くことを確認する。巾着縫合器を用いて口側結腸端のまつり縫合を行い，アンビルヘッドを挿入して固定する（図10a）。肛門よりサーキュラーステイプラーを挿入し，残存直腸断端に軽い緊張がかかる状況で保持する。トロカールが断端縫合線の中心付近を貫通するようにトロカールを出す（図10b）。トロカールが貫通しやすくするため，トロカールを出しながら貫通部位に電気メスで小切開を加える。トロカールが十分に出たあと，アンビルとトロカールをドッキングさせる。余分な周囲組織を挟み込まないように結腸と残存直腸を密着させるが，密着寸前に口側結腸の緊張を緩めてしっかりと締結させる（図10c）。その後一気にファイヤーを行って吻合・再建を完了させる（図10d，e）。締結を緩めゆっくりと吻合器を引き抜き，吻合で切除された全層の腸組織リングを確認する。このあとにリークテストを行い，吻合が完全であることを確認する。吻合がさまざまな要因により不安であると考える場合，回腸または右横行結腸に一時的人工肛門の造設を行う。ドレーンを吻合部付近の骨盤底に留置した後，後腹膜を可能なかぎり修復している。最後に閉腹して手術を終了する。

注意事項

- 吻合部に十分な血流が確保されていること，また緊張がないことが重要である。場合によりIMAの根部切離が必要となることもある。
- サーキュラーステイプラーを肛門より挿入しやすくするため，挿入前に肛門および肛門管を用手的にやさしく拡張させる。
- 吻合が不完全な場合は再吻合を行うか，追加縫合と一時的人工肛門造設を行う。

Ⅳ．LAR時の全体的な注意事項

前述したごとくLARではTMEまたはTSMEが基本操作であり，出血も少なく手術時間も短縮される。直腸固有筋膜を損傷しないように注意する。出血が多い場合は，holy planeを再確認して手術を進めるべきである。また腫瘍の進行度によってはさらに外側の層での手術の必要性もあり，安全なsurgical marginsを確保することが局所再発の減少につながる。またこの操作中では，自律神経損傷に注意を要する。TMEを伴うLARでは吻合部もかなり低位となり，縫合不全率が10％前後以上と高くなる[8]。このため吻合に不安因子のある場合は，一時的人工肛門造設を考慮すべきである[9]。

おわりに

低位進行直腸癌の肛門温存術はむずかしい手術であるが，機器や手技の発達により腹腔鏡を含めて積極的に施行されている。局所解剖の十分な理解と，TMEを主とした適切な剝離層，および安全なsurgical marginsの確保が重要となる。また通常のLARで肛門温存が困難な場合，経肛門的アプローチの手術法[10]も考慮すべきであろう。

文献

1) 大腸癌研究会編：大腸癌取扱い規約　第7版，金原出版，6-34，2006.
2) Heald RJ et al : The mesorectum in rectal cancer surgery-the clue to pelvic recurrence? Br J Surg 69 : 613-616, 1982
3) Scott N et al : Total mesorectal excision and local recurrence : a study of tumour spread in the mesorectum distal to rectal cancer. Br J Surg 82 : 1031-1033, 1995
4) Lowry AC et al : Consensus statement of definitions for anorectal physiology and rectal cancer : report of the Tripartite Consensus Conference on Definitions for Anorectal Physiology and Rectal Cancer. Dis Colon Rectum 44 : 915-919, 2001
5) Schiessel R et al : Intersphincteric resection for low rectal tumours. Br J Surg 81 : 1376-1378, 1994
6) Rullier E et al : Sphincter-saving resection for all rectal carcinomas : the end of the 2-cm distal rule. Ann Surg 241 : 465-469, 2005
7) 大腸癌研究会編：大腸癌治療ガイドライン―医師用2010年版，金原出版，13-15，2010
8) Peeters KC et al : Risk factors for anastomotic failure after total mesorectal excision of rectal cancer. Br J Surg 92 : 211-216, 2005
9) Hüser N et al : Systematic review and meta-analysis of the role of defunctioning stoma in low rectal cancer surgery. Ann Surg 248 : 52-60, 2008
10) Saito N et al : Intersphincteric resection in patients with very low rectal cancer : a review of the Japanese experience. Dis Colon Rectum 49 : S13-22, 2006

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特　集

直腸癌治療の最近の動向

5. 直腸癌に対する肛門温存手術

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キーワード　下部直腸癌，肛門温存手術，超低位前方切除術，経肛門吻合，肛門括約筋部分温存術

