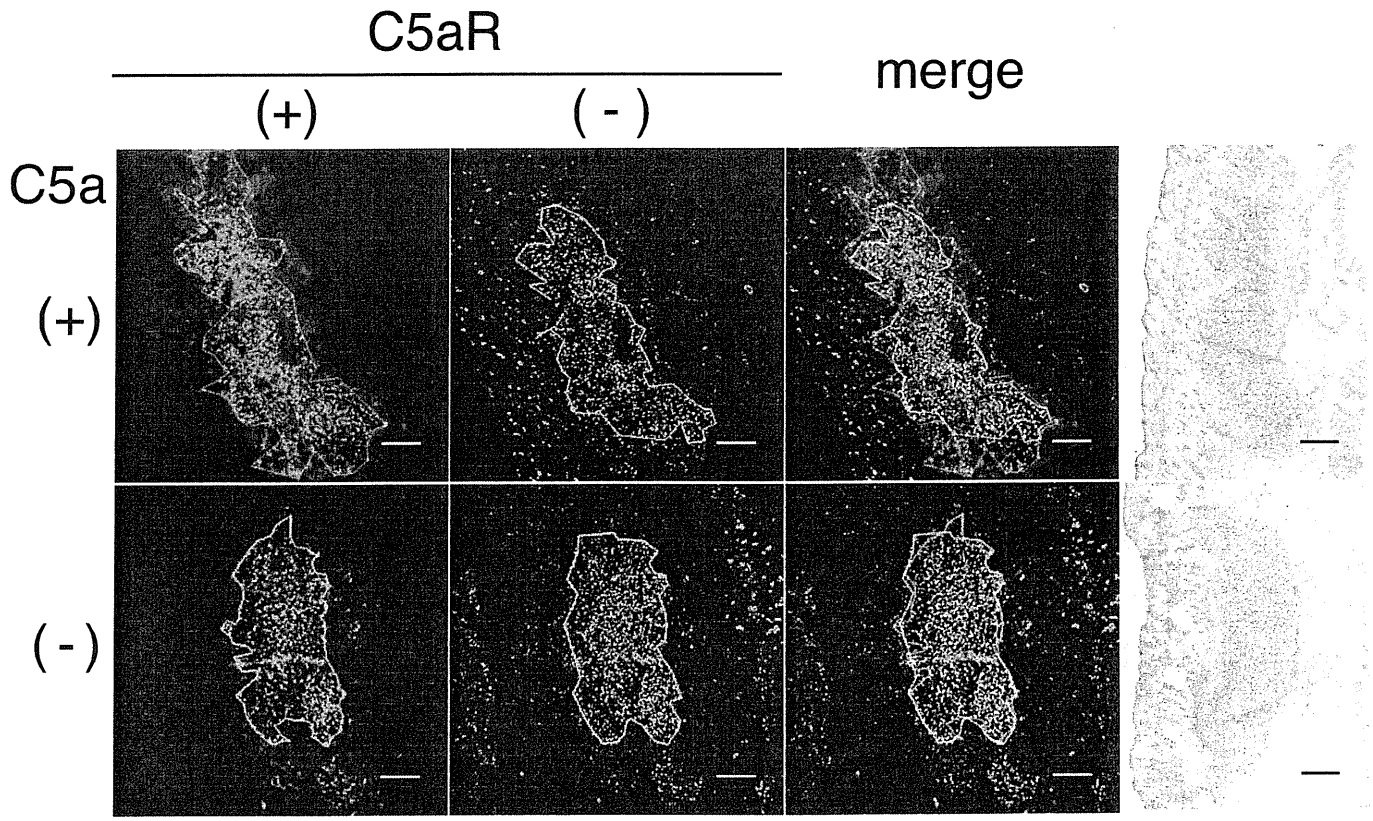


Figure 5

A



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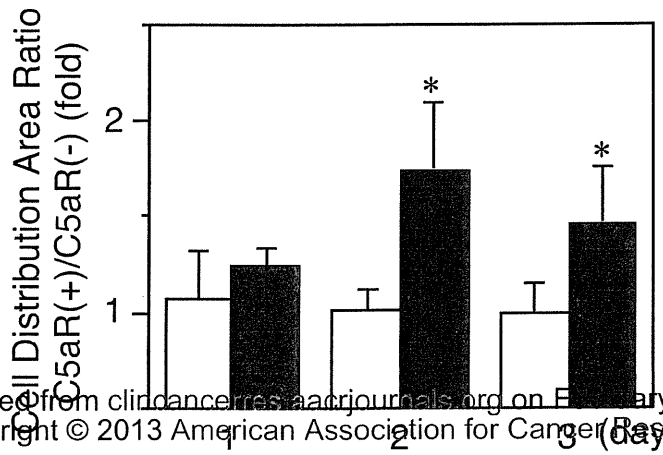

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Table 1. C5a-stimulated MMP release from C5aR expressing cancer cells.

