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A Nucleotide Substitution Responsible for the Tawny Coat Color Mutation Carried by the MSKR Inbred Strain of Mice

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"Tawny" is an autosomal recessive coat color mutation found in a wild population of *Mus musculus molossinus*. The inbred strain MSKR carries the mutation. The causative gene $Mc1r^{taw}$ of the tawny phenotype is the second recessive allele at the melanocortin 1 receptor locus and is dominant to the first recessive allele, "recessive yellow" ($Mc1r^{e}$). The $Mc1r^{taw}$ gene has six nucleotide substitutions, and its forecasted transcript has three amino acid substitutions (i.e., V101A, V216A, W252C). Though the nucleotide substitutions leading to V101A and V216A exist in various mouse strains, the nucleotide substitution leading to W252C exists in only tawny-colored mice. Thus this substitution is considered to be responsible for the expression of the tawny coat color. The frequency of the allele having this nucleotide substitution was 9.21% in the wild *M. m. molossinus* population inhabiting Sakai City, Osaka Prefecture, Japan, where the ancestral mice of the MSKR strain were captured.

The extension locus (*E*, now *Mc1r*) controls coat or feather color variants in animals. Pigmentation mutants resulting from the mutation of this locus have been found in several animals, for example, cattle, chicken, dog, fox, horse, and mouse (Adalsteinsson et al. 1995; Andersson and Sandberg 1982; Silvers 1979; Sponenberg and Bigelow 1987; Vage et al. 1997). In mammals, dominant alleles at this locus generally extend the black area of individual hair shafts (Doolittle et al. 1996). In 1993, Robbins et al. (1993) reported the nucleotide sequence of the mouse α-melanocyte stimulating hormone (α-MSH) receptor (now melanocortin 1 receptor, *Mc1r*) gene with four mutant alleles at the extension locus and proved that the extension locus encodes this receptor. The MC1R is a G protein coupled receptor consisting of seven transmembrane domains. Binding of α-MSH to its receptor stimulates melanocytes to synthesize cyclic adenosine monophosphate (cAMP) by signal transduction via G protein, and consequently the melanocytes produce black pigment (eumelanin) (Robbins et al. 1993). After the research of Robbins et al. (1993), nucleotide and amino acid sequences responsible for the melanocortin 1 receptor were reported in various kinds of animals, for example, cattle, horse, pig, dog, fox, guinea pig, and mouse (Adalsteinsson et al. 1995; Cone et al. 1996; Kijas et al. 1998; Lu et al. 1994; Mariani et al. 1996; Marklund et al. 1996; Newton et al. 2000; Vage et al. 1997; Valverde et al. 1995, 1996).

At the *Mc1r* locus of mice, three dominant mutant alleles (sombre, sombre-3J, and tobacco darkening) and one recessive mutant allele (recessive yellow) have been reported (Doolittle et al. 1996). Both sombre and sombre-3J mice have an entirely black coat, while the tobacco darkening mouse shows black fur in the dorsal region and agouti patterned fur on the flanks. The recessive yellow mouse has no black area on the individual hair shafts and shows a yellow coat everywhere over the body, except for a few black hairs appearing in juvenile mice (Doolittle et al. 1996; Robbins et al. 1993).

In 1999 we reported a new mutation, "tawny," at the *Mc1r* locus that was found in Japanese wild mice (*Mus*

musculus molossinus). The tawny ($Mc1r^{taw}$) mutation is a recessive allele dominant over the recessive yellow ($Mc1r^{e}$) allele. The tawny mouse shows light yellowish brown on the dorsal region with a white belly and black eyes (Figure 1). The dorsal hair shows so-called agouti pattern, consisting of a greatly lengthened subapical yellow region and reduced black region (Wada et al. 1999). The $Mc1r^{taw}$ mutation is maintained in the MSKR strain (Wada et al. 2000), its cognate MSKQ strain, and the Mmsw line.

In this article we researched nucleotide substitutions of the *Mc1r* gene in the tawny mutant (MSKR strain) and 24 other inbred strains of mice, along with mice from wild populations of *M. m. molossinus*, using nucleotide sequencing and/or the restriction fragment length polymorphism (RFLP) technique.

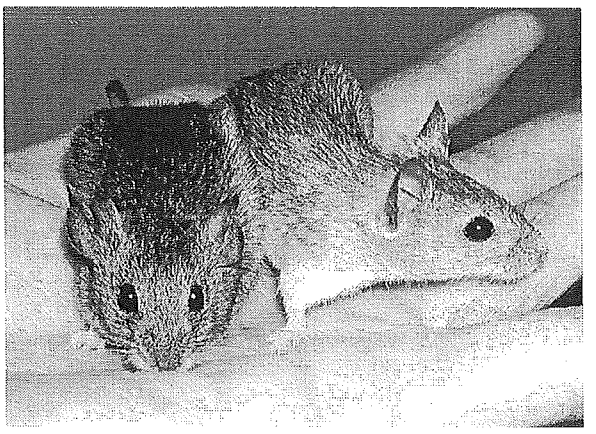

Figure 1. MSKR (right) and MSKR.B6-$Mc1r^{+}$ (left) mice. The MSKR mouse has a light yellowish-brown coat (tawny color).

Materials and Methods

Animals

The MSKR strain of mice (fixed for the tawny coat color mutation, $n = 4$) and 24 other inbred-strain mice were used. The 24 strains, which show nontawny coat colors, were A/J (3), AEJ/GnLe (2), BALB/cA (4), BFM/2 (2), C3H/HeJ-$Mc1r^{som}/Mc1r^{som}$ (2), C3H/HeN (4), C57BL/6J-$Mc1r^{e}/Mc1r^{e}$ (2), C57BL/6N (4), CASA/Rk (2), CAST/EiJ (2), CBA/N (3), DBA/2N (2), DDK/Nga (3), IS/Cam (1), MMNF (2), MOM (3), MSKA (4), MSKD (2), MSKM (2), MSKO (2), MSKZ (4), MSM/Msf (3), NC/Nga (4), and SM/J (3). The numeral in parentheses indicates the number of mice tested. All of these strains have been maintained in Osaka Prefecture University except the A/J, BFM, CASA, CAST, IS, MSM, and SM/J strains. The A/J, MSM, and SM/J strains have been maintained in the Institute for Laboratory Animal Research, Graduate School of Medicine, Nagoya University, Japan. The BFM/2, CASA, and CAST strains were kindly provided by the National Institute of Genetics (Shizuoka, Japan) and the IS strain was kindly provided by Wakayama Medical University (Wakayama, Japan). BFM/2 was derived from *M. m. brevirostris*, CASA and CAST were derived from *M. m. castaneus*, IS was established from the hybrid of *M. m. musculus* and *M. m. praetextus*. MMNF, MOM, MSKA, MSKD, MSKM, MSKO, MSKR, MSKZ, and MSM were established from *M. m. molossinus*.

In addition to these strains, we analyzed 105 wild mice (*M. m. molossinus*) captured in the Kinki-Shikoku area of Japan during the period from January 1991 to May 1992. Of the 105 mice, 23 were captured in Minoh City, Osaka Prefecture; 38 each were captured at the northern and southern parts of Sakai City, Osaka Prefecture; and 6 were captured in Wakimachi, Tokushima Prefecture (Figure 2).

Nucleotide Sequencing

Standard polymerase chain reaction (PCR) was performed with genomic DNA of MSKA, MSKM, MSKR, and MSM inbred mice using the GeneAMP 9700 PCR system (PerkinElmer, Wellesley, MA). A sense primer "mMc1r-1-for" (5′-TCTGAGGGATGTCAGAGACCC-3′) and an antisense primer "mMc1r-2-rev" (5′-GCAGTCACAGTTACCCT TTCTCC-3′) were originally designed based on the nucleotide sequence (accession no. X65635) reported by Mountjoy et al. (1992). The mMc1r-1-for and mMc1r-2-rev primers amplify a 1229 bp fragment including the entire coding region of the mouse *Mc1r* gene. PCR amplification was carried out under the following conditions: one cycle consisting of denaturation at 94°C for 5 min, 40 cycles consisting of denaturation at 94°C for 30 s, annealing at 61°C for 45 s, and extension at 72°C for 60 s in a reaction mixture containing 0.5 mM of each primer, 0.05 U/ml of *Taq* polymerase, 0.2 mM each of dNTPs, and 1.5 mM $MgCl_2$. The amplified fragments were purified by polyethylene glycol 6000 and cycle sequenced using the Dye Terminator Cycle Sequencing FS Ready Reaction Kit with the primers described above and additionally designed primers "mMc1r-2-for" (5′-CTCCATCTTCTATGCGCTGC-3′) and mMc1r-1-rev (5′-GAAAGTGACGAGGCAGAGCAG-3′), based on the manufacturer's instructions. The reactants were sequenced using an ABI model 373 automated DNA sequencer (Applied Biosystems, Foster City, CA).

