おわりに

局所再発で救命可能な例は希少であり以下の2つの場合である．中央再発で，pelvic exenterationが可能な症例と，未照射野内の表層性または径の小さな腫瘍で根治照射が可能な症例である．

pelvic exenterationは障害の大きな治療法であり，患者選択には十分な検討が必要である．しかしながら照射野内再発に対する唯一の根治可能な治療手段であり，慎重になり過ぎ適応のある患者に生存の機会を失わせてしまうことはしてはならない[2]．

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Obstetrical and Gynecological Practice Data Book

23．子宮頸癌Ⅰa期（Ⅰa1，Ⅰa2期）

笠松高弘

はじめに

子宮頸癌ⅠA期は摘出標本の病理学的診断でのみ確定するような微小病変であるので，その予後は極めて良好である。FIGOの年度報告[1]をみると手術単独の5年生存率はⅠA1期が99.3%（n＝810），ⅠA2期が98.7%（n＝178）である。しかし実地臨床においては，リスクに見合わない拡大手術を防ぎ，また患者の希望に沿って妊孕能を極力温存しようとすると，判断に迷う場合も少なくない。若年者のコルポスコピー適格例では比較的病変をとらえやすいが，閉経後や高齢者では病変が不可視領域の頸管内に後退するので，円錐切除をしても全病変を把握することが困難なことも多い。その結果，手術術式に迷い，心配のあまり不要な拡大手術を行ってリンパ浮腫などの術後障害を残してしまう場合も散見される。また，腺癌では標準術式がなお確立しているとはいえない。

なお，微小浸潤癌の概念とそれに基づく定義は従来いろいろ論じられてきたが，最も一般的なFIGO進行期分類[1]（表1）と，わが国の取扱い規約[2]（表2）とその計測の取り決め（図1）を示した。なお腺癌の場合，取扱い規約では計測法や病理学的診断基準等において，いまだ国際的にも合意が得られていないとして，再分類はせず独自の病理学的定義を定めている（表3）。

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Q-1・リンパ節転移率，再発率，生存率は？

1．扁平上皮癌

Ostor[3]は浸潤の深さが5mmまでの症例の極めて多数の文献調査を行った。これをさらにまとめてreviewしたMota[4]によると，表4のごとく浸潤が3mm以内であれば，リンパ節転移率，再発率ともに非常に低く，死亡例も極めて少ない。その治療法や再発部位，原病死した患者の再発形式などすべてに詳細な記述があるわけではないが，再発例のほとんどは保存的治療をした例であった。リンパ節転移と関係が深いとされる脈管侵襲は，浸潤の深さとともに増加し（表5），脈管侵襲があるほうが，リンパ節転移率は高くなる傾向がある（表6）。脈管侵襲がなく，3mm以内の浸潤では再発は極めて稀だが，脈管侵襲があって，3mmを超えると15.7%と無視し得なくなる（表7）。ⅠA1期の15%に脈管侵襲が認められている。

ⅠA2期のリンパ節転移率は2.1%，再発率は3.7%，再発の52%が原病死している。このうちの33%には拡大手術が行われていた。

わが国のTakesimaら[5]の5mm以内の浸潤例402例の報告では，ⅠA1期のリンパ節転移率は1.4%（1/72），ⅠA2期では3.4%（1/29）であった。傍結合織への転移例はなかった。広汎全摘を行ったⅠA2期のうち1人にVirchow節への再発を認めている（骨盤リンパ節転移はなく，脈管侵襲のみ）。

ⅠA1期，特に脈管侵襲がない場合は極めて低リスクであるが，ⅠA2期では，低いが無視し得ぬリスクが存在すると考えられる。

表1　FIGO ⅠA 期（1994）

The carcinoma is strictly confined to the cervix (extension to the corpus would be disregarded).

Ⅰa Invasive carcinoma which can be diagnosed only by microscopy. All macroscopically visible lesions—even with superficial invasion—are allotted to Stage Ⅰb carcinomas, invasion is limited to a measured stromal invasion with a maximal depth of 5.0 mm and a horizontal extension of not >7.0 mm, depth of invasion should not be >5.0 mm taken from the base of the epithelium of the original tissue—superficial or glandular. The involvement of vascular spaces—venous or lymphatic—should not change the stage allotment.

Ⅰa1 Measured stromal invasion of not >3.0 mm in depth and extension of not >7.0 mm.

Ⅰa2 Measured stromal invasion of >3.0 mm and not >5.0 mm with an extension of not >7.0 mm.

（文献1より引用）

表2　頸癌取扱い規約 ⅠA 期（1997）

Ⅰa期：組織学的にのみ診断できる浸潤癌。肉眼的に明らかな病巣はたとえ表層浸潤であってもⅠb期とする。浸潤は，計測による間質浸潤の深さが5 mm 以内で，縦軸方向の広がりが7 mm をこえないものとする。浸潤の深さは，浸潤がみられる表層上皮の基底膜(注2)より計測して5 mm をこえないものとする。脈管（静脈またはリンパ管）侵襲があっても進行期は変更しない。

Ⅰa1期：間質浸潤の深さが3 mm 以内で，広がりが7 mm をこえないもの。

Ⅰa2期：間質浸潤の深さが3 mm をこえるが5 mm 以内で，広がりが7 mm をこえないもの。

［注2］浸潤の深さについてFIGO分類では腺上皮の基底膜からの計測も併記されている。

（文献2より引用）

表3　頸癌取扱い規約　微小浸潤腺癌

正常の内頸腺領域に限局し，微小浸潤を示す腺癌である。微小浸潤とは腺癌上皮の間質への芽出を認め，その輪郭が滑らかなものをいう。Ⅰa期に分類する。

FIGOの臨床進行期分類では微小浸潤腺癌もⅠa1期，Ⅰa2期に分類されている。しかしわが国では浸潤の深さを計測する上での基点の設定，縦軸方向の広がりについて浸潤部位の計測法等種々の問題に関して合意が得られておらず，かつ国際的にも微小浸潤腺癌ならびにⅠa期の細分類に関する病理学的診断基準が確立されていないことから，本取扱い規約では微小浸潤腺癌をⅠa1期，Ⅰa2期に細分類しないことにした。

（文献2より引用）

表4　間質浸潤とリンパ節転移率・予後

間質浸潤	例数 n	リンパ節転移%	再発%	死亡%
<1 mm	2274	0.1	0.4	0.1
1～3 mm	1324	0.4	1.7	0.5
3.1～5 mm	674	2.1	3.7	1.9

（文献3より引用，改変）

2. 腺　癌

扁平上皮癌に比べ，臨床的に微小病変の段階でとらえるのが難しいこと，微小浸潤癌の病理学的概念にいまだ論議があることなどから，臨床病理学的に検討をした症例シリーズの報告数はとても少ない。また，もともとFIGOの臨床

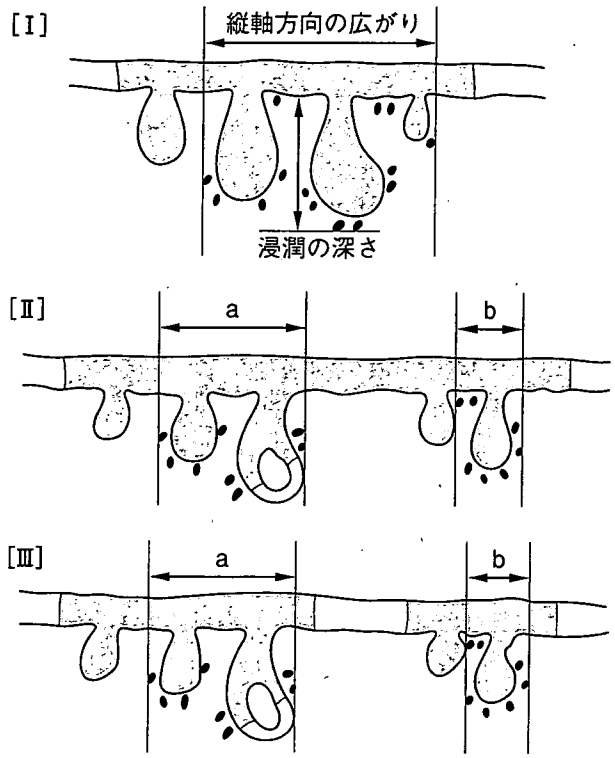

図１　微小浸潤扁平上皮癌の計測

浸潤の深さと縦軸方向の広がりはいずれも組織標本上での計測により mm 単位で記載する。浸潤の深さと縦軸方向の広がりは[Ⅰ]のように直交関係にある。

浸潤の深さは最深部の数値による。縦軸方向の広がりは浸潤巣の最大の幅を計測する。なお，[Ⅱ][Ⅲ]のように癌巣が skip lesion を形成している場合など，測定の対象部位が２カ所以上に及ぶ際には，それらのうちの最大値をもって当該症例の値とする。図では a >b なので，a を縦軸方向の広がりを評価するための値とする。