	MEC			HuCCT1/C5aR		
MMP	C5a (-)[a]	C5a (+)[a]	Ratio[b]	C5a (-)[a]	C5a (+)[a]	Ratio[b]
1	149332.6±17039	291140.4±5360.4*	1.95	88906.6±27436.8	129408±3485	1.46
2	32.5±11.3	116.9±67. 8	3.59	1220.4±184.3	870.7±73	0.71
3	26.8±12.0	134.7±42.1*	5.03	971.6±224.6	927.5±162.9	0.95
8	20.1±23.6	74.9±29.9	3.72	335.6±77.7	1128.3±120.9*	3.36
9	< 0.1[c]	12.3±3.6*	-	6105.1±2011.3	6213.4±4357.7	1.02
10	857.6±740.5	9301.4±641.8*	10.84	57641.4±5197.5	111666.4±7911.1*	1.94
13	359.2±118.7	1367.3±309.3*	3.80	1411.9±154.6	1642.8±287	1.16

[a] MMP concentrations (pg/mL) in culture supernatants of cancer cells cultured for 24 h in the presence (+) or absence (-) of C5a (100 nM). [b] ratio of C5a(+) versus C5a(-) in mean values. [c] below the detection limit. *$P < 0.01$ ($n = 4$).

JJCO Japanese Journal of Clinical Oncology

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Image of the Month

A Case of Peritoneal Dissemination of High-grade Small Round Cell Sarcoma

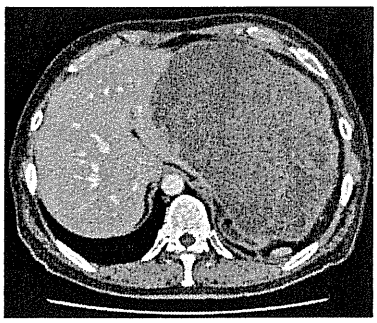

Figure 1.

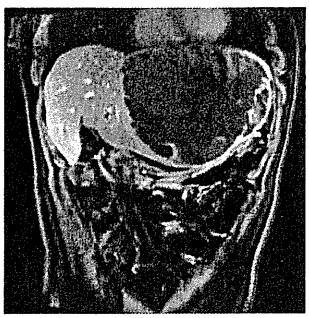

Figure 2.

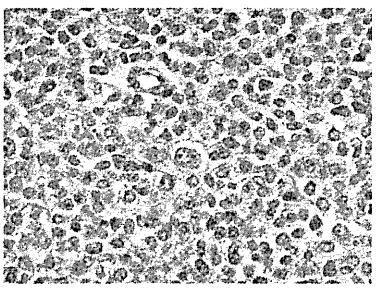

Figure 3.

A 46-year-old man, who had undergone resection of intra-abdominal tumor combined with part of the small bowel and ascending colon at another hospital 1 month before, was referred to our institution. After pathological examination of the resected specimen, the tumor was suspected to be high-grade small round cell sarcoma, presumably arising from the ileocecal mesentery. After referral to our hospital, six cycles of chemotherapy, composed of doxorubicin and ifosfamide, were administered, because imaging studies showed multiple residual intra-abdominal disseminations of the tumor. After chemotherapy, the size and number of the disseminative tumors had decreased. However, 6 months later, the patient complained of exaggerating left upper abdominal pain. Computed tomography (CT) and magnetic resonance imaging (MRI) demonstrated a rapidly enlarging mass of ~20 cm in diameter in the left subphrenic space (Fig. 1: axial CT image; Fig. 2: coronal MRI image). Intra-tumoral hemorrhage was suspected because laboratory tests showed progressive severe anemia (hemoglobin level declined to 5.9 g/dl). Although several other intra-abdominal tumors were recognized, excision of the largest subphrenic tumor was conducted in order to prevent tumor rupture and life-threatening bleeding. Total gastrectomy, left lateral bisegmentectomy (segments 2 and 3) of the liver and partial resection of the diaphragm were necessary for complete resection of the tumor.

Pathologically, the tumor consisted of small round cells with negative immunohistochemical reactivity for AE1/3, EMA and desmin. Finally, the diagnosis of high-grade small round cell sarcoma was made (Fig. 3).

The postoperative course was uneventful, but the patient died ~1 month after the surgery due to multiple liver metastases and rapid growth of residual tumors.