PCR-RFLP Analysis

In order to reveal the relationship between the nucleotide substitutions and the tawny phenotype, RFLPs of the *Mc1r* gene were compared between mice of the tawny mutation strain MSKR and the other 24 inbred strains. In addition, the incidence of RFLP identical to the tawny was investigated in wild populations of *M. m. molossinus* in the Kinki-Shikoku area of Japan. A 986 bp PCR product, which includes the entire 945 bp coding region of the mouse *Mc1r* gene, was amplified by a set of *Mc1r*-specific PCR primers described by Robbins et al. (1993). PCR amplification was carried out with the same methods mentioned above. In this experiment, however,

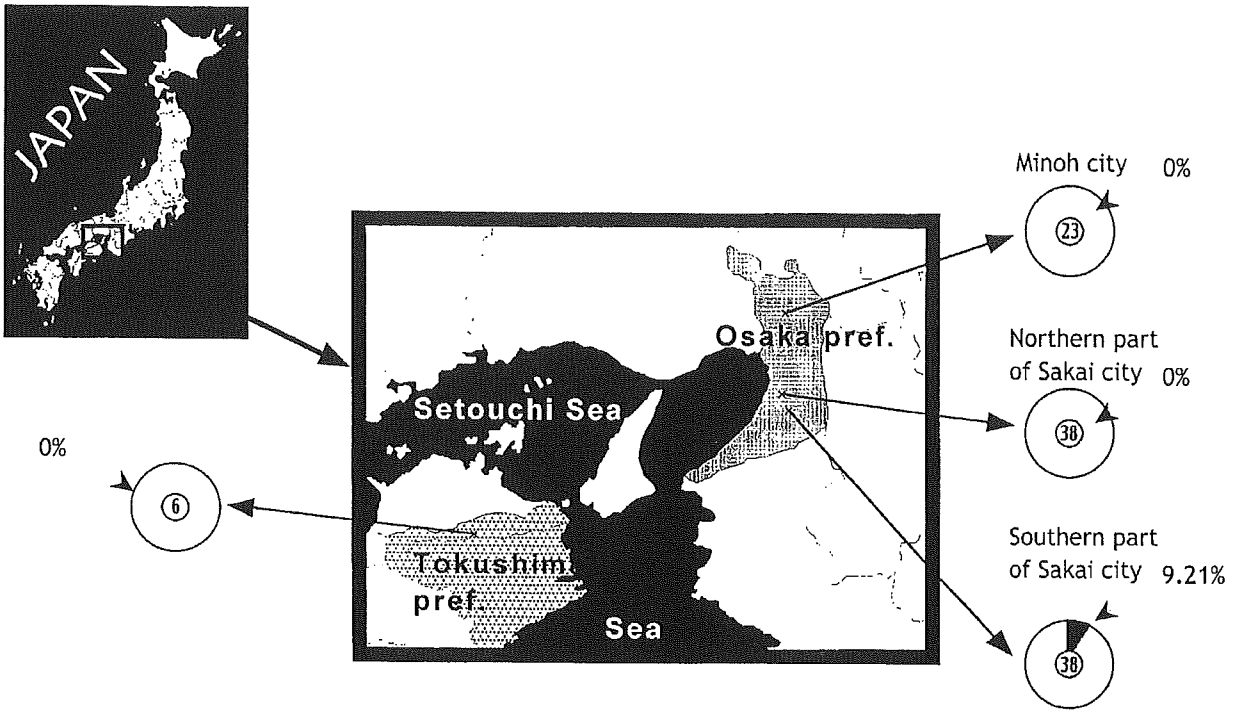

Figure 2. Sampling sites of wild *M. m. molossinus* in the Kinki-Shikoku area, Japan, and the frequency of the allele at the *Mc1r* locus that lacks the *Apa*I recognition site. The number within the small circles is the number of mice captured. The shaded part between the large and small circle indicates allele frequency.

annealing temperature was adjusted at 65°C and purification of the PCR products was carried out with ethanol. The purified PCR products were digested with endonucleases *Apa*I, *Cac*8I, and *Eco*RII, electrophoresed on 0.6% agarose gels, and visualized by ethidium bromide staining.

Results

Nucleotide Sequencing

Table 1 shows the nucleotide substitutions observed in the *Mc1r* alleles of four inbred strains of *molossinus* mice in comparison with the registered nucleotide sequence in GenBank (accession no. X65635, which was identified based on the Cloudman S91 melanoma cell line separated from a hybrid of BALB/cJ and DBA), along with their forecasted amino acids. Five nucleotide substitutions were identified at the positions listed; that is, substitution I (51T . . . C), II (302T . . . C), III (606G . . . A), IV (647T . . . C and 648C . . . T), and V (756G . . . T). The first four were observed both in wild-type and tawny-colored strains. The substitution V, however, was observed only in the MSKR strain that has the "tawny" coat color. According to the amino acid code, nucleotide substitutions I (AAT . . . AAC) and III (GCG . . . GCA) are silent. However, substitutions II, IV, and V cause amino acid substitutions; that is, 101 valine to alanine, 216 valine to alanine, and 252 tryptophan to cysteine, respectively (Table 1).

PCR-RFLP Analysis

The nucleotide substitutions II, III, IV, and V lead to recognition sites of endonucleases *Cac*8I, *Hinf*I, *Eco*RII, and *Apa*I, respectively (Table 1). Thus we investigated the distribution of these recognition sites across various mouse strains derived from some subspecies, the results of which are summarized in Table 2. The *Cac*8I recognition site was observed in all strains of *M. m. molossinus*. Other strains derived from other subspecies had or did not have the recognition site. The presence or absence of the *Hinf*I recognition site clearly distinguished the strains of *M. m. molossinus* from the strains of other subspecies; that is, the site was missing in the strains derived from *M. m. molossinus*. The *Eco*RII recognition site existed in all 25 strains investigated. The *Apa*I recognition site was missing in only the MSKR strain of *molossinus* mice that has the tawny ($Mc1r^{taw}/Mc1r^{taw}$) coat color.

In addition to the strains above, we performed RFLP analysis for wild mice. Among 38 wild mice captured in the southern part of Sakai City, where the original tawny mutant had been captured, 5 mice had no recognition site of *Apa*I—2 were homozygotes and 3 were heterozygotes—that is, the allele frequency of $Mc1r^{taw}$ was 9.21% in this population (Figure 2). The two homozygotes showed tawny coat color and the others (three heterozygotes) showed wild-type coat color. On the other hand, all 67 mice captured at other places had the recognition site for *Apa*I in their *Mc1r* genes.

Table 1. Nucleotide alligment around the substitution sites at the *Mc1r* gene and their forecasted amino acids

Arrangement number of substitution	I	II	III	IV	V
Nucleotide					
The ordinal number of substituted nucreotide from the start codon	51	302	606	647 & 648	756
GenBank	tcc aaT gcc gag gTg ggc gcG att[3] ctg gTC cag[4] ggc tgG ggc c[5]cc
Wild-type *molossinus* strains (MSM, MSKA, MSKZ)	- - - - - C - - - - - - -C- -[2]- - - -A - - - - - - -CT - - - - - - - - - - - - - - -
Tawny colored *molossinus* strain (MSKR)	- - - - - C - - - - - - -C- - - - - - -A - - - - - - -CT - - - - - - -T - - - - - -
Amino acid					
The ordinal number of marked amino acid from the start codon	17	101	202	216	252
GenBank	S N A E V G A I L V I L W G P
Wild-type *molossinus* strains (MSM, MSKA, MSKZ)	- -[1] - - A - -[1] - - A - - - - -
Tawny colored *molossinus* strain (MSKR)	- -[1] - - A - -[1] - - A - - C - -

[1] The nucleotide substitution is silent.

[2] The underlined sequence is *Cac*8I recognition site (GCN|NGC).

[3] The underlined sequence is alignment is *Hin*fI site (G|ANTC).

[4] The underlined sequence is *Eco*RII site (CC|WGG).

[5] The underlined sequence is *Apa*I site (GGGCC|C).

Discussion

In the tawny mutant, we found six nucleotide substitutions classifiable to five types (substitutions I–V in Table 1) at the *Mc1r* locus. The nucleotide substitutions at base pair positions 51 and 606 (substitutions I and III) are silent. Thus these two are not thought to be the cause of the tawny mutation. The nucleotide substitution at base pair 302 (substitution II) led to an amino acid substitution (Table 1). However, this substitution was also found in wild-type *molossinus* mice and other strains derived from other subspecies (Tables 1 and 2). Thus the nucleotide substitution at 302 is thought not to be the cause of the tawny coat color.