I. 内容要旨

直腸癌における肛門温存手術では，低位前方切除，超低位前方切除，従来の経肛門吻合，そして最近の Intersphincteric resection（ISR）±External sphincter resection（ESR），などが実施されている．とくに下部直腸癌では Double stapling technique による超低位前方切除が主であるが，機器使用の限界のため肛門温存を断念し直腸切断術に変更される事もある．しかし術式変更以前に，従来の経肛門吻合，さらには ISR を主とした肛門括約筋部分温存手術，などによる肛門温存の可能性を考慮する余地がある．超低位前方切除は低位前方切除の延長線上の手術で，厳密な意味での定義はない（本文を参考）．また従来の肛門吻合と ISR は，異なる手術法である．いずれの手術を実施するにも，各手術に対する操作の習熟が必要で，各術式の長所・短所をよく理解し，慎重な適応決定が要求される．もちろん直腸・肛門の解剖と生理に精通する必要がある．各術式により術後排便機能や QOL は異なるため，個々の症例の状況に応じなければならない．また肛門を温存するために，局所再発を助長することがあってはならない．本稿では，下部直腸癌に対する現時点での肛門温存手術について，各手術法の概要や重要な問題点について述べることにする．

II. はじめに

近年の器械吻合の進歩により，下部直腸癌の多くの症例で肛門温存が可能となった．また適正な肛門側断端（Distal margin）に関する臨床病理学的研究により，肛門温存手術の妥当性も示されている．直腸癌において肛門温存手術を実施する場合，低位前方切除（Low anterior resection：LAR），超低位前方切除（Very low anterior resection：v-LAR または Ultra-low anterior resection：U-LAR），経肛門吻合（Conventional coloanal anastomosis：CAA），さらに一部の施設では内肛門括約筋を切除して肛門吻合を行う Intersphincteric resection（ISR）を主とした肛門括約筋部分温存手術，などが行われている．これまでの肛門温存手術の変遷を表1に示す．永久人工肛門を伴う直腸切断術（Abdominoperineal resection：APR）の回避を目的とした Pull-through 術式が Babcock（1939），Bacon（1945），Black（1948），Turnbull-Cutait（1961），陣内（1961），らにより報告された．しかし Pull-through 手術例では合併症が多いこと，術後排便不良，などが示された．そこで前方切除術（手縫い法による）が主流となったが，低位前方切除（Low anterior resection：LAR）が不可能な場合，APR となっていた．本邦では今（1968），安富（1972）らにより LAR が普及された．この頃 Parks（1972）は，経肛門吻合による肛門温存手術を報告した[1]．これが今日における肛門吻合の土台となっている．一方，Androsov（1970），Fain（1975），Ravitch（1979）らにより自動縫合器を用いた器械吻合が導入され，Knight and Griffin（1983）により Double stapling technique（DST）が紹介された[2]．現在の下部直腸癌における肛門温存術の大半は，この DST による（超）低位前方切除術で実施されている．一般的に本法による肛門温存が不可能な場合，APR の適応とされがちであろう．これ

SPHINCTER-SAVING RESECTION FOR LOW RECTAL CANCER

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表1　直腸癌における括約筋温存手術の変遷

	1900	1940	1950	1970	2000
APR	Miles (1908)				
Pull-through		Babcoch (1939) Bacon (1945) Black (1948) Welch (1952) Turnbull-Cutait (1961)			
Anterior resection (手縫い)		Dixon (1939) end to end Baker (1950) end to side		Parks (1972) 経肛門吻合	
(器械)				Androsov (1970) Fain (1975) Ravitch (1979) Knight and Griffin (1983) *DST*	
Intersphincteric resection (ISR)				Shafik (1981) Parks (1977)	Braun (1991) Selraggi (1992) *Schiessel (1994)* Rullier (1999)