（文献２より引用）

進行期は腺癌を想定したものではないともいわれる。そこで，FIGO システムに従って，サイズの計測のみから分類し検討をした自験例[6]が表８である。浸潤 3 mm 以内に頸部外進展はなかった。ⅠA2 期に相当する病変は実際には非常に少ない。浸潤が 3 mm，縦軸 7 mm を超えると脈管侵襲，子宮外進展も無視できなくなり，広汎子宮全摘術を行っても 11.8％が再発し，原病死している。死亡例４例のうち１例にリンパ節転移があり，再発部位は３例が中央部，１例が腹膜であった。

表５　間質浸潤と脈管侵襲

間質浸潤	症例 n	脈管侵襲％
<1 mm	548	4.4
1～3 mm	596	16.4
3.1～5 mm	350	19.7

（文献４より引用）

表６　リンパ節転移と脈管侵襲・間質浸潤

脈管侵襲 n	間質浸潤	
	≤3 mm	3.1～5 mm
	リンパ節転移	
無（n＝479）	0.8％	8.3％
有（n＝102）	8.2％	7.5％

（文献４より引用）

表７　再発率と脈管侵襲・間質浸潤

脈管侵襲 n	間質浸潤	
	≤3 mm	3.1～5 mm
	再発	
無（n＝601）	0.6％	1.7％
有（n＝147）	3.1％	15.7％

（文献４より引用）

表９は浸潤 3 mm 以内の既知の報告をまとめたものである。リンパ節転移，子宮傍組織転移はなく，再発は１例（0.8％）のみである。この１例は自験例である[6][7]。ⅠA1 期相当の微小病変に対し（組織型は中分化の内頸部型，脈管侵襲なし），広汎子宮全摘術を行ったが（type 3），27 カ月後に中央部再発をした。骨盤内臓器全摘後 81 カ月無病生存したが，最終的に全身多発性転移をきたし 10 年目に原病死した症例である。経過から考え，極めて特殊な例と推測され，これをもってⅠA1 期の腺癌に縮小手術をしてはならないという症例には当たらないと考える。

なお卵巣に関しては，上記自験例 79 例のうち温存例 14 例に再発はなく，残りの摘出例に

表8　頸部腺癌の腫瘍径と脈管侵襲・リンパ節転移・傍組織浸潤・卵巣転移・予後（国立がんセンター・埼玉県立がんセンター　1969～97）

腫瘍径（n）		脈管侵襲 n	リンパ節転移	傍組織浸潤	卵巣転移	再発 n
深さ	縦径					
3 mm≧	7 mm≧*（24）	1/24（4.2%）	0	0	0	1/24（4.2%）
	7 mm＜（17）	1/17（5.9%）	0	0	0	0
3～5 mm	7 mm≧**（4）	0/4	0	0	0	0
	7 mm＜（34）	6/34（17.6%）	1（2.9%）	1（2.9%）	0	4/34（11.8%）

*ⅠA1，**1A2

表9　浸潤3 mm以下の頸部腺癌

	症例 n	手術 n	リンパ節転移 n	傍組織浸潤	再発
Nakajima（1983）	7	広汎 7	0/7	0/7	0/7
Matsukura（1989）	8	広汎 7　単純 4　円錐 1	0/3	0/3	0/8
Oster（1997）	43	広汎 10　単純 21　円錐 12	0/22	0/10	0/43
Shorge（1999）	21	広汎 16　単純 4　円錐 1	0/16	0/16	0/21
Kasamatsu（2002）	41	広汎 36　単純 5	0/36	0/36	1/41
	120		0/84	0/72	1/120

（文献6より引用，改変）

も転移はなかった。Brownら[8]の頸部腺癌Ⅰ期400例のreviewでは卵巣転移率は1.8%（1/400）である。

Q-2・標準的治療は？

1．扁平上皮癌

1）ⅠA1期

a）脈管侵襲のない場合：単純子宮全摘術*（卵巣温存可）。頸部円錐切除術（妊孕性温存希望時）。

*本稿でいう単純子宮全摘術とはいわゆるextrafascial simple hysterectomy with cuffを指す。腟壁は標準でも2 cmは切除する。

b）脈管侵襲のある場合：基本的にはa）に同じ。場合によっては広汎子宮全摘術も考慮。

ある程度の脈管侵襲であれば基本的には単純子宮全摘術でよいと考える。結果的に原病死に至る可能性は極めて稀と思われる。単純子宮全摘術＋骨盤リンパ節郭清は，肝心の傍子宮結合織，基靱帯周囲リンパ節を考慮していない術式と考えられる。ただし，1視野に多量にみられる場合は，特殊例として，本当にⅠA1期としてよいか，通常の扁平上皮癌と考えてよいか再評価をし，場合によっては広汎全摘（type 2）も考慮する必要があるかもしれない。

妊孕性温存目的の円錐切除は，当然ながら断端陰性，温存頸管の掻爬で病巣がないことの確認が必要である。

2）ⅠA2期

a）広汎子宮全摘術

単純子宮全摘術を行ってもおそらく大部分の場合根治すると考えられるが，リンパ節転移率など考慮すると，やはり広汎子宮全摘術の適応である。ただし末梢に至る十分な神経温存を考慮した術式（type 2相当）を用いる。

強い妊孕性温存希望がある場合，試験的治療ではあるが広汎子宮頸部摘出術も検討される。

b）放射線治療

以上はすべて手術療法であるが，合併症など

により外科治療ができない場合などは，摘出範囲相当をカバーする放射線治療（高線量率腔内照射，または腔内照射＋外照射）で同等の成績が挙げられる。

2. 腺　癌

1）ⅠA1期

単純子宮全摘術（卵巣温存可）

コルポスコピー，頸部生検，円錐切除による全病巣把握が扁平上皮癌に比べ困難なこと，既知の症例シリーズ報告数が非常に少ないことなどから，一律に標準といえる術式は確定されていない。自験例[6]では微小浸潤腺癌は，頸管の高位方向へ進展する傾向があり，ⅠA1期相当病変の14%は子宮摘出標本上，外子宮口から水平方向に20 mm以上高位に位置しており，円錐切除での全病巣評価の困難さが窺われた。しかし，それでも浸潤3 mm以内の腺癌の頸部外進展は皆無で，術前の病巣評価の精度を高めれば，上記術式も可能と考える。もし，摘出標本で広汎子宮全摘術を必要とする病巣と診断された場合は，再手術を行う。

円錐切除による妊孕性温存は腺癌の場合推奨されない。円錐切除をした上皮内腺癌でも29%に遺残があり，うち67%は頸管掻爬が陰性であったとする報告[9]がある。試験的な症例シリーズはあるが，安易に行ってはならない。

2）ⅠA2期

広汎子宮全摘術

Q-3・臨床上の注意点は？

1）治療前の病巣評価を正確に行う

腺癌は別として，コルポスコピー適格例では比較的容易であり，コルポスコピーのできる婦人科腫瘍専門医，婦人科病理・細胞診に長けた病理専門医のそろった施設では，すべてに円錐切除を行う必要はもちろんない。

円錐切除による評価が必要な場合，その手技に習熟する必要がある。特に病巣が頸管内主体の場合，十分深い切除が必要である。このような場合，安易な浅い切除では意味がない。レーザーやいわゆるloop円錐切除でも術者によっては可能だが，一般的にはcold-knifeを用い手技も必要である。特に腺癌にはcold-knifeを用いた深い切除が要求される。

また，高齢者の萎縮した頸部では頸管内の微小病変の生検は大変難しく，円錐切除でも診断ができないことが多い。細胞診による評価が主体とならざるを得ないので，高齢者の頸部細胞診評価が可能な施設での診療が望まれる。

2）治療の個別化

微小病変であり，本来死亡することがまずない対象だけに，過剰治療や過小治療は避けねばならない。例えば，扁平上皮癌で浸潤3 mm以内，縦軸10 mm，脈管侵襲なし，という症例にⅠB期として準広汎または広汎子宮全摘を施行する婦人科医はいないであろう。単純子宮全摘術の適応である。また，浸潤3.3 mm，縦軸5 mmでは，やはり単純子宮全摘術であろう。疑問症例では，婦人科腫瘍専門医，頸部癌に精通した細胞診専門医，病理専門医でグループカンファレンスを行い，治療方針を決定することが肝要である。もちろん，患者にはその過程を説明し，呈示した治療法のリスク等を詳しく説明することはいうまでもない。

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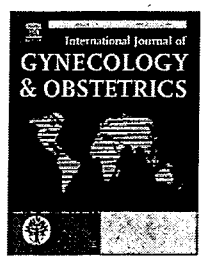

CLINICAL ARTICLE

Surgical treatment for neuroendocrine carcinoma of the uterine cervix

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KEYWORDS
Neuroendocrine carcinoma;
Radical hysterectomy;
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Abstract

Objective: To identify the best operative approach for neuroendocrine cervical carcinoma (NECC). *Methods:* The records of surgically treated patients with stages IB to IIB NECC were reviewed. *Results:* Of 10 patients who met the study criteria for NECC and underwent radical hysterectomy, 4 had pT1bN0, 4 had pT1bN1, 1 had pT2aN0, and 1 had pT2bN1 disease. Those with pT1bN1 or pT2bN1 disease received postoperative adjuvant radiotherapy and/or chemotherapy, and recurrence occurred in 7 patients (70%). Among these 7 patients, 5 (71%) had a primary NECC tumor with deep stromal invasion and 5 (71%) had extrauterine disease (parametrium and/or lymph node). The recurrences in 6 patients (86%) were located outside the pelvis (lung, liver, or brain). Stromal invasion was 6 mm or less in the 3 patients who did not experience disease recurrence. *Conclusions:* Pelvic control by radical hysterectomy may not be beneficial for patients with NECC except for those with an early invasive lesion.