As shown in substitution IV of Table 1, the nucleotides 647 and 648 of the mouse *Mc1r* gene reported by Mountjoy et al. (1992) were T and C, respectively, while those of our results from *molossinus* strains were C and T, forecasting alanine at 216. Nucleotide 648 results in the recognition site of *Eco*RII (Table 1). In addition to *molossinus* mice, all 24 strains derived from other subspecies also had no recognition site for *Eco*RII (Table 2). This result suggests that no mice have a nucleotide sequence identical to that reported by Mountjoy et al. (1992) at the position. Furthermore, the alanine at 216 has been found in many kinds of mammals that have different phenotypes from the tawny mouse (Adalsteinsson et al. 1995; Cone et al. 1996; Kijas et al. 1998; Lu et al. 1994; Mariani et al. 1996; Marklund et al. 1996; Newton et al. 2000; Vage et al. 1997; Valverde et al. 1995, 1996). Thus the nucleotide substitutions at 647 and 648 are thought not to be responsible for the tawny coat color.

Although substitutions I–III described above were observed both in wild-type and tawny-colored mice, substitution V (756 guanine to thymine), which leads to the 252 tryptophan to cysteine substitution, was observed only in tawny-colored mice (Table 1). This result strongly suggests that this substitution results in the tawny phenotype.

Table 2. Classification of mouse strains based on RFLP at the *Mc1r* gene

Arrangement number of substitution[1]	II	III	IV	V
Endonucleases	*Cac*8I	*Hin*fI	*Eco*RII	*Apa*I
GenBank	lost	exist	lost	exist
Common type A[2]	exist	exist	exist	exist
Common type B[3]	lost	exist	exist	exist
Molossinus type[4]	exist	lost	exist	exist
MSKR type	exist	lost	exist	lost

[1] Identical to the arrangement number in Table 1.

[2] Including A/J, AEJ, DDK, BALB/cA, C57BL/6N, and C57BL/6J-*Mc1r*^{e}/*Mc1r*^{e}.

[3] Including BFM, C3H/HeN, C3H/HeJ-*Mc1r*^{som}/*Mc1r*^{som}, CASA, CAST, CBA/J, DBA/2N, IS, SM/J, and NC/Nga.

[4] Including MMNF, MOM, MSKA, MSKD, MSKM, MSKO, MSKZ, and MSM.

Amino acid 252 is conserved as tryptophan in all reported animals (Klungland et al. 1995; Marklund et al. 1996; Mountjoy et al. 1992; Robbins et al. 1993; Vage et al. 1997; Valverde et al. 1995). Amino acid 252 is involved in the sixth transmembrane domain of MC1R (Mountjoy et al. 1992; Robbins et al. 1993). The replacement of a hydrophobic amino acid residue (tryptophan) with a hydrophilic one (cysteine) would change the α-helix structure of the transmembrane domain of MC1R. Moreover, the sixth transmembrane domain forms an α-MSH binding pocket with first and third transmembrane domains (Prusis et al. 1995). In site-specific mutant research using recombinant COS7 cells (from African green monkey), replacement of an amino acid residue in the sixth domain has been reported to decrease α-MSH binding affinity (Frandberg et al. 1994). The tawny-type MC1R with W252C also may decrease α-MSH binding affinity. The altered affinity of MC1R to its ligand might lead to extension of yellow color at the subapical region of the hair shaft. Another possibility is that a decrease in constitutive activity and/or stability of the MC1R protein might result in the tawny phenotype. A ligand binding affinity test will solve this problem.

The allele frequency of $Mc1r^{taw}$ was 9.21% in the wild *molossinus* mice captured in the southern part of Sakai City, Osaka Prefecture, Japan (Figure 2), where the original tawny mutant had been captured. The $Mc1r^{taw}$ mutation is thought to have originally occurred in the population at the southern part of Sakai City, because the allele frequency of $Mc1r^{taw}$ was 0% in other areas adjacent to the southern part of the city.

The amino acid substitution W252C is quite likely to be the cause of the tawny coat color, as mentioned. However, there remains a possibility that some other abnormality occurring at or around the *Mc1r* coding region might give rise to the mutant color.

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●婦人科〔悪性腫瘍〕

IV. 卵巣がん
1. 手術療法
2) 標準的手術

東京慈恵会医科大学産婦人科
山田恭輔，岡本三四郎，高尾美穂，上田 和，斎藤元章，茂木 眞，落合和徳

はじめに

卵巣癌治療における標準的手術は，腫瘍の広がりを決定する（staging laparotomy）目的を含み重要な意義を持つ．具体的な手技としては，両側付属器摘出術，子宮全摘術，大網切除術が基本術式に含まれ，さらにstaging laparotomyの一つとして後腹膜リンパ節郭清が行われる．インフォームド・コンセントを得るに際しては，肉眼的に浸潤や転移がなくても原発腫瘍のほかに大網切除やリンパ節を郭清あるいは生検する必要性があること，進行卵巣癌においては，播種や周囲臓器への浸潤により，多量出血や副障害が起こり得ることなどをふまえ，十分説明することが重要である．

卵巣癌手術の背景

日本婦人科腫瘍学会の卵巣癌治療ガイドラインによる卵巣癌手術の目的は，①卵巣腫瘍の確定診断すなわち悪性腫瘍か否かを知ること，②悪性腫瘍ならばその組織型と進行期の確定（surgical staging），③病巣の完全摘出または最大限の腫瘍減量（maximum debulking），④後療法のための情報を得ること，とされている（表1）．

これらの目的を満たす標準的手術としては，両側付属器摘出術，子宮全摘術，大網切除術を含む基本術式に加え，staging laparotomyの

表1 卵巣癌手術の目的

1. 卵巣腫瘍の確定診断すなわち悪性腫瘍か否かを知ること
2. 悪性腫瘍ならばその組織型と進行期の確定
3. 病巣の完全摘出または最大限の腫瘍減量
4. 後療法のための情報を得ること

（卵巣がん治療ガイドライン2004年版 日本婦人科腫瘍学会編より）

表2 上皮性卵巣癌の具体的手術手技

基本手術に含まれる手技	両側付属器摘出術・子宮摘出術・大網切除術
Staging laparotomyに含まれる手技	腹腔細胞診 腹腔内各所の生検 後腹膜リンパ節（骨盤・傍大動脈）郭清術または生検
Cytoreductive surgeryに含まれる手技	腹腔内各所の播種病巣の切除

（卵巣がん治療ガイドライン2004年版 日本婦人科腫瘍学会編より）

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表3　手術説明同意書（実例）

1. 現在の診断名，病状：　進行卵巣癌

2. 予定している手術の名称と方法：
　両側付属器摘出術，子宮全摘術，大網切除術
　リンパ節郭清術（骨盤・傍大動脈）
　播種病巣の切除
　ただし，腫瘍の広がりが強く適切な腫瘍減量ができない場合は
　試験開腹術にとどめ，化学療法を先行させる。

3. 予想される合併症や偶発症と危険性：
　術中多量出血→輸血の可能性
　他臓器損傷（腸管，尿管，膀胱，神経）
　術後血栓症，肺塞栓症
　リンパ浮腫
　腸閉塞
　創部感染
　術後出血
　腸閉塞，創部感染，術後出血などによる再手術

一つとして後腹膜リンパ節（骨盤・傍大動脈）郭清が行われる（表2）．

腹腔内臓器に浸潤や播種病巣が認められる場合，さらに病巣切除が必要となる場合がある．卵巣癌の手術療法に関する用語にはさまざまなものがあり，腫瘍減量または縮小を意味する用語としてcytoreduction, debulkingが使用されているが，必ずしも定義が一定とはいえず，標準的手術の位置づけが混乱されることがある．

しかし，インフォームド・コンセントに際しては，標準的手術にはステージングと腫瘍減量の二つの目的が含まれることをわかりやすく説明すると理解を得られやすい．肉眼的に浸潤や転移がなくても，治療方針決定のために原発腫瘍のほかに，大網切除やリンパ節を郭清あるいは生検する必要性があることを説明する．

一方，初回腫瘍減量手術（primary debulking surgery）は，初回手術時に病巣を完全摘出または可及的に最大限の腫瘍減量を行う手技と定義されている．進行癌における腫瘍減量手術のインフォームド・コンセントは他稿を参照されたいが，卵巣癌が疑われる患者および家族への手術説明に際しては，早期癌・進行癌それぞれに応じた，卵巣癌の特性を理解してもらうよう努めることが重要である．