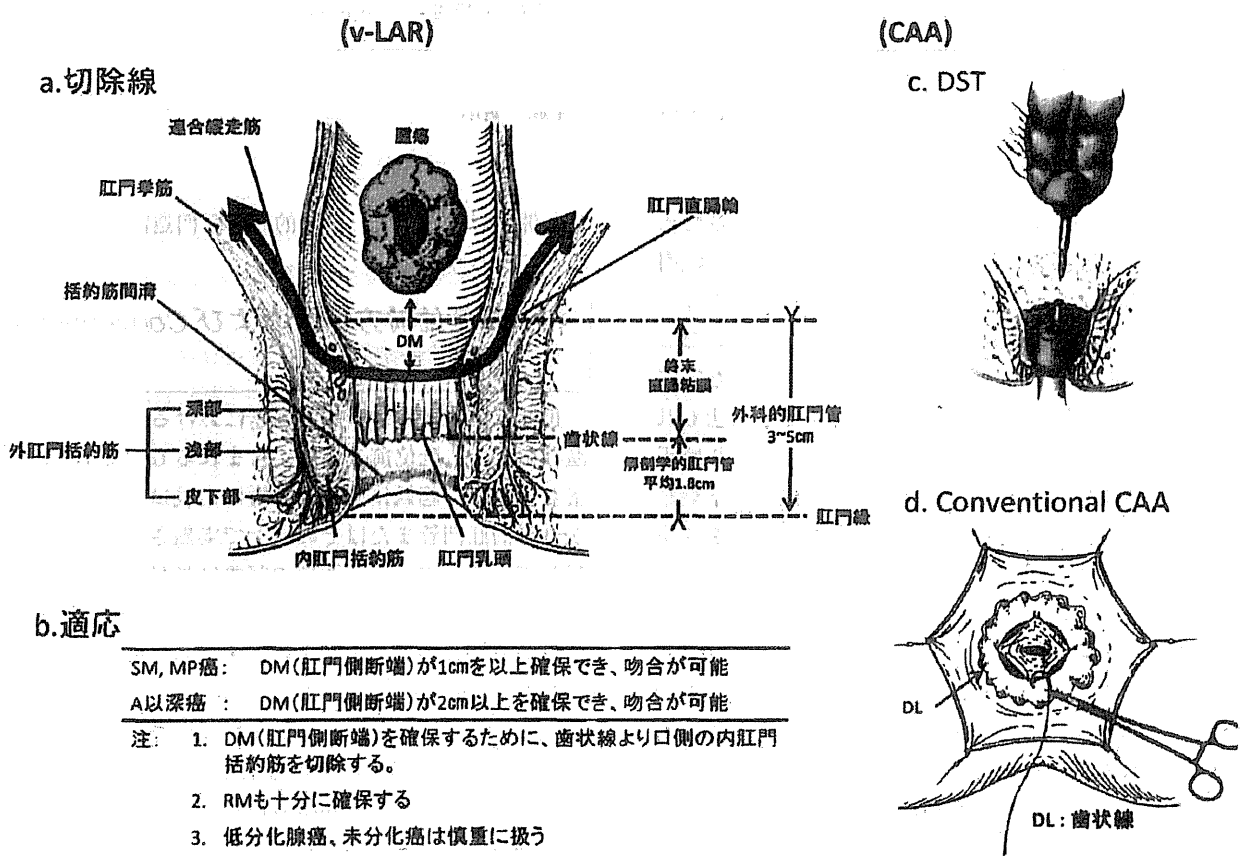
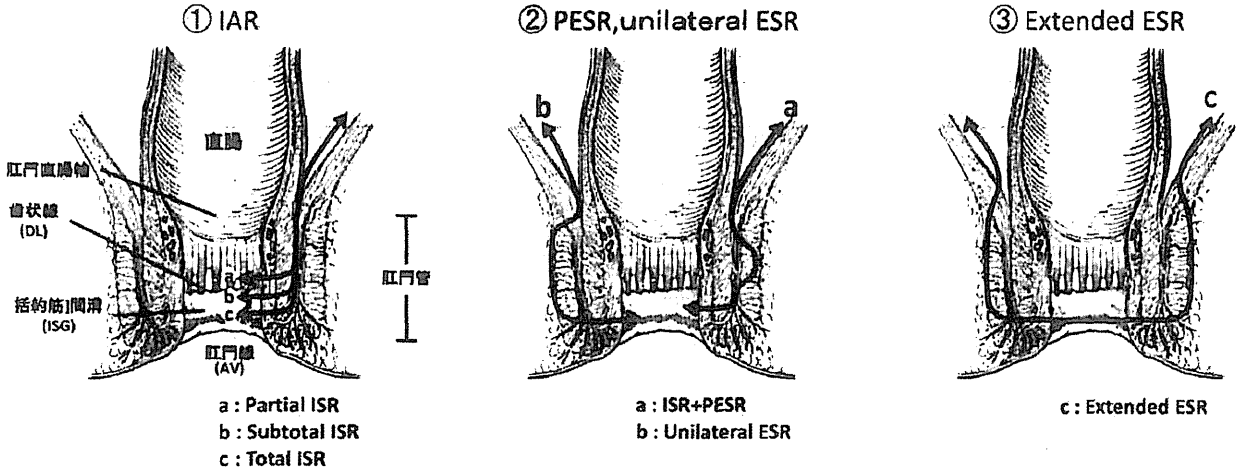