1. Introduction

Neuroendocrine carcinoma arising from the uterine cervix is an uncommon malignancy comprising less than 5% of all cervical malignancies [1]. Histopathologically, neuroendocrine cervical carcinoma (NECC) resembles small cell carcinoma of the lung and is classified as small cell carcinoma of the cervix in the World Health Organization International Histologic Classification of Tumors. It is noted for its very aggressive behavior and has the poorest prognosis of the various cervical carcinomas, even after multimodal therapy. In a recent study, the 5-year survival rate of patients with International Federation of Gynecologists and Obstetricians (FIGO) stage IB1 disease was between 50% and 60%, which was significantly poorer than the 90% rate for patients with stage IB1 squamous cell carcinoma [2]. In that study, none of the patients whose disease was more extensive than stage IB1 or who had clinical evidence of lymph node metastasis survived

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their disease. It has been suggested that the poor outcome in patients with NECC is due to early and frequent metastasis.

Owing to the rarity of NECC, no multicenter study has been conducted on the disease and the optimal initial therapeutic approach has not been clarified. On the other hand, in patients with stages IB to II cervical carcinoma of the ordinary histologic type, radical hysterectomy followed by adjuvant pelvic radiation is the standard surgical approach. Radical hysterectomy has also been employed for the treatment of NECC. The present retrospective study was carried out to assess the efficacy of surgical treatment for NECC; establish a framework for designing new therapeutic strategies; and improve prognosis.

2. Patients and methods

The medical records and pathologic materials of 2096 patients with cervical carcinoma who were treated at the Gynecology Division and Diagnostic Pathology Division of the National Cancer Center Hospital in Tokyo, Japan, between 1980 and 2004 were reviewed. The study criteria were the following: having a lesion that fulfilled the histologic criteria for neuroendocrine carcinoma according to the WHO International Histologic Classification of Tumors and the Armed Forces Institute of Pathology; having stage IB to IIB disease; and having undergone primary radical hysterectomy. Patients in whom only a portion of the tumor showed neuroendocrine features were excluded. The biopsy materials were immunohistochemically stained for keratin, carcinoembryonic antigen, chromogranin, synaptophysin, and neuron-specific enolase (NSE). For this study, a gynecologic pathologist re-examined all hysterectomy materials. All patients were staged according to the 1994 FIGO staging system. Those treated before 1994 were retrospectively restaged based on their clinical records and pathologic findings.

Every 2 to 6 months, the patients found to be asymptomatic after the primary radical hysterectomy underwent a pelvic examination and a chest radiograph, and had a cervical smear taken and tumor markers measured (chiefly, NSE). Symptomatic patients then underwent appropriate examinations by ultrasound, computed tomography, and/or magnetic resonance imaging. Follow-up continued through March 2006. Survival curves were obtained by the Kaplan–Meier method.

3. Results

Among the 2096 patients with cervical carcinoma, 10 met the study criteria and were diagnosed as having pure NECC. Their median age was 41 years (range, 28–61 years) and median follow-up time (or time to death) was 25 months (range, 8–204 months). No patient was lost to follow-up. Eight patients had FIGO stage IB1 disease, one had stage IB2, and one had stage IIB. Table 1 shows the clinical characteristics of the 10 patients. All underwent radical hysterectomy with bilateral salpingo-oophorectomy and pelvic lymphadenectomy. All tumors were completely removed. Adjuvant radiotherapy or chemotherapy was administered to the 5 patients in whom lymph node metastasis or parametrial invasion was diagnosed from the surgically resected materials. Two of these 5 patients received radiotherapy to the whole pelvis and the para-aortic field, for a total dose of 45 to 50 Gy; 1 received radiotherapy to the whole pelvis alone; and the remaining 2 were treated with a chemotherapy regimen (one was treated with a combination of cisplatin, doxorubicin, and cyclophosphamide and the other with cisplatin and etoposide).

Eight patients had stage pT1b disease and 2 patients had stage pT2 disease. Lymph node metastasis was found in 4 (50%) and lymph-vascular space invasion was found in 7 (88%) of the 8 patients found from the surgically resected materials to have stage pT1b disease. The primary lesions of the 2 patients with stage pT2 disease showed lymphovascular invasion.

Disease recurred in 7 patients (70%) at a median interval of 8 months following initial surgery (range, 4–26 months). Of these 7 patients, 6 (86%) died at a median interval of 16 months after the onset of recurrence despite aggressive multimodal therapy (systemic chemotherapy, radiation, and surgery). For the 10 patients, the cumulative 5-year survival rate was 43% and the median survival time was 29 months. The disease-free survival rate was 50% at 24 months and 30% at 36 months.

In the 3 patients in whom disease did not recur, the primary tumor was an early invasive lesion of 6 mm or less (3, 5, and 6 mm, respectively). In the 7 patients in whom disease recurred, it was a deeply invasive lesion (median, 19 mm [range, 6–40 mm]). Pelvic lymph node metastasis was found in the surgically resected materials of 1 (33%) of the 3 patients with no recurrence and in 4 (57%) of the 7 patients with recurrence.

Table 1 Clinical characteristics of the patients with NECC and their status

Patient no.	Postsurgical stage	Tumor size, mm		Adjuvant therapy	Initial failure sites	Status (no. of months)
		Depth	Length			
1	pT1b1N0	3	14	None	NA	NED (29)
2	pT1b1N0	5	40	None	NA	NED (122)
3	pT1b1N0	15	28	None	Liver, lung	DOD (65)
4	pT1b1N0	18	29	None	Pelvic wall	DOD (18)
5	pT1b1N1	6	20	Radiotherapy	Liver, lung	DOD (29)
6	pT1b1N1	6	38	Chemotherapy	NA	NED (204)
7	pT1b1N1	20	24	Radiotherapy	Liver	DOD (8)
8	pT1b2N1	40	80	Radiotherapy	Liver, lung	DOD (22)
9	pT2aN0	10	20	None	Pelvic wall, PALN	DOD (22)
10	pT2bN1	25	50	Chemotherapy	Pelvic wall, Brain	AWD (21)

Abbreviations: AWD, alive with disease; DOD, dead of disease; NA, not applicable; NED, no evidence of disease; PALN, para-aortic lymph node.

The initial recurrence sites were located outside the pelvis in 6 (86%) of these 7 patients and in the pelvic sidewall of the remaining patient. In the 9 patients with distant metastasis the most frequent site was the liver (in 4 patients [44%]), followed by the lung (in 3 [33%]), the brain (in 1 [11%]), and para-aortic lymph nodes (in 1 [11%]).

4. Discussion

NECC has the poorest prognosis of the various cervical carcinomas owing to early and frequent metastasis. Because of the rarity of the disease, no large-scale multicenter study has been performed and the optimal initial therapeutic approach to NECC has not been determined. Radical hysterectomy, which is the standard surgical procedure for stages IB to II cervical carcinoma of the ordinary type, has been adopted for the treatment of NECC. Sevin et al. [3] reported a 5-year survival rate of 36.5% for patients with stages IB to IIA NECC who underwent radical hysterectomy followed by adjuvant chemotherapy, compared with 71.6% for patients with cervical carcinoma of other histologic subtypes. At our institute, the cumulative 5-year survival rate was 43% for patients with stages IB to IIB NECC, whereas it was 84%, 78%, and 65%, respectively, for stages IB, IIA, and IIB cervical carcinoma of the ordinary type [4]. The traditional surgical approach therefore does not appear to be effective in patients with NECC.