手術説明の実際

手術説明の実例を表3・図3に示すが留意点を以下に解説する．

1．現在の診断名，病状

卵巣腫瘍には，良性・境界悪性・悪性腫瘍と悪性度に幅があり，術前の良悪性鑑別が困難なことも少なくない．明らかに良性腫瘍と診断される場合を除き，境界悪性を含み悪性が少しでも疑われる場合には，術中迅速病理検査を行うことのできる施設での手術が望ましい．

すなわち，開腹所見，術中迅速病理検査の結果によって，診断がより確実になることを説明する．術前に腹水貯留や画像診断において，リンパ節腫大や腹膜播種の所見を認め，さらに腫

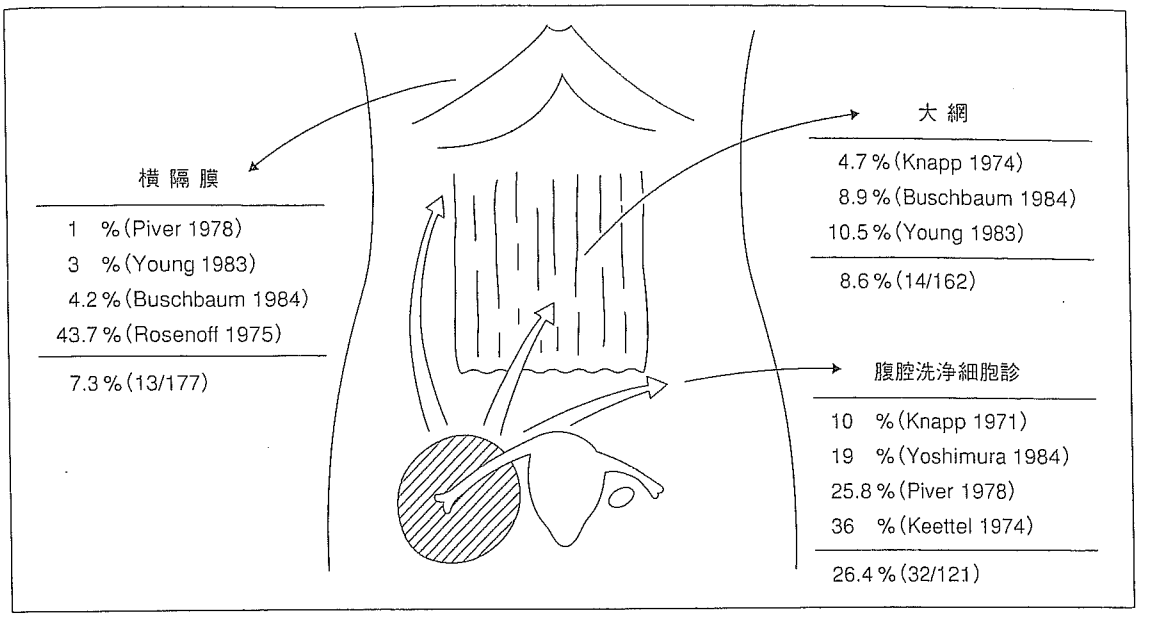


図1 卵巣に限局していると思われた卵巣癌の広がり 〔文献3）より〕

瘍マーカーの異常高値などで進行癌が疑われる場合には，その病状に対し，手術療法を基本とした適切な治療がなされることを説明する．

2．予定している手術の名称と方法

標準的手術（卵巣癌根治手術）としては，基本術式に加え，staging laparotomy に含まれるリンパ節郭清術（傍大動脈～骨盤）を行う施設が多い．しかし，術前に早期癌が考えられる場合と進行癌が考えられる場合では，結果的に術式が同じでもインフォームド・コンセントに際しては，強調して説明すべき部分が異なる．それぞれに適切な内容にすべきであり，以下の点をふまえて患者および家族へ説明をわかりやすく行う．

組織型と進行期の確定を行うための staging laparotomy は，卵巣癌治療において早期癌，進行癌にかかわらず重要な意義を持つ．早期癌ではステージングの正確さを期するためだけではなく，後療法を省略できる症例を抽出する観点からも，広範囲にわたる系統的な腹腔内および後腹膜腔の検索を行うことが推奨されている．肉眼的に腫瘍が卵巣に限局していると思われる症例でも，staging laparotomy を正確に施行することにより，腹腔内の細胞診陽性例が 10～36%，大網転移 5～11%，横隔膜転移 1～44%に発見されると報告されている（図1）．

卵巣癌 pT1 期でのリンパ節転移頻度を表4に示すが，系統的リンパ節郭清術によって up staging されることになる．腹腔内および後腹膜腔の検索によって決定されたステージに基づいて治療方針の決定，予後推定がなされることは重要な意義がある．

進行癌においては，基本術式ならびに staging laparotomy に加えて腹腔内播種や転移病巣の可及的摘出を行うが，完全摘出ができない場合でも，できるだけ小病巣 optimal になるよう努める．すなわち初回手術時の残存腫瘍の大きさが直接予後に反映されることから，腫瘍組織の減量はきわめて重要な意義を持つ．残存腫瘍径が 2cm 以下に縮小された症例の予後は比較的良好とされる．

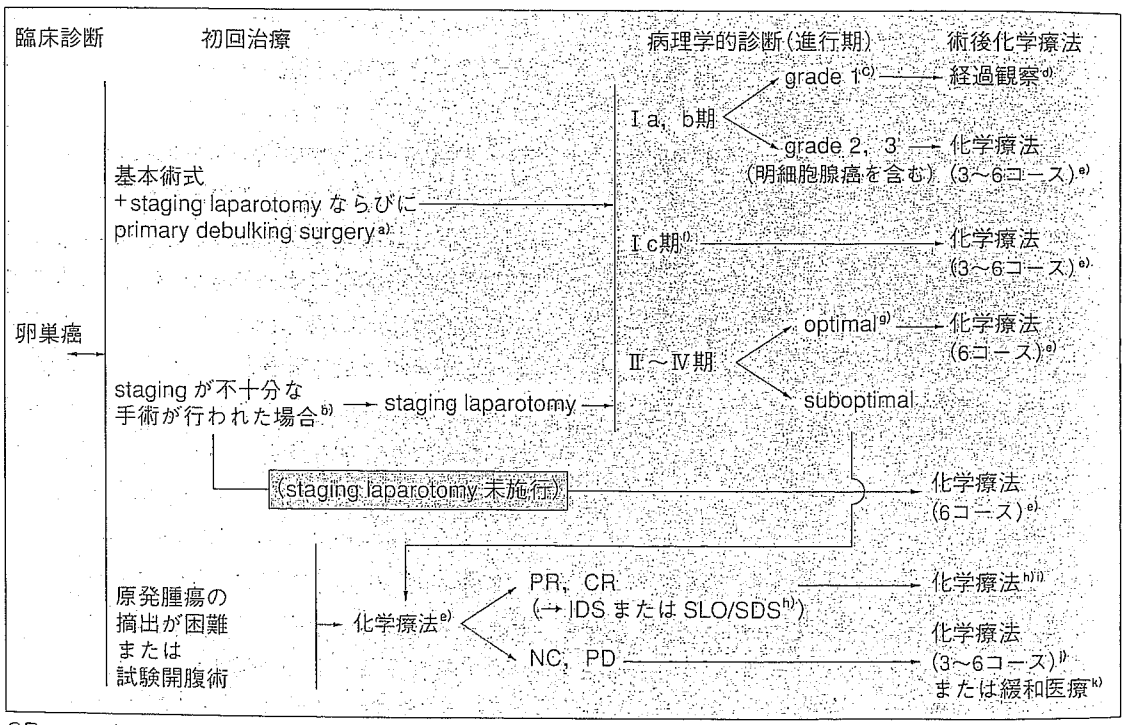
しかし一方，進行卵巣癌においては，術野

表4　卵巣癌pT1期でのリンパ節転移頻度（系統的PALA＋PLA）

著者	発表年	症例数	転移陽性率（%）	進行期亜分類陽性率 Ia	Ib	Ic
DiRe	1989	128	12.5			
Pickel	1989	28	25.0	25.0		20.0
Burghardt	1991	37	24.0			
Benedetti	1993	35	14.0			
Petru	1994	40	23.0			
Onda	1996	33	21.0			
Baiocchi	1998	242	13.2	120	14.7	13.6
Kanazawa	1999	44	11.4			
Sakuragi	2000	78	5.1	3.2		6.4
Suzuki	2000	47	10.6	5.6		13.8
Total		712	14.1%	11.7% (29/247)		11.5% (13/113)