図1　Very Low Anterior Resection, Conventional Colo-anal Anastomosis



適応

肛門管内腫瘍最深部	DM(1~2cm)の位置
～T2・・・・・ISR±PESR T3～・・・・・PESR Unilateral ESR Extended ESR	歯 状 線 ・・・Partial ISR±α 中　間 ・・・Subtotal ISR±α 括約筋間溝・・・Total ISR±α

α:各ESR

図2　ISR±ESRの切除線，適応

は安全な肛門側切離縁の距離（1～2cm）確保のため肛門括約筋や肛門管に切り込む必要がある場合，肛門温存の適応ではないと考えられていたからであろう．肛門管切除は，排便機能の面から禁止とされていたことも理由であろう．しかし最近，ISRを主とした肛門括約筋の部分切除を行って肛門を温存する手術法も試みられている．ISRという術式はParksらにより提唱され，腹腔操作に加え肛門側操作により内・外括約筋間溝の剝離を行い，内肛門括約筋とともに標本を切除し，手縫いによる結腸肛門吻合を行う術式である[3]．本術式の成績は，その後Schissel[4]やRullier[5]により報告された．本邦からも寺本[6]，白水[7]，著者[8]，山田[9]らにより報告されるようになったが，まだまだ長期的な腫瘍学的及び機能的予後については不明なところも多い．もちろん直腸癌の治療では根治性と機能温存が重要であり，肛門温存のために局所再発の増加があってはならない．また術後排便機能不良のため，QOLを損なってもならない．本稿では下部直腸癌における超低位前方切除を含む最近の肛門温存手術について述べることにする．各術式の適応は腫瘍の局在と進行度，および患者本人の希望も考慮して決定される．また合併症対策として，一時的人工肛門造設も増加することになろう．

III. 超低位前方切除およびConventional CAA

超低位前方切除は下部直腸癌における標準的な肛門温存術式で，低位前方切除に含まれるものである．厳密な意味での超低位前方切除術の定義はないが，吻合が外科的肛門管またはその直上で実施されたものと理解している．従って肛門側の剝離は外科的肛門管に及び，大半がDSTにより再建される．標準的な（超）低位前方切除の適応を図1に示す．本法の適応は，安全なSurgical margins（Distal margin：DM，Radial margin：RM）が確保され吻合が可能であるかどうかで決定される．手術の実際はLARに準ずるものであり，上方郭清，Total mesorectal excision（TME）±自律神経温存，±側方郭清，吻合，で構成される．詳述は省略するが，安全なDMの確保は重要であり，T3以深症例では病理組織学的検討により2cmとされている．またT2までの症例では，DMは最短1cmでよいと考えている．T4症例でも合併切除によりRM

表2 ISR±PESRの予後

Reference	Patient No. (T3 or stage Ⅲ)	Preoperative radiotherapy or radiochemotherapy (RCT)	5-year Survival	Local Recurrence Rate
Schiessel R. et al. Dis Colon Rectum 2005 : 48		No	—	5.3%
☆ Rullier E. et al. Ann Surg. 2005 : 241	58 (78%) [2y～]	Yes (T3～) [54Gy]	OS : 81% DFS : 70%	2%
☆ Hohenberger W. et al. Colorectal Disease 2006 : 8	53 (34%) [1995-2001]	Yes (65%) [50.4gy]	OS : 85.1%	25.1% 14.2% (RCT)
Saito N. et al. Dis Colon Rectum 2006 : 49	225 (45%)	(T3, 25%) [45Gy]	OS : 91.9% DFS : 83.2%	5.3%
☆ Portier G. et al. Br J Surg 2007 : 94	173 (31%, 44%)	Yes (53%)	OS : 86.1%	10.6%
☆ Chamlow R. et al. Ann Surg. 2007 : 246	90 (36%)	Yes (41%) [25-45gy]	OS : 82% DFS : 75%	8.8%
☆ Akasu T. et al. J Am coll Surg 2007 : 205	108 (40%)	No	3y OS : 95%	5.7% (3-year) 0% : T1-T2 15% : T3
☆ Weiser MR. et al. Ann Surg 2009 : 249	44 (86%)	Yes	5y-DFS : 83%	0%
Yamada K. et al. Dis Colon Rectum 2009 : 52	107 (55%)	No	Stage Ⅲ OS : 75% DFS : 72% Stage Ⅱ OS : 100% DFS : 83.5%	2.5%
Saito N. et al. World J Surg 2009 : 33	132 (76%)	113 (31%, 37%)	OS : 80.0% DFS : 69.1%	10.6%