Using as search words *small cell carcinoma* and *uterine cervix* as well as *neuroendocrine carcinoma*, we conducted a Medline search of the articles on NECC published in English from January 1976 to July 2006, selecting those reporting on more than 5 patients and specifying both sites of recurrence and outcomes. This literature provided information on a total of 49 patients, including our own, who underwent radical hysterectomy for stages IB to IIB disease. The clinical

Table 2 Outcome and patterns of recurrence in patients with neuroendocrine cervical carcinoma who underwent radical hysterectomy

Author	No. of patients	Adjuvant therapy (no. of patients)	No. of recurrent sites	Status (no. of patients)
Perrin and Ward [12]				
IB	4	Chemoradiotherapy (n=4)	Locoregional (n=2)	NED (n=1)
IIA	1	None (n=1)	Lung (n=2)	DOD within 12 months (n=4)
			Liver (n=1)	
			Brain (n=1)	
			Thoracic spine (n=1)	
Chang et al. [13]				
IB	19	Chemotherapy (n=23)	Locoregional (n=5)	NED (n=13)
			Lung (n=5)	DOD within 10 months of recurrence
II	4		Liver (n=5)	(n=10)
			Brain (n=3)	
			Distant node (n=3)	
			Bone (n=2)	
			Kidney (n=2)	
			Breast (n=1)	
			Spleen (n=1)	
			Adrenal gland (n=1)	
Viswanathan et al. [2]				
IB	6	Chemotherapy (n=4)	Locoregional (n=2)	NED (4)
		None (n=2)	Distant node (n=1)	DOD (2)
			Liver (n=1)	
			Bone (n=1)	
			Breast (n=1)	
Tsunoda et al. [14]				
IB	3	Chemotherapy (n=2)	Locoregional (n=1)	NED (n=2)
IIB	2	Radiotherapy (n=2)	Lung (n=1)	DOD within 16 months (n=3)
		None (n=1)	Liver (n=1)	
			Brain (n=1)	
			Kidney (n=1)	
Present study				
pT1b	8	Chemotherapy (n=2)	Locoregional (n=3)	NED (n=3)
pT2	2	Radiotherapy (n=3)	Liver (n=4)	AWD (n=1)
		None (n=5)	Lung (n=3)	DOD within 65 months (n=6)
			Distant node (n=1)	
			Brain (n=1)	

Abbreviations: AWD, alive with disease; DOD, dead of disease; NED, no evidence of disease.

characteristics of these 49 patients are summarized in Table 2. Forty patients (82%) had stage IB or pT1b disease; 31 (63%) received adjuvant chemotherapy; 9 (18%) received radiotherapy or chemoradiotherapy; and the remaining 10 (20%) did not receive adjuvant therapy. Fourteen patients were treated with combination chemotherapy using vincristin, doxorubicin, and cyclophosphamide alternating with cisplatin and etoposide; 8 were treated with cisplatin, vinblastin, and bleomycin; 3 were treated with cisplatin and etoposide; and 3 were treated with cisplatin, doxorubicin, and etopocide. Recurrence occurred in 26 patients (53%) and 25 patients (51%) died of the disease. Among those in whom disease recurred, 23 (88%) had extrapelvic metastasis. Of the 45 distant sites of recurrence, the most frequently reported were the lung (27%) and liver (27%), followed by a distant node (11%), and the brain (13%). Based on these findings, the development of widespread hematogenous metastasis is the most important pattern in NECC, and controlling hematogenous spreading should be a top priority in the attempt to improve the survival of patients with this type of cervical carcinoma.

In comparison, the prognosis for patients with cervical squamous cell carcinoma who are treated with radical hysterectomy is good. Recurrence develops in 10% to 15% of patients with stages IB or IIA disease who undergo radical hysterectomy, with or without postoperative radiation of the whole pelvis [5]. Following radical hysterectomy, the difference in outcome among patients with squamous cell carcinoma and those with NECC may be due to differences in the biologic behavior of the carcinomas. In patients with NECC, pelvic control alone usually does not lead to a good outcome because of the high incidence of distant metastasis in the early stage.

Lymphedema and bladder dysfunction develop in almost all patients who undergo radical hysterectomy [6]. Morbidities associated with radical hysterectomy include chronic bladder dysfunction (in 3% of patients), ureterovaginal or vesicovaginal fistula (in 1%–2%), lymphocele formation (in 5%), small bowel obstruction (in 1%), pulmonary embolism (in 1%–2%), injury to the obturator or genitofemoral nerve, and blood loss requiring transfusion [7]. These complications may interfere with systemic postoperative adjuvant therapy for the control of distant metastasis. Thus, radical hysterectomy does not appear to be beneficial in patients with NECC, and indications for this treatment should be limited.

In the present study, the stromal invasion of the primary tumor was 6 mm or less in patients who did not experience recurrence and a median of 19 mm in those who experienced recurrence. The incidence of pelvic lymph node metastasis was higher among patients who experienced recurrence than in those who did not. And 2 series of meta-analyses demonstrated that the presence of lymph node metastasis was the most important factor for a poor prognosis [8,9]. Viswanathan et al. [2] reported that none of their patients with clinical evidence of lymph node metastasis survived their disease. In patients with NECC, radical hysterectomy may be indicated only in cases of early invasive lesion with no lymph node metastasis.

Theoretically, to reduce the incidence of widespread distant metastasis after hysterectomy, adjuvant systemic chemotherapy is indicated. As no large-scale, multicenter study has been conducted with patients diagnosed as having NECC, no optimal regimen has been established for treating the disease. A regimen originally developed for the treatment of small cell carcinoma of the lung, which includes cisplatin and etoposide, has been tried. Although several studies have suggested the combination of cisplatin and etoposide to be beneficial, they reported on small numbers of patients [8,10,11]. Multicenter randomized controlled trials are needed.

In conclusion, pelvic control by radical hysterectomy does not appear to be generally beneficial for patients with NECC, and it should be limited to those with an early invasive lesion without obvious lymph node metastasis. Rather, nonradical hysterectomy followed by new, aggressive adjuvant chemotherapy may be considered following surgery.

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Analysis of the clinicopathological prognosis of stage IVb cervical carcinoma

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Abstract. The aim of this study was to evaluate the clinicopathological prognostic factors in patients with stage IVb cervical carcinoma (CC). All patients with stage IVb CC included in the study were diagnosed from 1997 to 2006 at the National Cancer Center Hospital. We retrospectively examined clinicopathological parameters in these patients, including the efficacy of chemotherapy. Survival was evaluated using Kaplan-Meier curve analysis and log-rank test. The independent prognostic factors found to be predictive of survival in univariate and multivariate analysis were evaluated using a Cox's proportional hazard model. Thirty-six patients (median age 54 years) were diagnosed with stage IVb CC. The median progression-free survival and overall survival were 3.8 and 11.1 months, respectively. As initial treatment, 4 patients underwent hysterectomy, 13 received chemotherapy, 17 received radiotherapy, and the remaining 2 patients refused treatment. A total of 21 patients received chemotherapy, of which 13 were initial cases, 7 were persistent/recurrence cases, and 1 was a postoperative adjuvant case; 15 patients were never treated with chemotherapy. On univariate analysis, poor performance status (PS) and non-chemotherapy groups were considered poor prognostic factors, respectively. On multivariate analysis, poor PS (p=0.007; hazard ratio, 2.64) and non-chemotherapy (p=0.016; hazard ratio, 6.03) were independent prognostic factors of survival, respectively. Poor PS and non-chemotherapy groups were found to have poor prognosis in patients with stage IVb CC. Chemotherapy may improve the survival for stage IVb CC.

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Key words: stage IVb cervical carcinoma, prognostic factor, chemotherapy, performance status

Introduction

Cervical carcinoma is the main cause of death in females throughout the world, despite the fact that a useful screening method has been established (1). In stage I/II patients, conventional treatments such as surgery and radiotherapy have achieved good results. In stage III/IV patients, various treatments such as the combination of surgery and radiotherapy, radiotherapy, and chemoradiation therapy are being examined, though their long-term results are still poor (2,3). The 5-year survival of stage IVb patients ranges from 0 to 44%, and approximately 50% of these patients show a fatal outcome within 1 year (4-6). No standard therapy has been established, and palliative surgery, radiotherapy, and best supportive care (BSC) have been performed as initial treatment. However, since stage IVb cervical carcinoma is a systemic disease, surgery and radiotherapy are useful for local control, but are insufficient. In addition, BSC is not effective for the severe local pain characteristic of this disorder (7). Since 1990, chemotherapy has been employed as a type of BSC in patients with good general condition and organ function (8). However, as this therapy targets the relief of symptoms and improvements in quality of life (QOL), regimens with less toxic low-dose agents were initially administered (9). No randomized comparative study has examined whether chemotherapy for stage IVb cervical carcinoma prolongs survival compared to BSC.