PALA：para-aortic lymphadenectomy，PLA：pelvic lymphadenectomy

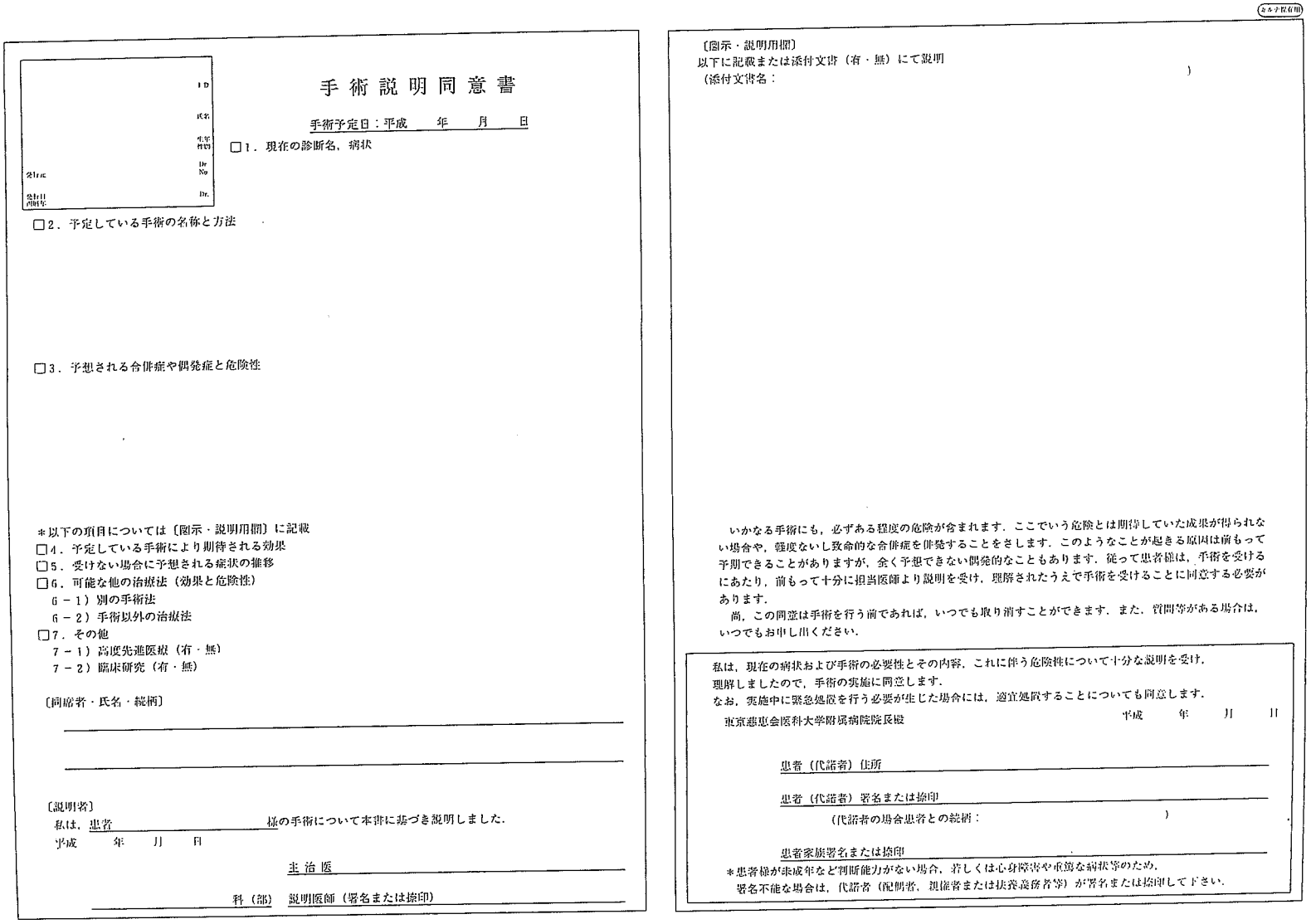
（卵巣がん治療ガイドライン2004年版 日本婦人科腫瘍学会編より）



CR：complete response，PR：partial response，NC：no change，PD：progressive disease

（卵巣がん治療ガイドライン 2004年版 日本婦人科腫瘍学会編より）

図2　治療フローチャート



手術説明同意書

ID
氏名
生年月日
Dr No
発行元
発行日
Dr.

手術予定日：平成　　年　　月　　日

□1．現在の診断名，病状

□2．予定している手術の名称と方法

□3．予想される合併症や偶発症と危険性

＊以下の項目については〔図示・説明用紙〕に記載
□4．予定している手術により期待される効果
□5．受けない場合に予想される症状の推移
□6．可能な他の治療法（効果と危険性）
　6－1）別の手術法
　6－2）手術以外の治療法
□7．その他
　7－1）高度先進医療（有・無）
　7－2）臨床研究（有・無）

〔同席者・氏名・続柄〕

〔説明者〕
私は，患者　　様の手術について本書に基づき説明しました．
平成　　年　　月　　日

主治医

科（部）　説明医師（署名または捺印）

（カルテ保存用）

〔図示・説明用紙〕
以下に記載または添付文書（有・無）にて説明
（添付文書名：　　）

いかなる手術にも，必ずある程度の危険が含まれます．ここでいう危険とは期待していた成果が得られない場合や，軽度ないし致命的な合併症を併発することをさします．このようなことが起きる原因は前もって予期できることがありますが，全く予想できない偶発的なこともあります．従って患者様は，手術を受けるにあたり，前もって十分に担当医師より説明を受け，理解されたうえで手術を受けることに同意する必要があります．

尚，この同意は手術を行う前であれば，いつでも取り消すことができます．また，質問等がある場合は，いつでもお申し出ください．

私は，現在の病状および手術の必要性とその内容，これに伴う危険性について十分な説明を受け，理解しましたので，手術の実施に同意します．
なお，実施中に緊急処置を行う必要が生じた場合には，適宜処置することについても同意します．

東京慈恵会医科大学附属病院院長殿　　平成　　年　　月　　日

患者（代諾者）住所

患者（代諾者）署名または捺印
（代諾者の場合患者との続柄：　　）

患者家族署名または捺印

＊患者様が未成年など判断能力がない場合，若しくは心身障害や重篤な病状等のため，署名不能な場合は，代諾者（配偶者，親権者または扶養義務者等）が署名または捺印して下さい．

図３　東京慈恵会医科大学附属病院における手術説明同意書

表5　婦人科手術における静脈血栓塞栓症の予防

リスクレベル	産婦人科手術	予防法
低リスク	30分以内の小手術	早期離床および積極的な運動
中リスク	良性疾患手術 (開腹，経腟，腹腔鏡) 悪性疾患で良性疾患に準じる手術 ホルモン療法中の患者に対する手術	弾性ストッキング あるいは 間欠的空気圧迫法
高リスク	骨盤内悪性腫瘍根治術 (静脈血栓塞栓症の既往あるいは血栓性素因のある) 良性疾患手術	間欠的空気圧迫法 あるいは 低用量未分画ヘパリン
最高リスク	(静脈血栓塞栓症の既往あるいは血栓性素因のある) 悪性腫瘍根治術	(低用量未分画ヘパリンと間欠的空気圧迫法の併用) あるいは (低用量未分画ヘパリンと弾性ストッキングの併用)

が広範であり，播種や周囲臓器への浸潤により多量出血や副障害が起こり得る．腫瘍の広がりが強度で，手術侵襲がQOLを低下させるおそれがある場合は，試験開腹術にとどめ，化学療法を先行させる判断を行うことを説明する．図2に卵巣癌治療フローチャートを示すが，担当医師が治療の流れを順序よく説明するのに役立つ．

3．予想される合併症や偶発症と危険性

実例（表3）に示した予想される合併症や偶発症と危険性は，卵巣癌の標準的手術に特有なものではないが十分な説明を要する．卵巣癌手術は腹腔内や後腹膜腔の広範囲な操作が求められ，消化管や尿路系への術後合併症，リンパ節郭清操作に伴うリンパ浮腫，さらに血管の損傷が生じるおそれがある．特に進行癌が疑われる症例では，副障害に対する対応策を含めわかりやすく説明を行う．

静脈血栓塞栓症予防ガイドラインによると，卵巣癌手術は危険因子にあげられ，その予防法を表5に示す．特に巨大腫瘍や多量腹水貯留を認める症例は，術前に深部静脈血栓の有無を診断する必要があり，血栓を認める場合は，下大静脈フィルターを挿入したうえで手術を行うことが望ましい．