☆ Preoperative RCT is recommended in T3 patients

APRの成績…OS : 75-85%, LR : 14-23%

が確保できれば，可能な限り肛門温存を行って良いと考える．低分化腺癌や未分化癌では，肛門温存は慎重に決定されるべきである．本法の殆どはDSTにより実施されるが，安全なmarginsが確保されても機器の使用に限界がある場合もあり，術式をAPRに変更することがある（男性例に多い）．このような場合は器械吻合に頼らず，Parksの手縫いの経肛門吻合(Conventional CAA）を行うと肛門温存は可能となる．このConventional CAAは後述するISRとは別の術式であり殆どの内肛門括約筋が残存する．本術式により自然肛門から排便できることの恩恵は大きいが，LAR症候群と呼ばれる排便障害を認めることもしばしばある[10)～12)]．術前より，これらについて説明する必要がある．また吻合部が低位になるほど縫合不全の危険が増加し，一時的人工肛門造設も考慮したい．

IV. ISRを主とした肛門括約筋部分温存手術

外科的肛門管およびその近傍に癌腫が存在または進展する超低位直腸癌では，永久人工肛門を伴うAPRが標準手術である．この様な症例で可能な限りAPRを回避する手術法が，ISRを主とした肛門括約筋部分温存手術である．ISRは現時点で究極的な肛門温存手術であり，Parksらにより最初に報告された術式である．超低位前方切除およびConventional CAAで肛門温存が不可能な症例で，ISRにより安全なSurgical Marginsが得られ，肛門温存が可能な場合に実施される．最近，本邦ではISRに加えて外肛門括約筋を部分合併切除（External sphincter resection : ESR）するISR+ESRの報告も白水[7)]や著者[13)]らにより報告されている．これらの術式の適応と切除線を図2に示

表3 術後排便機能

Reference	Patients No.	Normal Continence (%)	Major Incontinence (%)	Colostomy for Incontinence (%)
Schiessel R, et al. Br J Surg. 1994 ; 81	37	67.5	0	0
Teramoto T, et al. Dis Colon Rectum 1997 ; 40	10	20	—	0
Rullier E, et al. Ann Surg. 2001 ; 224	21	57	9.5	0
Tiret E, et al. Colorectal Disease 2003 ; 5	25	54	0	0
Shirouzu K, et al. Tech Coloproctol 2003 ; 7	16	37.5	—	0
Bittorf B, et al. EJSO 2004 ; 30	31	29	25.8	—
Saito N, et al. Dis Colon Rectum 2004 ; 47	35	27.3 [1Y]	0	0
Schiessel R, et al. Dis Colon Rectum 2005 ; 48	101	86.3	—	(0.8)
Saito N, et al. Dis Colon Rectum 2006 ; 49	181	68 [2Y]	7 [2Y]	0
Yamada K, et al. Br J Surg. 2007 ; 94	35	60	2.9	0
Ito M, et al. Dis Colon Rectum 2009 ; 52	90	77 [2Y]	23 [2Y]	0
Yamada K, et al. Dis Colon Rectum 2009 ; 52	107	70 [1-2Y]	1.9 [2Y]	—

[year]

す．本法は比較的難易度が高く，安全な Surgical margin の確保や機能保持のための手術手技，肛門および肛門管の解剖・生理の理解，などが要求される．ISR 手術は，肛門側より内外括約筋間の intersphincteric plane を利用して安全な margin を確保し，切離を行って標本を切除し，結腸肛門吻合による再建を行う方法である．従って腫瘍の存在部位により，様々な切除線が想定される．このため ISR は，内肛門括約筋の切除量（肛門側切除線）によりいくつかの Type に分類される．partial ISR は歯状線直上で切除される場合で，Subtotal ISR では肛門側切除線が歯状線と括約筋間溝の間となり，Total ISR は肛門側切除線が括約筋間溝となり内肛門括約筋が全切除される．これらの術式は，腫瘍下縁の位置と安全な DM（1～2cm）の確保の関係で決定される．ISR の安全な適応は，外科的肛門管内の腫瘍の最深部が T2 までとなる．しかし T2 の場合でも，T3 と同様に安全な RM を確保するために一部の外肛門括約筋の合併切除が必要となる場合もある（Partial ESR：PESR）．肛門挙筋浸潤所見や外肛門括約筋浸潤所見を認める症例は，ISR の適応ではない．一方，外科的肛門管における腫瘍最深部が T3，T4（外肛門括約筋浸潤，肛門挙筋浸潤）の場合，RM 確保のためかなりの部分の外肛門括約筋の合併切除（ESR）が必要となる．図2に示すように，Unilateral ESR や Extended ESR の切除線となる．これらにより安全な Margins が確保不能の場合，APR の絶対的適応となる．これらの究極的肛門温存手術では，肉眼型の Type 4 は除外され，Type 3，および組織型が低分化型を示す場合は慎重に適応決定を行う必要がある．まだ腫瘍学的および機能的予後の不明な面も多く，無理は禁物である．