Several studies have investigated single-agent chemotherapy for cervical carcinoma, and reported that the response rates to cisplatin, ifosfamide, paclitaxel, vinorelbine and topotecan of 20-30% (5,8,10-12), 14-40% (13-15), 17% (16), 15% (17,18) and 12-19% (19,20), respectively. Cisplatin has been the most frequently used agent, and has achieved the highest response rate. Therefore, cisplatin has been employed as a key drug for more than 20 years. However, the response to single-agent cisplatin has been limited, and combination chemotherapy with other agents has been administered to achieve improvement in prognosis, exceeding the enhancement of its toxicity. Result of recent phase III studies have indicated that combination regimens with cisplatin/paclitaxel (21) or cisplatin/topotecan (22) are more effective than single-agent cisplatin.

A few studies have reported that factors affecting the prognosis of stage IVb cervical carcinoma include main organ

metastases, multiple lymph node metastases, poor performance status (PS), and non-squamous cell carcinoma (23-29). According to some studies, the results of surgery combined with radiotherapy or radiotherapy alone are relatively good in stage IVb cervical carcinoma patients with para-aortic lymph node metastases alone (30-33). However, chemotherapy for stage IVb patients with cervical/mediastinal lymph node or main organ metastases, without surgery and radiotherapy, has been reported to have only slight effect.

In this study, we retrospectively investigated the clinicopathological features of stage IVb cervical carcinoma, and evaluated the efficacy of chemotherapy for this stage of cancer.

Patients and methods

Patients with stage IVb cervical carcinoma were diagnosed and treated in the National Cancer Center Hospital between April 1997 and March 2006. Stage was evaluated according to the FIGO staging. We rectrospectively reviewed the medical chart of these patients.

Treatment. Therapeutic strategies were selected for individual patients. For surgery, total hysterectomy (radical hysterectomy in some patients) and bilateral salpingo-oophorectomy were performed. Pelvic and/or para-aortic lymphadenctomy were performed in some patients. For radiotherapy, the area of external irradiation was established as the entire pelvic region from the closed pore to the L4/5 lumbar vertebrae, with a radiation dose of 2 Gy per treatment (total dose, 50-60 Gy). When the cumulative dose reached 20-30 Gy, external irradiation was combined with high-dose intra-cavity irradiation, with a central shield, at a radiation dose of 5 Gy (total dose, 20-25 Gy). When imaging findings suggested para-aortic lymph node metastases, biopsy was performed. After a definitive diagnosis of metastases was made, the irradiation field was extended to include the para-aortic node. For chemotherapy, eligible patients participated in a phase II clinical study with an in-house protocol that we previously reported, including paclitaxel (PTX)/carboplatin (CBDCA) therapy (Kitagawa R, *et al*, Proc ASCO 22: abs. 5048, 2004) (PTX, 175 mg/m^2, CBDCA AUC5, day 1, every 3 weeks for 6 cycles), and carboplatin (CBDCA)/irinotecan (CPT) therapy (Hori S, *et al*, Proc ASCO 21: abs. 835, 2002) (CBDCA AUC5, day 1, CPT 60 mg/m^2, days 1, 8 and 15, every 4 weeks for 6 cycles). For patients with PS of 3, weekly PTX/CBDCA therapy (PTX 80 mg/m^2, CBDCA AUC2, continuous administration for 20 weeks) was administered. In 1 patient with small cell carcinoma, cisplatin (CDDP)/CPT therapy (CDDP, 60 mg/m^2, day 1, CPT 60 mg/m^2, days 1, 8 and 15, every 4 weeks for 6 cycles) was administered as postoperative adjuvant therapy.

Best supportive care (BSC) was defined as treatment targeting the relief of symptoms without surgery, radiotherapy or chemotherapy, as described above.

Evaluation. Pretreatment clinical evaluation was repeated before each treatment cycle with the exception of radiography or CT/MRI imaging, which was repeated at least every other treatment cycle. Treatment was continued until disease progression or adverse effects precluded further administration. The response to treatment, in terms of the best response achieved in a given patient, was assessed using standard clinical criteria. A complete response (CR) was defined as the disappearance of all gross evidence of disease for at least 4 weeks. A partial response (PR) was defined as a >50% reduction in the product of perpendicular diameters obtained from the measurement of each lesion, sustained for at least 4 weeks. Progressive disease (PD) was defined as a >50% increase in the product of perpendicular diameters of any lesion documented within 2 months of study entry or the appearance of any new lesion within 8 weeks of study entry. Stable disease (SD) was any condition not meeting any of the above three criteria. Overall survival was measured as the observed length of life from protocol entry to death or (for living patients) date of last contact. Progression-free survival was measured from the date of initiation of protocol to the first progression or death, or to the date of last contact for patients who were alive and progression-free.

Persistent disease was defined as carcinoma at a pelvic site known to be previously involved within 6 months of staging. Recurrent disease was classified as a new tumor in the extrapelvic area or pelvic disease >6 months after staging in a location previously tumor-free. Persistent or recurrent disease was documented by surgical exploration, biopsy or progression on imaging studies. The time of recurrence or death was calculated from the date of original staging. The end of the follow-up period was March 2006.

Statistical analysis. Statistical analysis was performed using SPSS. The impact of clinical and pathologic risk factors on survival was evaluated using Kaplan-Meier curve analysis and log-rank test. The independent prognostic factors found to be predictive of survival in univariate and multivariate analysis were evaluated using Cox's proportional hazard model. P-values <0.05 were considered significant.

Results

Thirty-six patients were treated between April 1997 and March 2006. Table I shows the patient characteristics. The median age was 54 years. In 34 patients, PS was almost 0, 1 or 2. In the remaining 2 patients, PS was 3. As initial treatment, surgery was performed in 4 patients, radiotherapy in 17, and chemotherapy in 13. BSC was performed in two patients who did not wish to receive aggressive treatment. Histopathologically, 18 patients had squamous cell carcinomas, 16 had adenocarcinomas and 2 had small cell carcinomas. The median primary tumor diameter was 4.1 cm, with a maximum of 7.7 cm. In addition, a bulky mass was detected in 28 patients. In 13 patients, hydronephrosis was noted, with 8 of these having bilateral hydronephrosis. The number of distant metastases was 1 in most patients, but 3 or 4 in some patients. The metastatic lesion sites included the para-aortic node in 7 patients and the main organs in 8 patients. Table II shows the sites of distant metastases (including duplicating patients). In the abdominal cavity, para-aortic lymph node metastases were detected in 18 patients (50%), comprising the highest percentage. In the extraperitoneal region, supraclavian lymph node metastases were detected in 13 patients (36%). Among main organ metastases, liver metastases were detected in 7

Table I. Patient characteristics.

Age (year), median (range)	54 (28-77)
PS 0/1/2/3	5/18/11/2
No. of patients	36
Initial treatment	
Surgery	4
Radiotherapy	17
Chemotherapy	13
Best supportive care	2
Pathology	
Squamous cell carcinoma	18
Adenocarcinoma	16
Small cell carcinoma	2
Primary tumor size (cm), median (range)	4.1 (2.1-7.7)
Bulky mass >4 cm	
Negative	8
Positive	28
Hydronephrosis	
Negative	23
Unilateral	5
Bilateral	8
No. of distant metastases	
1	20
2	13
3	2
4	1
Site of distant metastases	
Para-aortic lymph node only	7
Distant lymph node only	7
Organ metastases only	1
Para-aortic lymph node + Distant lymph node	10
Para-aortic lymph node + Organ metastases	1

Table II. Distant metastases in patients.

Metastatic sites	n (%)
Intra-abdominal metastases	
Para-aortic lymph node	18 (50)
Liver	7 (19)
Spleen	2 (5.5)
Small intestine	1 (2.7)
Extra-abdominal metastases	
Lung	4 (11)
Bone	2 (5.5)
Supraclavicular lymph node	13 (36)
Mediastinal lymph node	2 (5.5)
Inguinal lymph node	2 (5.5)

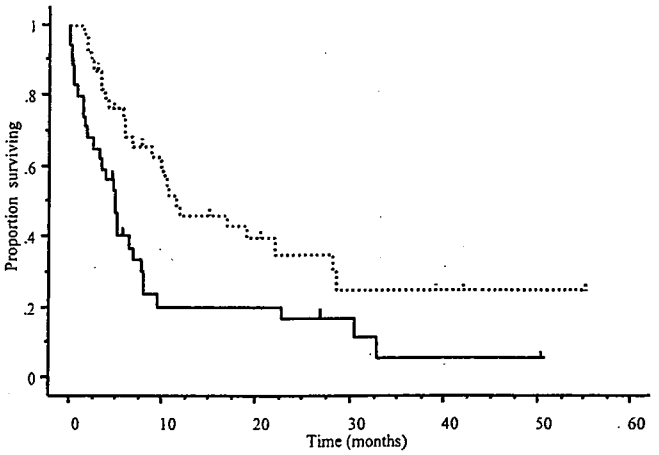

Figure 1. Kaplan-Meier analysis of progression-free survival (solid line) and overall survival (dotted line). Vertical bars indicate censored cases.