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卵巣癌腫瘍減量手術における消化管合併切除

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落合和徳　田中忠夫

はじめに

卵巣癌治療において初回手術時の残存腫瘍の大きさが，直接予後に反映されることから，腫瘍組織の減量はきわめて重要な意義をもつ。しかし，進行卵巣癌手術においては，術野が広範であること，播種や周囲臓器への浸潤があることから多量出血や副障害の発生などの問題点がある。残存腫瘍径は数ある予後因子のなかでも，手術療法が直接関与しうる因子であり，これらの問題点をいかに克服していくかが課題である。本稿では骨盤内原発巣の切除，特に腫瘍が消化管と癒着を認める場合の手術手技に焦点を当て解説する。

卵巣癌手術の背景と問題点

卵巣癌手術においては，まずステージングが重要であり，病巣の広がりを確認してから腫瘍組織の摘出が引き続いて行われる。卵巣癌の手術療法に関する用語にはさまざまなものがあり，一次的および二次的腫瘍減量または縮小を意味する用語としてcytoreduction, debulking が使用されているが，必ずしも定義が一定とはいえない。卵巣癌手術は，その目的や化学療法との組み合わせにおける施行時期によって，一つの分類として表1のように区分される[1]。日本婦人科腫瘍学会の卵巣癌治療ガイドラインによると初回腫瘍減量手術(primary debulking surgery) は初回手術時に病巣を完全摘出または可及的に最大限の腫瘍減量を行う手技と定義されている[2]。基本術式には両側付属器摘出術，子宮摘出術，大網切除術が含まれるが，腹腔内臓器に浸潤や播種病巣が認められる場合，さらに病巣切除が必要となる。

ダグラス窩の腫瘍が直腸に及ぶ場合，直腸漿膜のみに浸潤・転移しているのか直腸筋層・粘膜まで達しているのかで術式が異なる（図1）[3]。しかし，実際には術前に正確な評価が困難な場合が多く，開腹時でさえ腫瘍がS状結腸・直腸と癒着していると，浸潤の深さを正確に判断したうえで術式を選択するのは難しいことがある。その一つの理由は，腫瘍が直腸・S状結腸あるいは子宮後壁と強固に癌性癒着している場合は，はじめに癒着剥離を行うと予想以上に多量出血をみることがあるからである[4]。進行卵巣癌では付属器が腫瘍に置換され，なおかつ卵巣の解剖学的位置関係から，しばしばダグラス窩に癌性癒着し小骨盤腔は凍結骨盤になっていることがあり，慎重な剥離操作と止血操作に加え術式の工夫が要求される。われわれは浸潤性癒着が疑われる場合には積極的に腸管切除を行っているが，進行癌，大きな腫瘍では術前に消化管切除のインフォームドコンセントを得ておくこと，外科医師にスタンバイを依頼しておくことが重要である。

本稿ではcytoreductive surgery に含まれる，腹腔内各所の播種病巣の切除手技については割愛す

る。リンパ節郭清術はstaging laparotomy に含まれるが，手術の流れのなかでその要点を後述する。

消化管合併切除の適応

消化管合併切除の適応については明確な基準があるわけではないが，われわれの教室では，腸切除によって残存腫瘍を2cm以下にできる症例，腸閉塞が切迫している症例を目安にしている。人工肛門造設術に関しては，可能であれば同意を得ておくが，器械吻合の術式の発達，QOLの観点から初回手術においては行わないことが多くなっている。しかし，その決定には年齢や組織型が考慮されることもあり，また，試験開腹術において造設されることもある。消化管切除を行うかの判断は腫瘍の広がりを考慮して慎重に個別化されるべきである[5]。

手術のストラテジー

進行卵巣癌手術は，子宮筋層病変，特に子宮肉腫・癌肉腫の開腹時において，子宮後壁とS状結腸・直腸が癒着を認める臨床像と共通点がある。癒着剥離が可能と思われても子宮後壁・直腸前面からのアプローチは多量出血をきたすという経験を生かし，われわれは，卵巣癌手術に子宮悪性腫瘍，広汎子宮全摘術あるいは骨盤除臓術を応用した方法を行い出血量の軽減に努めている。その術式のポイントは，腫瘍周囲の癒着剥離は行わず，壁側腹膜を切開することから始まり卵巣動静脈・子宮動脈本幹を結紮する。卵巣・子宮動静脈を早期に結紮することで，血流が一気に遮断される。ダグラス窩の腫瘍が大きいと視野が狭いが，後壁の癒着剥離は行わず，腫瘍を対側に圧排しつつ図

表1　卵巣癌の手術療法

Initial Surgery	
	Staging Laparotomy（腹腔細胞診、後腹膜リンパ節郭清術など）
	Primary Debulking Surgery（初回腫瘍減量手術）
Second Surgery	
	Interval Debulking Surgery *
	Secondary Debulking Surgery
	Second Look Operation

＊通常2-3コースの化学療法後に施行される。

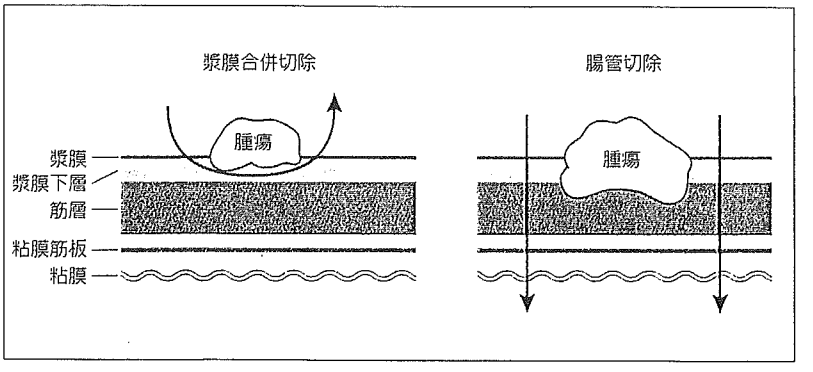


図1　腫瘍の浸潤程度と切除の適応
漿膜のみに播種上に転移している場合には漿膜切除で十分であるが，筋層・粘膜まで及ぶ場合には直腸の合併切除が必要である。

2に示す骨盤漏斗靱帯を腸腰筋に沿って切開を進めると壁側・後腹膜腔が展開され視野が確保される。基靱帯，膀胱子宮靱帯の処理後，腟切断を行う。この時，直腸前面を露出し腸管切断位置を決める。

主病巣は壁側腹膜ごと切除するようなアプローチで中心に向かうと，腫瘍周囲からの出血の減少につながる（図3）。

従来，リンパ節郭清は腫瘍切除の操作とは独立して進められる。しかし，進行卵巣癌手術においては，リンパ節切除の手順は柔軟に捉え，操作を進めるのに必要と思われるリンパ節切除を適宜行うこともある。腫瘍摘出後，改めてリンパ節郭清を行うのが合理的と考える。残存する脂肪組織，リンパ節を吸引管にて吸引する操作が後腹膜腔のオリエンテーションを明らかにするのに有効である。

図2　骨盤漏斗靱帯の局所解剖
(Garrey, Govan, Hodge, Callander：Gynaecology Illustratedより)
腫瘍が大きいと視野が狭いが，後壁の癒着剥離は行わず壁側（後腹膜腔）を展開していくと視野が確保される。