最近になり ISR±PESR の腫瘍学的予後，および術後排便機能の状況が判明しつつある．現在までに報告されている，主な腫瘍学的予後を表2に示す．本法による5年 overall survival（OS）は，80%～90%，Disease free survival（DFS）は 69%～83%，局所再発率は 10% 前後以下の報告が多い．しかし T3～症例では，局所再発率が高くなる傾向である[14]．一方 APR の OS は 65%～85%，DFS は 60%～70% 程度，局所再発率は 10%～20% の報告が多い．Background の相違や補助療法の進歩などもあり単純に比較することはできないが，ISR±PESR の手術成績が APR に比較して低下することはない[15]．また本法の術後排便機能について，主な報告を表3に示す．Occasional minor

soilingを含んだNormal Continence症例の割合は，60%～86%であると報告されている．しかし真の意味でのPerfectの症例の割合は，20%～40%程度と推察される．失禁のためストーマ造設が必要となった報告は非常に少ない．しかし著者らの経験では，術前にChemoradiation治療（CRT）を行った50例中2例（4%）に就労の関係でストーマ造設を必要とし，CRT例で排便機能は悪い傾向にあった[16]．詳細なアンケート調査では排便機能障害は経時的改善を認めるが，夜間のsoilingは遷延する傾向を示した．術後排便機能は多くの症例で許容範囲内であるが，排便機能不良例も実在する．QOLについては大腸癌研究会のプロジェクト研究（寺本班）で調査され，ISR±ESR群とAPR群間にQOLの差を認めなかった[17]．このように肛門括約筋部分温存手術の長所・短所の実態が解明されつつある．

V. おわりに

近年の肛門温存手術は，先達の外科医の努力，機器の発達，技術改良，などにより進歩してきた．各術式の長所，短所を理解し，個々の症例に応じた最善の術式を選択したい．また新しい手術法では，慎重な症例選択と根治性・機能およびQOLを考慮して適切な切除範囲を決定し，注意深く手術をすすめたい．「温故知新」という言葉が心にしみる．

文　献

1) Parks AG: Transanal technique in low rectal anastomosis. Proc R Soc Med, 65: 975-926, 1972.
2) Knight CD, Griffen FD: Techniques of low rectal reconstruction. Curr Probl Surg, 20: 387-456, 1983.
3) Lyttle JA, Parks AG: Intersphincteric excision of the rectum. Br J Surg, 64: 413-416, 1977.
4) Schiessel R, Karner-Hanusch J, Herbst F, et al.: Intersphincteric resection for low rectal tumours. Br J Surg, 81: 1376-1378, 1944.
5) Rullier E, Zerbib F, Laurent C, et al.: Intersphincteric resection with excision of internal anal sphincter for conservative treatment of very low rectal cancer. Dis Colon Rectum, 42: 1168-1175, 1999.
6) Teramoto T, Watanabe M, Kitajima M: Per anum intersphincteric rectal dissection with direct coloanal anastomosis for lower rectal cancer: the ultimate sphincter-preserving operation. Dis Colon Rectum, 40(Suppl): S43-S47, 1997.
7) Shirouzu K, Ogata Y, Araki Y, et al.: A new ultimate anus-preserving operation for extremely low rectal cancer and for anal canal cancer. Tech Coloproctol, 7: 203-206, 2003.
8) Saito N, Ono M, Sugito M, et al.: Early results of intersphincteric resection for patients with very low rectal cancer: an active approach to avoid a permanent colostomy. Dis Colon Rectum, 47: 459-466, 2004.
9) Yamada K, Ogata S, Saiki Y, et al.: Functional results of intersphincteric resection for low rectal cancer. Br J Surg, 94: 1272-1277, 2007.
10) Karanjia ND, Schache DJ, Heald RJ: Function of the distal rectum after low anterior resection for carcinoma. Br J Surg, 79: 114-116, 1992.
11) Paty PB, Enker WE, Cohen AM, et al.: Long-term functional results of coloanal anastomosis for rectal cancer. Am J Surg, 167: 90-94, 1994.
12) Graf W, Ekström K, Glimelius B, et al.: A pilot study of factors influencing bowel function after colorectal anastomosis. Dis Colon Rectum, 39: 744-749, 1996.
13) Saito N, Moriya Y, Shirouzu K, et al.: Intersphincteric resection in patients with very low rectal cancer: a review of the Japanese experience. Dis Colon Rectum, 49(Suppl): S13-22, 2006.
14) Akasu T, Takawa M, Yamamoto S, et al.: Incidence and patterns of recurrence after intersphincteric resection for very low rectal adenocarcinoma. J Am Coll Surg, 205: 642-647, 2007.
15) Saito N, Sugito M, Ito M, et al.: Oncologic outcome of intersphincteric resection for very low rectal cancer. World J Surg, 33: 1750-1756, 2009.
16) Ito M, Saito N, Sugito M, et al.: Analysis of clinical factors associated with anal function after intersphincteric resection for very low rectal cancer. Dis Colon Rectum, 52: 64-70, 2009.
17) Hashimoto H, Shiokawa H, Funahashi K, et al.: Development and validation of a modified fecal incontinence quality of life scale for Japanese patients after intersphincteric resection for very low rectal cancer. J Gastroenterol, 45: 928-935, 2010.