Table III. Characteristics of 21 patients with chemotherapy.

	n=21
Indication for therapy	
Initial case	13
Persistent/recurrence case	7
Postoperative case	1
Regimens	
Paclitaxel/carboplatin	9
Irinotecan/carboplatin	9
Weekly paclitaxel/carboplatin	2
Irinotecan/cisplatin	1

patients, comprising the highest percentage, followed by lung metastases in 4 patients. The median progression-free survival and overall survival were 3.8 months and 11.1 months, respectively (Fig. 1).

We examined the effects of chemotherapy on stage IVb cancer (Table III). Chemotherapy was administered to 21 patients, 13 of whom were undergoing initial treatment, 7 of whom had persistent/recurrence, and 1 of whom was undergoing postoperative therapy. The regimens consisted of paclitaxel/carboplatin in 9 patients, irinotecan/carboplatin in 9, weekly paclitaxel/carboplatin in 2, and cisplatin/irinotecan in 1. In 2 patients, including 1 undergoing postoperative adjuvant therapy, chemotherapy was discontinued due to adverse effects. For lesions that could be measured, the response rate was 61.9% (95% CI, 41.1-82.6) including 4 patients with CR and 9 patients with PR (Table IV).

We compared survival in the chemotherapy and non-chemotherapy groups. The median survivals of the chemotherapy and non-chemotherapy groups were 11.1 and 5.1 months, respectively, with a significant difference (p=0.0055) (Fig. 2).

We also compared survival between initial chemotherapy and initial other treatment groups. The median survivals in the initial chemotherapy and initial other treatment groups

Table IV. Response rate of chemotherapy (n=21).

Response (%)					
CR	PR	SD	PD	NE	RR
4	9	4	1	3	61.9% (95% CI, 41.1-82.6%)

CR, complete response; PR, partial response; SD, stable disease; PD, progression disease; NE, not evaluable; RR, response rate.

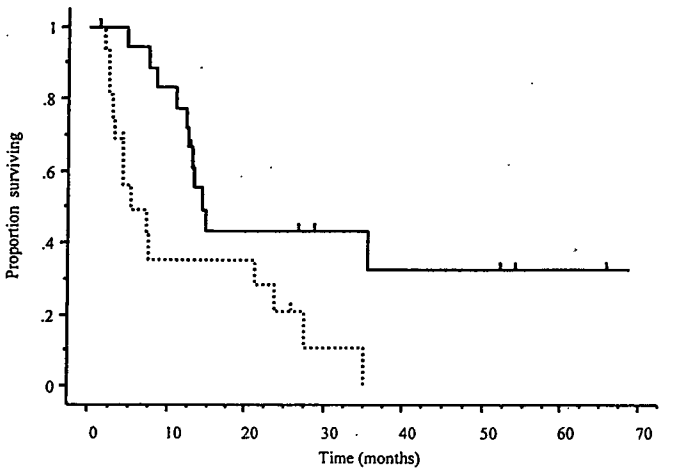

Figure 2. Kaplan-Meier analysis of overall survival according to with/without chemotherapy in stage IVb cervical carcinoma. Chemotherapy group (solid line) is significantly better prognosis (p=0.0055) than non-chemotherapy group (dotted line). Vertical bars indicate censored cases.

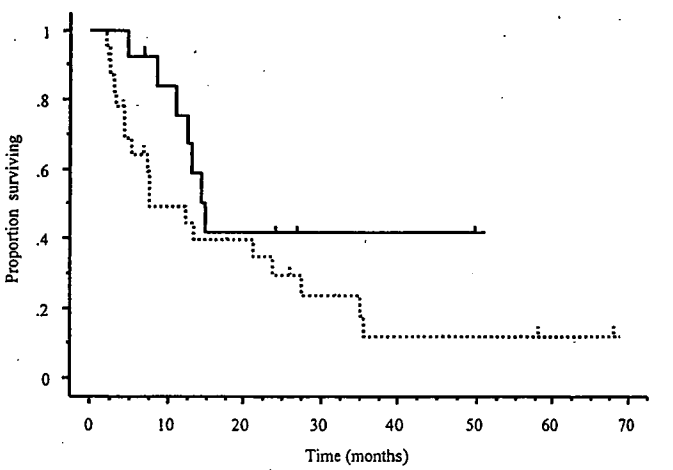

Figure 3. Kaplan-Meier analysis of overall survival according to with/without initial chemotherapy in stage IVb cervical carcinoma. There are no statistical differences (p=0.09) between initial chemotherapy group (solid line) and other initial treatment group (dotted line). Vertical bars indicate censored cases.

were 13.2 and 7.5 months, respectively, but it did not reach statistical significant (p=0.09) (Fig. 3). Two patients treated by chemotherapy alone as an initial treatment have survived

Table V. Prognostic factors of overall survival.

	Univariate	Multivariate		
Factor	P-value	P-value	HR	95% CI
Age ≥50	0.171	0.506	1.36	0.54-3.43
PS (0 and 1 vs. 2 and 3)	0.005	0.007	2.64	1.42-4.91
Pathology (SCC vs. non-SCC)	0.638	-	-	-
Organ metastases (0 vs. ≥1)	0.792	-	-	-
No. of distant metastases (1 vs. ≥2)	0.109	0.546	1.22	0.63-2.35
Bulky mass	0.478	-	-	-
Chemotherapy	0.011	0.016	6.03	1.97-18.37

disease-free for 51.8 and 68.6 months, respectively. One patient had stage IVb CC with para-aortic lymph node metastases while the other had stage IVb CC with subclavian lymph node metastases and mediastinal lymph node metastases. Both patients were administered paclitaxel/carboplatin for 6 cycles. After 6 cycles, the primary lesion and metastatic site exhibited complete response.

We analyzed chemotherapy, age, PS, histological type, main organ metastases, number of distant metastases, and bulky masses as prognostic factors. On univariate analysis, poor PS and non-chemotherapy groups were prognostic factors. On multivariate analysis, a poor PS (p=0.007; hazard ratio, 2.64; 95% CI, 1.42-4.91) and non-chemotherapy groups (p=0.016; hazard ratio, 6.03; 95% CI, 1.94-18.37) also affected overall survival (Table V).

Discussion

The prognosis of stage IVb cervical carcinoma is poor in patients with systemic metastases. No treatment has been established. In the NCI-PDQ, it is described that therapeutic strategies for this stage of cancer include palliative radiotherapy, chemotherapy as a regimen designed by a clinical study, and chemotherapy with cisplatin, which has previously been reported (34).

In stage IVb patients with para-aortic lymph node metastasis alone, surgery with postoperative radiotherapy and extended radiotherapy achieved a 5-year survival rate of 50% (30-33), and radical surgery may also be an option. However, since most metastases involve the main organs, it is difficult to control them by local treatment, and chemotherapy is indicated for most patients (4).

Various regimens of chemotherapy for this stage of cancer, including single-agent, have been investigated. In particular, cisplatin has most frequently been employed, and yields the highest response rate as a single-agent. It has therefore been

used as a key drug for more than 20 years (5,8,10-12). However, since the efficacy of cisplatin as a single-agent persists for only 6 months, combination regimens have been administered to improve in the prognosis to an extent exceeding the enhancement of its toxicity. In the 1990s, many phase II clinical studies investigated combination regimens with 2-4 agents including cisplatin. Cisplatin with ifosfamide (IFM) yielded the second highest response rate, and bleomycin (BLM), which has commonly been employed to treat other cancers due to its similar high response rate and low toxicity. The usefulness of IP (IFM + CDDP) (35) and BIP (BLM + IFM + CDDP) (36) regimens has also been examined. Some regimens have achieved a response rate of 60% or higher; however, these regimens for the non-advanced and locally advanced stages are quite toxic and shorten the survival of some patients. In addition, no comparative study has been conducted, and the evaluation of each regimen has been insufficient. In the latter half of the 1990s, combination regimens with new agents were designed, and the need for a standard therapy was emphasized.

Recently, carboplatin (37-39), topotecan (19,20) and paclitaxel (40-42) have also been reported to be tolerable and efficacious. Complete responses have also been observed with topotecan and paclitaxel. However, topotecan has greater toxicity than carboplatin or paclitaxel. Therefore, palliation with single-agent cisplatin, carboplatin, paclitaxel or topotecan is a reasonable approach in patients with recurrent disease. A phase II study evaluating the effectiveness of docetaxel in patients who have persistent or recurrent cervical cancer is ongoing (GOG-0127S).