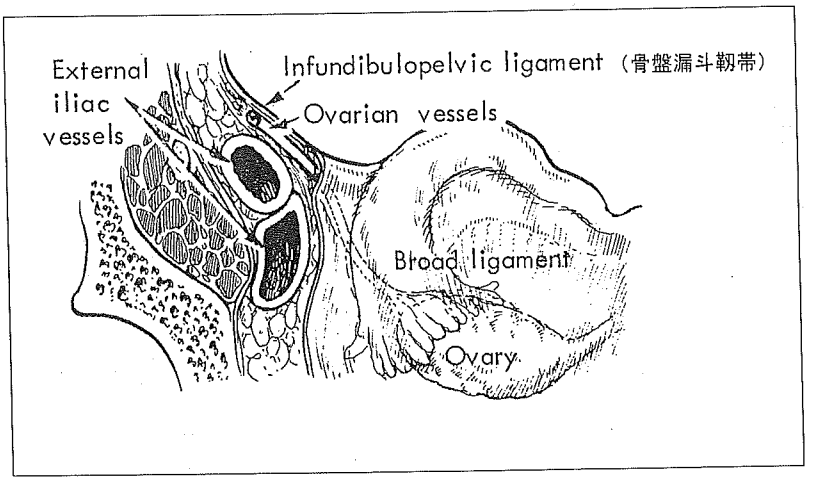
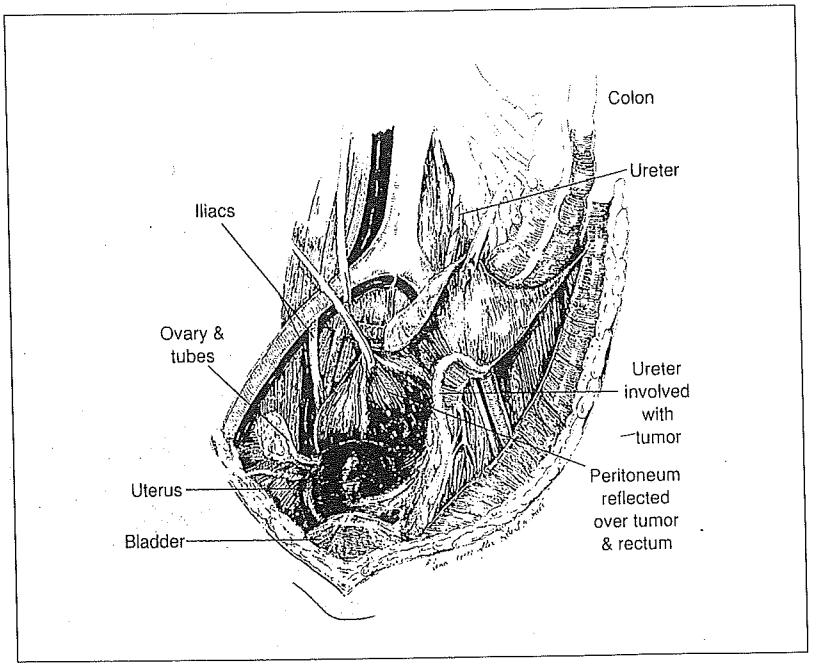


図3　後腹膜からのアプローチ
(John A. Rock, John D. Thompson：Operative Gynecologyより)
側壁腹膜ごと切除するようなアプローチで中心に向かう。
左卵巣腫瘍が腹膜ごと持ち上げられている。



進行卵巣癌手術の実際

1 壁側腹膜の切開

側壁からアプローチすることが大切で後壁からのアプローチはしない。卵巣腫瘍が中心にあり，右後腹膜腔が展開されているところを示す（図4）。

2 卵巣動静脈の結紮

尿管の走行を確認したうえで卵巣動静脈を結紮する（図5）。

3 子宮動脈本幹の結紮（図6）

4 基靱帯の処理

基靱帯を結紮・切断には場合によりEndo-GIAによる器械吻合を行っている（図7）。

5 膀胱子宮靱帯の処理

尿管を尿管トンネル入口部まで遊離し，膀胱を子宮頸部・腟壁より剥離し膀胱子宮靱帯前層を切

図4　壁側腹膜の切開
右後腹膜腔を展開している。

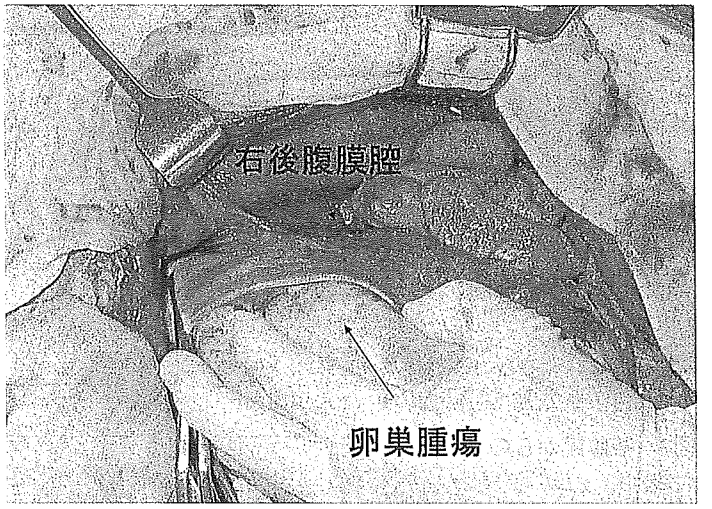


図5　卵巣動静脈の結紮
尿管の走行を確認し，卵巣動静脈を結紮する。

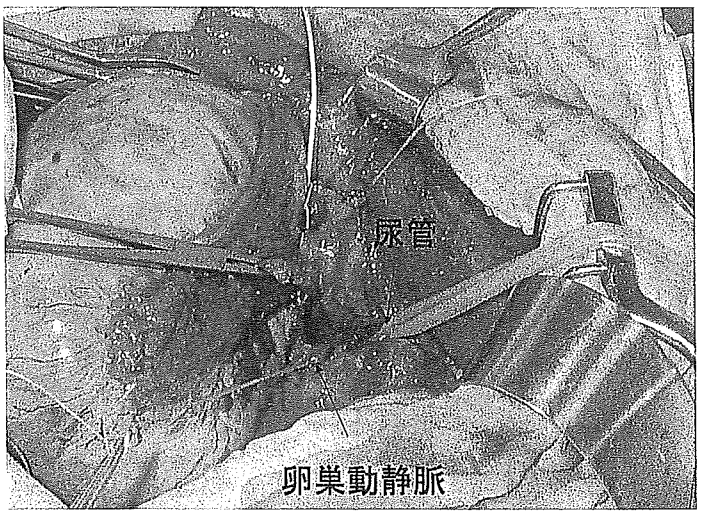


図6　子宮動脈本幹の結紮
ケリー鉗子を用いて子宮動脈を分離結紮、血液供給が一気に遮断される。

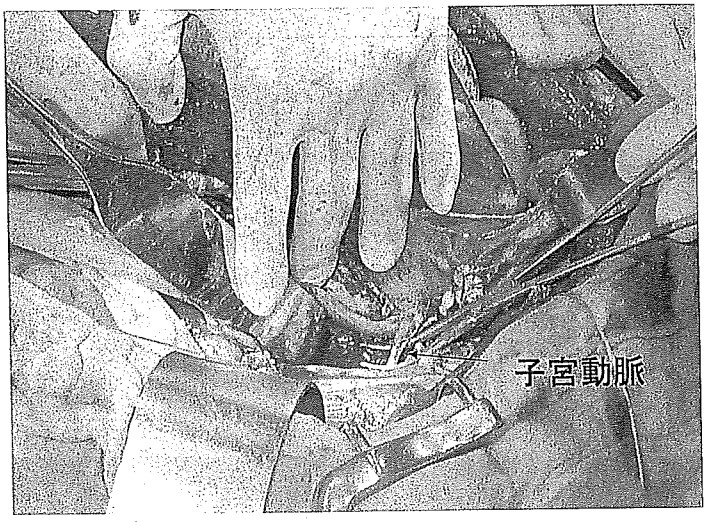


図7　基靱帯の処理
場合により器械（Endo-GIA）を使用する。

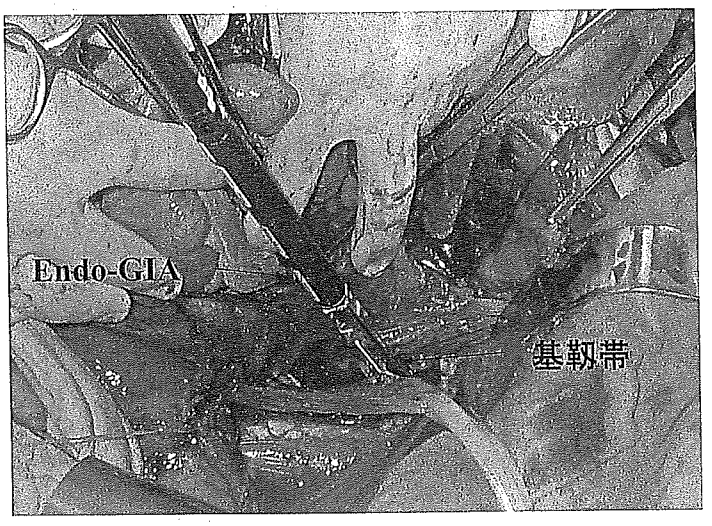
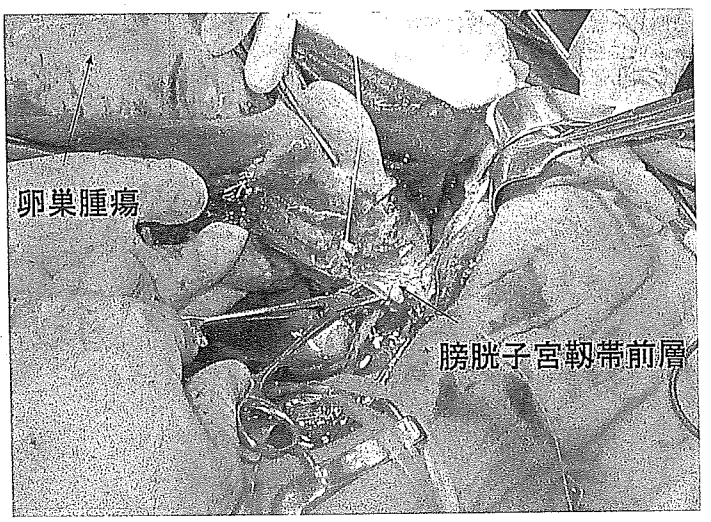