利益相反：なし

SPHINCTER-SAVING RESECTION FOR LOW RECTAL CANCER

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R0 resection, preservation of the anal sphincter, and local control are considered to be the most important target criteria in rectal cancer surgery. Many efforts have been made in recent years to increase the rate of sphincter preservation by performing pull-through operations, ultra-low anterior resection (U-LAR), and intersphincteric resection (ISR). U-LAR is the standard surgery for patients with lower rectal cancer to preserve anal function. Reconstruction in U-LAR is mainly performed using stapled anastomosis. Although conventional coloanal anastomosis makes it possible to preserve the anal sphincter, the mechanical methods are difficult. In that case, almost all the internal sphincter is preserved. The final options for preserving the sphincter are ISR and external sphincter resection (ESR). Although the internal sphincter is sacrificed partially, subtotally, or totally in ISR, and the external sphincter is resected partially or extensively in ESR, complete or incomplete anal function is maintained. However, the literature is not clear regarding long-term oncologic outcome and anal function after these procedures. The application of these surgical techniques can reduce the rate of abdominoperineal resection in very low rectal cancer. The indications for these procedures must be carefully determined based on tumor site and stage as well as the patient's own preference.

Cyclin D1 predicts the prognosis of advanced serous ovarian cancer

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Abstract. We previously reported that cyclin E (*CCNE1*) amplification is strongly associated with resistance to treatment in serous ovarian cancer by high-resolution oligonucleotide copy number analysis. Dysregulation of cell cycle control has been implicated as the key event in human oncogenesis, and aberrant expression of G1-S phase-related genes in particular has been reported in epithelial ovarian cancer (EOC). Nevertheless, there are conflicting results concerning the prognostic values of these abnormalities in EOC. This study focused on advanced serous EOC cases and investigated the association between the expression of G1-S phase-regulatory proteins and clinicopathological parameters. The utility of these proteins as prognostic factors was assessed, and whether these targets reflect chemoresistance of advanced serous EOC was investigated. A total of 66 patients treated by primary surgery were evaluated in this study. Immunohistochemical analysis for cyclin D1, pRb, p16, p53, $p27^{Kip1}$, $p21^{Waf1/Cip1}$ and cyclin E was performed on formalin-fixed tissue sections collected from primary surgical specimens. The correlations between the expression of these proteins and the clinicopathological parameters, including progression-free survival (PFS), overall survival (OS) and chemosensitivity, were examined. Upon univariate analysis, overexpression of cyclin D1 was positively correlated with reduced PFS (p=0.00062) and OS (p=0.00037). Reduced expression of $p27^{Kip1}$ was associated with shorter OS (p=0.064). Upon multivariate analysis, overexpression of cyclin D1 (p=0.0019), reduced expression of $p27^{Kip1}$ (p=0.042) and residual tumor volume (p=0.0092) were identified as independent predictors of OS. Overexpression of cyclin D1 (p=0.011) as well as residual tumor volume (p=0.006) were significantly associated with first-line chemosensitivity. In advanced serous EOC, overexpression of cyclin D1 contributed largely to poor prognosis, and this may have been in part mediated by chemoresistance. Cyclin D1 is a possible target for overcoming the refractory nature of advanced serous EOC.