Cisplatin-based combination chemotherapy regimens such as cisplatin/paclitaxel (21) and cisplatin/topotecan (22) have been extensively investigated in clinical studies. A randomized phase III study comparing paclitaxel and cisplatin versus cisplatin alone showed that the two-drug combination yielded a higher response rate (36 versus 19%) and improved progression-free survival (4.8 versus 2.8 months; $p<0.001$), although no improvement has been seen in median survival (21). Another randomized phase III GOG study investigated the combination of cisplatin and topotecan versus cisplatin alone for persistent/recurrent cervical cancer. In this study of 294 eligible patients, the topotecan combination regimen was superior to single-agent cisplatin with respect to overall response rate (27 versus 13%; $p=0.004$), progression-free survival (4.6 versus 2.9 months; $p=0.014$), and median survival (9.4 versus 6.5 months; $p=0.017$) (22). A phase II study assessed cisplatin and gemcitabine in patients with advanced, persistent/recurrent cervical cancer; 17 patients were evaluated (43). The response rate was 57% in patients who had not previously received radiotherapy, and there was 1 complete response of 14 months. Paclitaxel and carboplatin have recently been assessed for recurrent or persistent cancer of the cervix; 4 of 15 patients had a complete response and 5 showed a partial response for an overall response rate of 60% (39). The median survival of all 15 patients treated was 17 months (range, 4-39 months). The combination of vinorelbine and cisplatin has also been assessed in 42 patients with recurrent or metastatic cervical cancer; the overall response rate was 48% (44). The GOG is currently conducting a phase III trial (GOG204) to assess 4 cisplatin-doublet regimens in patients with advanced metastatic or recurrent cancer (cisplatin/paclitaxel, cisplatin/topotecan, cisplatin/gemcitabine, versus cisiplatin/vinorelbine).

In our hospital, we conducted an in-house clinical study. For eligible patients, paclitaxel/carboplatin or irinitecan/carboplatin therapy was administered. Adverse effects were within the permissible ranges, and there were no treatment-related deaths, as reported in other studies. Response rate as an end-point was also similar to or exceeded that previously reported, suggesting the usefulness of these treatment options in chemotherapy for cervical carcinoma. In patients with poor PS, weekly paclitaxel/carboplatin therapy was safe. Several reports have indicated that the hematological toxicity of this therapy is lower than that of tri-weekly therapy, and that the therapeutic effects of these two regimens are similar (45,46). Weekly paclitaxel/carboplatin therapy may be useful for treating stage IVb cancer patients with poor PS.

In patient with this stage of cancer, nephropathy is frequent, making cisplatin administration difficult in many cases. Carboplatin can be administered to patients with nephropathy, without hydration. Considering the adverse effects, less toxic agents should be reviewed.

In this study, two patients treated by chemotherapy alone as an initial treatment have survived disease-free for 51.8 and 68.6 months, respectively. For patients with recurrence who desired sequential treatment, chemotherapy was administered when we considered them eligible. Considering that the prognosis was significantly better than that in the non-chemotherapy group, chemotherapeutic intervention may be useful in stage IVb patients who have undergone initial treatment and in those with persistent/recurrent metastases.

Eligible, consenting patients should be enrolled in clinical trials employing new drugs and/or strategies. Since there is as yet no evidence for the curative potential of chemotherapy in cervical cancer and no established survival benefit, and uncertainty exists as to how often response translates into symptom relief ('palliation'), non-protocol therapy should not be encouraged. Nevertheless, for a patient who is ineligible or unwilling to participate in a study but who wants treatment, there may still be an indication for chemotherapy giving 'psychological support' or hope. When such a patient insists on treatment and seeks untested remedies rather than a hospice if orthodox chemotherapy is not offered, single-agent cisplatin or carboplatin may be justified, with due attention being paid to contraindications and the toxic side effects. An interval response assessment and finite period of treatment are indicated. Objective benefit is possible, but not likely.

Prognostic factors for stage IVb cervical carcinoma include PS, age, histological type, main organ metastases, and distant metastases (23-29). In this study, univariate and multivariate analysis revealed that non-chemotherapy and poor PS influenced prognosis. In patients with poor PS, it is difficult to continue treatment, and chemotherapy may exceed cancer control due to systemic disease. However, we can not conclude the efficacy of chemotherapeutic intervention, as this study was a retrospective study and involved only a small number of patients. Previously, surgery and radiotherapy have been selected for this stage of cancer. The results of chemotherapy for initial treatment were similar to those for conventional treatment, suggesting the efficacy of chemotherapy as initial

treatment. However, a randomized comparative study should be conducted to demonstrate its efficacy.

In conclusion, the prognosis of stage IVb cervical carcinoma remains poor. Chemotherapy may improve the survival of patients with stage IVb CC.

Acknowledgments

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Promoter Hypermethylation Contributes to Frequent Inactivation of a Putative Conditional Tumor Suppressor Gene *Connective Tissue Growth Factor* in Ovarian Cancer

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Abstract

Connective tissue growth factor (CTGF) is a secreted protein belonging to the CCN family, members of which are implicated in various biological processes. We identified a homozygous loss of *CTGF* (6q23.2) in the course of screening a panel of ovarian cancer cell lines for genomic copy number aberrations using in-house array-based comparative genomic hybridization. *CTGF* mRNA expression was observed in normal ovarian tissue and immortalized ovarian epithelial cells but was reduced in many ovarian cancer cell lines without its homozygous deletion (12 of 23 lines) and restored after treatment with 5-aza 2′-deoxycytidine. The methylation status around the *CTGF* CpG island correlated inversely with the expression, and a putative target region for methylation showed promoter activity. *CTGF* methylation was frequently observed in primary ovarian cancer tissues (39 of 66, 59%) and inversely correlated with *CTGF* mRNA expression. In an immunohistochemical analysis of primary ovarian cancers, CTGF protein expression was frequently reduced (84 of 103 cases, 82%). Ovarian cancer tended to lack CTGF expression more frequently in the earlier stages (stages I and II) than the advanced stages (stages III and IV). CTGF protein was also differentially expressed among histologic subtypes. Exogenous restoration of CTGF expression or treatment with recombinant CTGF inhibited the growth of ovarian cancer cells lacking its expression, whereas knockdown of endogenous CTGF accelerated growth of ovarian cancer cells with expression of this gene. These results suggest that epigenetic silencing by hypermethylation of the *CTGF* promoter leads to a loss of CTGF function, which may be a factor in the carcinogenesis of ovarian cancer in a stage-dependent and/or histologic subtype-dependent manner. [Cancer Res 2007;67(15):7095–105]

Introduction

Epithelial ovarian cancer is the leading cause of death from gynecologic tumors (1), due to its aggressive nature and the fact that the majority of patients are diagnosed in advanced stages of the disease. The lack of preventive strategies, early diagnostic methods, and effective therapies to treat recurrent ovarian cancer creates a pressing need to understand the molecular mechanisms responsible for the development and progression of ovarian cancer and to identify molecular markers and targets for diagnosis as well as therapy (2). Sporadic ovarian cancers display defects in many genes, including *AKT, EGFR, ERBB2, RAS, PIK3CA, MYC, DOC-2/DAB2, SNCG*, and *TP53*, as well as a myriad of cytogenetic abnormalities (3). These defects result from both genetic and epigenetic changes and can occur at varying frequencies in different pathologic subtypes, which are morphologically and biologically heterogeneous, both early and late in the transformation process (2). Because there have been no known tumor suppressor genes (TSG) other than *TP53* showing high frequencies of somatic mutations in ovarian cancer, further efforts for the identification of putative TSGs are needed.

Several typical TSGs were originally pinpointed by mapping regions of biallelic loss in cancer cells (4–6), although the homozygous deletion of those genes is a rare event and other mechanisms, including aberrant methylation of CpG sites within the promoter region (7), may predominantly contribute to their functional inactivation. Therefore, scanning the entire genome for homozygous deletions with high resolution is believed to be useful for a precise and rapid identification of tumor suppressors. Indeed, we have applied in-house bacterial artificial chromosome (BAC)–based arrays (8) for an array-based comparative genomic hybridization (array-CGH) analysis of various human cancers and identified candidate TSGs mainly inactivated through homozygous loss or promoter hypermethylation from homozygously deleted regions (9, 10).