図8　膀胱子宮靱帯の処理
鋸歯型鉗子を用いて尿管トンネルをつくる。



断する（図8）。

❑膣切断

膣壁より十分尿管を剥離し傍子宮結合織を切断する。膣壁を前壁から後壁へと切開し直腸を露出し直腸切断部位を確認するとともに膣断端を縫合閉鎖する（図9）。

❑消化管合併切除

膣切断後，卵巣腫瘍と子宮を可及的に摘出するか，あるいは腫瘍を腸切除とともに子宮と一塊に摘出する（図10）。骨盤壁からのoozing に対してはアルゴンビームコアギュレーターを使用している。

図9　膣切断
膣切断後，直腸前面を露出し直腸切断部位を確認する。

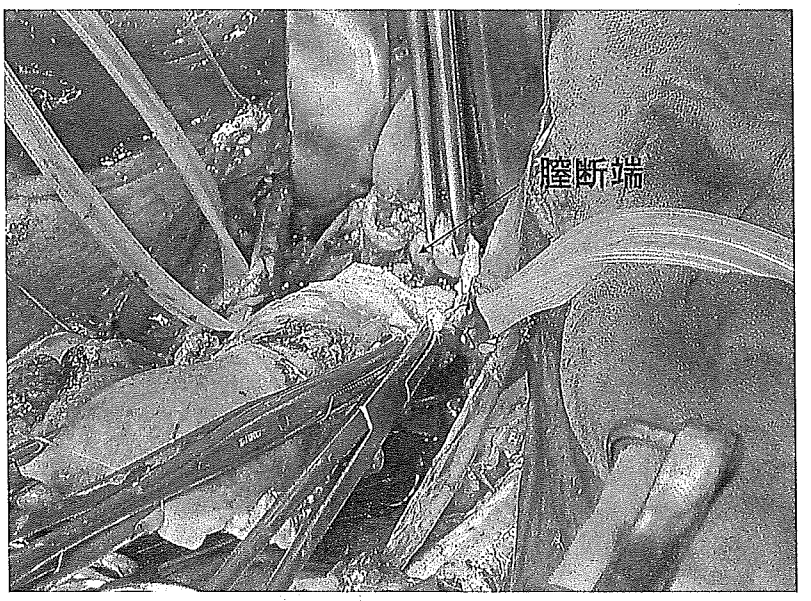
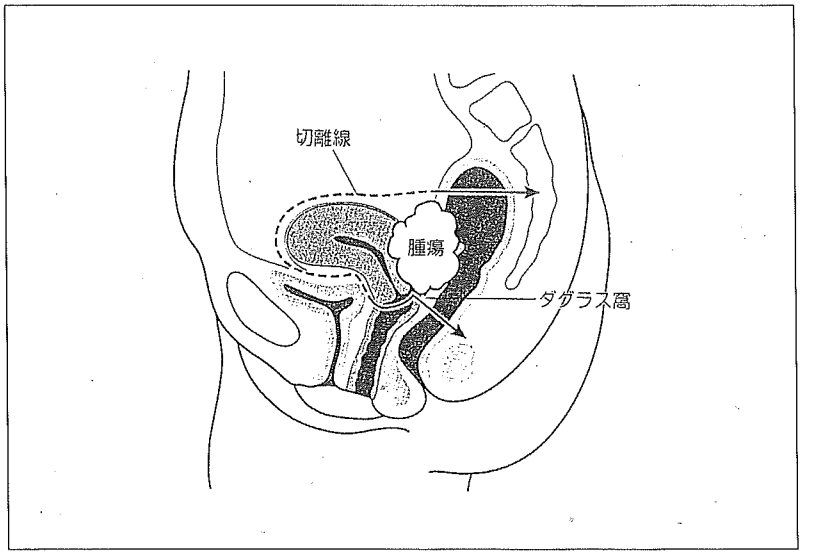


図10　消化管合併切除
（文献3より）
腫瘍・子宮・直腸を一塊に切除，あるいは腫瘍・子宮を直前に摘出し消化管を切除する。



結語

卵巣癌主病巣が直腸・S状結腸あるいは子宮後壁と強固に癌性癒着している場合は，側壁からアプローチすることが大切である。壁側腹膜ごと切除するように中心に向かうと，腫瘍周囲からの出血の減少につながる。壁側腹膜を切開することから始まり，卵巣・子宮動静脈を早期に結紮することで，血流が一気に遮断される。さらに基靱帯，膀胱子宮靱帯の処理後，腟切断を行い腫瘍と子宮を可及的に摘出するか，あるいは腫瘍を腸切除とともに子宮と一塊に摘出する。卵巣癌の手術において，十分なインフォームドコンセントと外科医師との緊密な連絡体制のもと，標準術式以外に子宮悪性腫瘍の手術手技を応用することで，出血量の減少を含め，より確実な手術が期待できると考えられた。

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Indoleamine 2,3-Dioxygenase Serves as a Marker of Poor Prognosis in Gene Expression Profiles of Serous Ovarian Cancer Cells

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Abstract

Purpose: We aimed to find key molecules associated with chemoresistance in ovarian cancer using gene expression profiling as a screening tool.

Experimental Design: Using two newly established paclitaxel-resistant ovarian cancer cell lines from an original paclitaxel-sensitive cell line and four supersensitive and four refractory surgical ovarian cancer specimens from paclitaxel-based chemotherapy, molecules associated with chemoresistance were screened with gene expression profiling arrays containing 39,000 genes. We further analyzed 44 genes that showed significantly different expressions between paclitaxel-sensitive samples and paclitaxel-resistant samples with permutation tests, which were common in cell lines and patients' tumors.

Results: Eight of these genes showed reproducible results with real-time reverse transcription-PCR, of which *indoleamine 2,3-dioxygenase* gene expression was the most prominent and consistent. Moreover, by immunohistochemical analysis using a total of 24 serous-type ovarian cancer surgical specimens (stage III, $n = 21$; stage IV, $n = 7$), excluding samples used for GeneChip analysis, the Kaplan-Meier survival curve showed a clear relationship between indoleamine 2,3-dioxygenase staining patterns and overall survival (log-rank test, $P = 0.0001$). All patients classified as negative survived without relapse. The 50% survival of patients classified as sporadic, focal, and diffuse was 41, 17, and 11 months, respectively.

Conclusion: The indoleamine 2,3-dioxygenase screened with the GeneChip was positively associated with paclitaxel resistance and with impaired survival in patients with serous-type ovarian cancer.

Ovarian cancer is one of the primary causes of death related to gynecologic malignancies (1). Nearly 65% of ovarian cancer patients die from their disease within 5 years (2). Although ovarian cancer is considered highly responsive to combination therapy with paclitaxel and carboplatin (3), cancer recurs rapidly in >50% of responsive patients, and in many cases, the recurring cancer cells develop chemoresistance (4). Therefore, countering chemoresistance is essential for ovarian cancer management.

Properties within tumor cells that may lead to drug resistance in ovarian cancer include multidrug resistance proteins and mismatched repair processes (e.g., alterations in the p53 pathway; refs. 5–7). In addition, various molecules have been documented as candidates for chemoresistance in ovarian cancer (8–12). However, molecular targeting to overcome chemoresistance has not yet been delineated in ovarian cancer.

The development of microarray methods for large-scale analysis of gene expression makes it possible to search systematically for key molecules that may be involved in chemoresistance (13). We have already applied this approach to ovarian cancer (14) as well as to other cancers (15, 16). In previous works on ovarian cancer, gene expression profiling was used to distinguish types of ovarian cancer (17), malignant transformation from normal tissue (18, 19), serous uterine from ovarian cancers (20), or metastatic from nonmetastatic disease (21). Although some advances have been seen in chemoresistance of childhood acute lymphoblastic leukemia as well as other types of cancers (22–24), the technology has not elucidated a set of genes associated with chemoresistance, a critical factor for improving prognosis in most cancers.

In this experiment, GeneChip was applied to screen molecules expressed differentially between chemoresistant and chemosensitive cell lines as well as cancer cells derived from patients who were either clinically sensitive or resistant to chemotherapy. The clinical significance of a prominent molecule was further confirmed with immunohistochemical analysis to predict recurrence after chemotherapy.

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