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doi:10.1093/jjco/hys198

S3-7　GEST試験（膵癌の第III相試験）の追跡調査結果報告

横浜市立大学附属市民総合医療センター消化器病センター[1]，国立がん研究センター中央病院肝胆膵腫瘍科[2]，静岡県立静岡がんセンター消化器内科[3]，大阪府立成人病センター消化器検診科[4]，神奈川県立がんセンター消化器内科[5]，愛知県がんセンター中央病院消化器内科[6]，九州大学病院がんセンター[7]，杏林大学腫瘍内科[8]，福岡山王病院膵臓内科[9]，東京女子医科大学消化器外科[10]，千葉県がんセンター消化器内科[11]，東北大学大学院消化器外科[12]，昭和大学病院腫瘍内科[13]，東京大学大学院医学系研究科公共健康医学専攻生物統計学[14]，九州大学大学院臨床・腫瘍外科[15]
杉森　一哉[1]，奥坂　拓志[2]，福冨　晃[3]，
上野　秀樹[2]，井岡　達也[4]，大川　伸一[5]，
朴　成和[3]，山雄　健次[6]，水元　一博[7]，
古瀬　純司[8]，船越　顕博[9]，羽鳥　隆[10]，
山口　武人[11]，江川　新一[12]，佐藤　温[13]，
大橋　靖雄[14]，田中　雅夫[15]

【目的】切除不能進行膵癌に対してゲムシタビン（GEM）単独療法を対照として，TS-1単独療法の生存期間における非劣性，ゲムシタビン＋TS-1（GS）併用療法の生存期間における優越性を検証した．本試験結果は2011年のASCOおよび本学会にて報告を行ったが，今回追跡調査を実施したので報告する．

【方法】GEM群，TS-1群は添付文書の用法・用量に従い投与が行われた．GS群は1,000mg/m^{2}のGEMを1，8日目に点滴静注し，TS-1は体表面積に合わせた規定量（60mg，80mg，100mg/日）を14日間連続投与し，その後7日間休薬する21日を1コースとしたスケジュールで実施した．2011年7月までのデータを使用して解析した．

【成績】本追跡調査解析時のイベント数は832例（FAS）中795イベントであった．

(1) MSTはGEM群8.8ヵ月（95% CI：8.0-9.7），TS-1群9.7ヵ月（95% CI：7.6-10.8）であった（OS HR＝0.96，97.5% CI：0.79-1.17，p<0.001非劣性）．

GS群のMSTは9.9ヵ月（95% CI：9.0-11.2）であった（OS HR＝0.91，97.5% CI：0.75-1.11，p＝0.28優越性）．

(2)サブグループ解析では，局所進行例とPS1症例でGS群は良好な傾向を示した．局所進行例のMSTはGEM群12.7ヵ月（95% CI：9.7-14.9），GS群15.9ヵ月（95% CI：13.0-19.7，HR＝0.72，95% CI：0.51-1.03），PS1のMSTはGEM群6.2ヵ月（95% CI：4.9-8.3），GS群9.6ヵ月（95% CI：8.0-10.9，HR＝0.62，95% CI：0.46-0.83）であった．

【結論】TS-1のGEMに対する非劣性の証明が再確認された．GS療法は更なる検討の余地がある．

S3-8　進行膵癌に対するレニンアンギオテンシン系（RA）抑制による治療成績向上の試み

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【目的】血管新生など腫瘍増殖を促すRA系は，膵癌における新たな治療標的として期待されている．我々は，Gemcitabine（GEM）療法を施行した進行膵癌155例の後向き解析にて，RA系の抑制による良好な治療成績の可能性を報告した（Br J Cancer. 2010；103：1644-8）．今回GEM・Candesartan（Can）併用療法の第1相試験・多施設共同第2相試験を報告する．【方法】[phase1]正常血圧の進行膵癌に対するGEM・Can併用療法の第1相試験として，GEM1000mg/m2 3投1休に加えてCan 4，8，16，32 mgの4 Levelをday1-28に1日1回内服のスケジュールとした．Grade4骨髄抑制，grade2血圧低下・腎機能障害，その他のgrade3非骨髄抑制の有害事象をDLTとした．[phase2] phase1で決定した推奨投与量（RD）のスケジュールに基づき，高血圧症例を含めた進行膵癌を対象に，安全性・有効性を評価した．Canは，正常血圧例で16mg，高血圧例で8mgを開始，血圧低下がない場合16mgに増量した．【結果】[phase1] 09年7月～10年10月に局所進行7例/遠隔転移7例の14例を登録（Level 1 3例，Level2 3例，Level3 6例，Level4 2例），Level 3にてgrade4好中球減少（1/6例），Level4にてgrade 2血圧低下（2/2例）を認め，Canの推奨投与量は16mgに決定．奏効率（RR）は0%であったが，Disease control rate（DCR）は79%，無増悪生存期間7.6カ月，全生存期間22.9カ月と良好な結果が得られた．[phase2] 11年5月～11年12月に進行膵癌35例（局所進行9例/遠隔転移26例）を登録．grade3以上の主な重篤な有害事象は，骨髄抑制（好中球減少24%/血小板減少18%）を認め，Canによる有害事象として，grade2血圧低下2例を2コースで認めたが，Can減量により軽快．観察期間3.1カ月と短いものの，RRは13%，DCR63%と期待できる結果が得られた．【結語】進行膵癌に対するGEM・Can併用療法は安全かつ有効な可能性が示唆され，RA系抑制は予後改善へ向けた有効な戦略となりうると考えられた．