In ovarian cancer, the aberrant DNA methylation of known TSGs, such as *p16INK4A* (11), *RASSFIA* (12), *BRCA1* (13), and *hMLH1* (14), has been reported. However, the importance of epigenetic changes to TSGs in ovarian cancer remains largely unknown, and it is possible that more genes frequently inactivated through DNA methylation and involved in the pathogenesis of ovarian cancer will be identified. In the report presented here, during the course of a program to screen a panel of ovarian cancer cell lines for copy number aberrations in a genome-wide manner using our in-house BAC array (8), we have identified a homozygous loss of *connective tissue growth factor* (*CTGF/CCN2*) at 6q23.1, whose expression was absent in some ovarian cancer cell lines without homozygous loss, although it

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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was present in normal ovary. Because (*a*) reduced expression of CTGF and its clinicopathologic significance (15, 16) and DNA methylation of the genomic sequence around *CTGF* (17–19) in several cancers were reported but (*b*) a detailed target sequence for DNA methylation contributing gene silencing has never been shown and (*c*) the effect of down-regulated CTGF expression on ovarian carcinogenesis remains unknown, we further determined the expression and methylation status of *CTGF* and their clinicopathologic and functional significance using cell lines and primary tumors of ovarian cancer.

Materials and Methods

Cell lines and primary tumors. Of the 24 ovarian cancer cell lines used (Supplementary Table S1), ES-2 was obtained from the American Type Culture Collection; OVISE, OVMANA, OVTOKO, OVKATE, OVSAHO, and RMUG-S were from the Japanese Collection of Research Bioresources (Osaka, Japan); and HT, MH, KK, KF28, and KFr13 were from the National Defense Medical College (20, 21). Other lines (HTOA, HUOA, HMKOA, MCAS, HMOA, HNOA, RMG-I, RMG-II, RMUG-L, W3UF, HIOAnu, and HTBOA) were described previously (22). As a control, the normal ovarian epithelial cell–derived cell line OSE-2a (23) was kindly provided by Dr. Hidetaka Katabuchi (Kumamoto University School of Medicine, Kumamoto, Japan). All cell lines were maintained in appropriate medium supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 μg/mL streptomycin. The status of the *TP53* gene (exons 5–8) mutation was determined as described previously (24). To analyze restoration of genes of interest, cells were cultured with or without various concentrations of 5-aza 2′-deoxycytidine (5-aza-dCyd) for 5 days and/or 100 ng/mL trichostatin A (TSA) for the last 12 h.

Primary tumor samples were obtained during surgery from 114 patients being treated at the National Cancer Center Hospital in Tokyo, with written consent from each patient in the formal style and after approval by the local ethics committees. Samples from 66 of these patients were frozen immediately in liquid nitrogen and stored at −80°C until required, whereas samples from 103 of the patients were embedded in paraffin for immunohistochemistry. None of the patients had received preoperative radiation or immunotherapy. All patients underwent complete surgical staging, including i.p. cytology, bilateral salpingo-oophorectomy, hysterectomy, omentectomy, and pelvic/para-aortic lymphadenectomy. Aggressive cytoreductive surgery was done in patients with advanced disease. Surgical staging was based on the International Federation of Gynecology and Obstetrics (FIGO) staging system: stage I, 57 patients; stage II, 11 patients; stage III, 34 patients; and stage IV, 12 patients.

Array-CGH analysis. A MCG Cancer Array-800 (8) was used for the array-CGH. Hybridizations were carried out as described elsewhere (9). Hybridized slides were scanned with a GenePix 4000B (Axon Instruments), and acquired images were analyzed with GenePix Pro 6.0 imaging software (Axon Instruments). Average ratios that deviated significantly (>2 SD) from 0 (log 2 ratio, <−0.4 and >0.4) were considered abnormal.

Screening for homozygous deletions by genomic PCR using cell lines. We screened DNAs from 24 ovarian cancer cell lines for homozygous losses by genomic PCR. All primer sequences used in this study are listed in Supplementary Table S2.

Reverse transcription-PCR and quantitative real-time reverse transcription-PCR. Single-stranded cDNAs were generated from total RNAs and amplified with primers specific for each gene (Supplementary Table S2). The *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) gene was amplified at the same time to allow estimation of the efficiency of cDNA synthesis. For reverse transcription-PCR (RT-PCR), PCR products were electrophoresed in 3% agarose gels (9). Quantitative real-time RT-PCR experiments were done with an ABI Prism 7900 Sequence Detection System (Applied Biosystems) as described previously (24). Each assay was done in triplicate.

Methylation analysis. Genomic DNAs were treated with sodium bisulfite and subjected to PCR using primer sets designed to amplify regions of interest (Supplementary Table S2). For the combined bisulfite restriction analysis (COBRA; ref. 26), PCR products were digested with *Bst*UI and electrophoresed. For bisulfite sequencing, PCR products were subcloned and then sequenced.

For the methylation-specific PCR (MSP) analysis, sodium bisulfite–treated DNAs were subjected to PCR using primers specific to the methylated (MSP) and unmethylated (unmethylated specific PCR) forms of DNA sequences of interest (Supplementary Table S2), and PCR products were visualized on 3% agarose gels. DNAs from cell lines recognized as unmethylated by bisulfite sequencing were used as negative controls for methylated alleles, whereas those from cell lines recognized as methylated or CpGenome Universal Methylated DNA (Chemicon International) were used as positive controls.

Promoter reporter assay. DNA fragments around the *CTGF* CpG island were obtained by PCR and ligated into the vector pGL3-Basic (Promega). Reporter assay was done as described elsewhere (9) using each construct or a control empty vector and an internal control pRL-hTK vector (Promega).

Immunohistochemistry and scoring method. Indirect immunohistochemistry was done with formalin-fixed, paraffin-embedded tissue sections as described elsewhere (26). Briefly, antigens were retrieved by autoclave pretreatment in high pH buffer (DAKO) for 10 min at 95°C. After blocking in 2% normal swine serum, the slides were incubated with an anti-CTGF antibody (1:100 dilution; Santa Cruz Biotechnology) overnight at 4°C and then reacted with a Histofine simple stain, MAX PO(G) (Nichirei). Antigen-antibody reactions were visualized with 0.2% diaminobenzidine tetrahydrochloride and hydrogen peroxide. The slides were counterstained with Mayer's hematoxylin.

A formalin-fixed HTBOA cell line expressing *CTGF* mRNA, in which >50% of cells showed cytoplasmic staining of CTGF protein, and RMUG-S cell line lacking *CTGF* mRNA expression, in which none of the cells showed cytoplasmic staining of CTGF protein, were used as positive and negative controls, respectively. Specificity of the antibody was verified by Western blotting (9) as well as an absorption test using synthetic peptide (Santa Cruz Biotechnology; Supplementary Fig. S1). The percentage of the total cell population that expressed CTGF was evaluated for each case at ×200 magnification. Expression of CTGF was graded as either positive (≥10% of tumor cell cytoplasm showing immunopositivity) or negative (<10% of tumor cell cytoplasm showing immunopositivity or no staining). Two observers, who were blinded to the clinical outcomes of the patients, evaluated the slides independently; if a significant discrepancy emerged between their judgments, a consensus was reached after discussion.

Transient transfection, Western blotting, and colony formation assay. A plasmid expressing COOH-terminal 3× Myc-tagged CTGF (pCMV-3Tag-4-CTGF) was obtained by cloning the PCR product of the full coding sequence of *CTGF* in-frame along with the Myc epitopes into the vector pCMV-3Tag-4 (Stratagene). pCMV-3Tag-4-CTGF, or the empty vector (pCMV-3Tag-4-mock), was transfected into cells for colony formation assays (9). The expression of CTGF protein in transiently transfected cells was confirmed 48 h after transfection by Western blotting as described elsewhere (9). Cells were stained with crystal violet after 2 weeks of incubation in six-well plates with appropriate concentrations of G418.

Treatment with recombinant CTGF. To assess the effect of CTGF on growth of ovarian cancer cell lines, cells were treated with 2.5 μg/mL of recombinant human CTGF (Peprotech EC) or PBS for 72 h. The numbers of viable cells after transfection were assessed by a colorimetric water-soluble tetrazolium salt (WST) assay (24). The cell cycle in CTGF-treated cells was analyzed using fluorescence-activated cell sorting (FACS) as described elsewhere (24). For Western blotting, 24-h serum-starved cells were pretreated with or without 2.5 μg/mL CTGF for 1 h and then stimulated with 25 ng/mL of recombinant human epidermal growth factor (EGF; Sigma) for 15 min. Phosphorylation status of extracellular signal-regulated kinase 1/2 (ERK1/2) was evaluated using anti–phospho-ERK1/2 (P-ERK1/2) and anti-ERK1/2 antibodies (Cell signaling Technology).

Transfection with synthetic small interfering RNA. *CTGF*-specific small interfering RNA (siRNA; *CTGF*-siRNA) was purchased from Santa Cruz Biotechnology. A control siRNA for the luciferase gene (CGUACGCG-GAAUACUUCGA, *Luc*-siRNA) was synthesized by Sigma. Each siRNA