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Key words: ovarian cancer, cyclin D1, p27, prognosis, immunohistochemistry

Introduction

Ovarian cancer is the most lethal gynecological malignancy in developed countries and is the 9th most common cancer in Japanese females. An estimated 8,304 new cases and 4,467 mortalities occurred in 2005 (Center for Cancer Control and Information Services, National Cancer Center, Japan). Although ovarian cancer patients respond to cytoreductive surgery and combination chemotherapy satisfactorily, advanced cases exhibit a high level of recurrence, and the overall survival (OS) rate has not significantly changed for decades. However, clinical trials have been undertaken to improve prognosis. Since there are different clinical behavior patterns for certain histopathological subgroups, separate trials have been developed for clear cell (1) and mucinous carcinomas (2). The alteration of dose/schedule and the use of intraperitoneal therapy have been shown to be superior in at least one trial (3,4).

A number of clinicopathological factors, including the volume of residual tumor after primary surgery, FIGO stage and tumor grade, are reported to be the key prognostic factors (5,6). Cytoreduction to a non-macroscopic residual tumor is the ultimate goal, and it improves prognosis (7-9). However, in order to improve the prognosis of advanced epithelial ovarian cancer (EOC) cases, other predictive biomarkers should also be elucidated.

We previously described that cyclin E (*CCNE1*) amplification was strongly associated with resistance to treatment in serous ovarian cancer by high-resolution oligonucleotide copy number analysis (10). Therefore, the amplification status of cyclin E has potential for therapeutic exploitation, whereby patients exhibiting cyclin E amplification may benefit from novel, cyclin-related targeted treatments. Dysregulation of cell cycle control has been implicated as the key event in human oncogenesis, and aberrant expression of G1-S phase-related genes in particular has been reported in a number of human cancers, including EOC (5,11). Aberrant expression of the p16-cyclin D1-CDK4/6-pRb and p21-p27-cyclin E-CDK2 pathways have been reported to correlate with prognosis (5,11-14).

Barbieri *et al* reported in their series of 70 EOC cases that overexpression of cyclin D1 was associated with a shorter

OS. In particular, among patients with stage III/IV tumors and residual disease greater than 2 cm, cyclin D1 expression significantly influenced clinical outcome (5). Bali *et al* reported on 134 serous ovarian cancers for which molecular markers predicted reduced OS in univariate analysis, which included overexpression of cyclin D1 and p53, and reduced expression of $p27^{Kip1}$ and $p21^{Waf1/Cip1}$ (11). In contrast, it was reported that low nuclear p27 expression was associated with improved 3-year OS and progression-free survival (PFS) in 150 advanced stage (FIGO stages II, III and IV) EOC patients (12). Kommoss *et al* carried out immunohistochemical analysis of $p16^{Ink4a}$ and pRb expression levels and found that they correlated with survival in a series of 300 patients with FIGO stage II-IV ovarian carcinoma. They reported that $p16^{Ink4a}$-negative tumors had a significantly worse prognosis in both univariate and multivariate analyses. High expression levels of pRb were associated with an incremental deterioration of prognosis, which was also the case in the subgroup of optimally debulked patients (15). Meanwhile, Khouja *et al*, using immunohistochemistry, evaluated 171 primary stage III ovarian carcinoma tumors for expression of Ki-67, p16, p14 and p57. High expression of p16 was correlated with poor differentiation and survival in univariate analysis. However, in multivariate analysis, p16 expression was not significantly associated with shorter survival (13). Some of the results are contradictory, probably due to the variety of histotypes and stages of EOC as well as disease heterogeneity, different research methodologies or the sample sizes of the studies. As serous ovarian cancer is the most common histological type of EOC and the prognosis of advanced cases remains poor, we limited our analysis to the serous histotype and advanced cases to eliminate such bias.

We focused on advanced serous EOC (stage III/IV) cases in particular and investigated the association between the expression of G1-S phase-regulatory proteins and the clinicopathological parameters. We aimed to identify the utility of these proteins as prognostic factors and to evaluate whether these targets reflect chemoresistance of advanced serous EOC.

Patients and methods

Patients and tumor specimens. The Jikei University School of Medicine Ethics Review Committee approved the study protocol, and informed consent was obtained from the patients. The tumor specimens were surgically obtained from a group of 66 patients with advanced primary ovarian serous adenocarcinoma who were treated at the Department of Obstetrics and Gynecology, The Jikei University School of Medicine, and Jikei University Kashiwa Hospital. The tumors were staged in accordance with the International Federation of Gynecology and Obstetrics (FIGO) system (1988). The clinical and pathological characteristics of the patient cohort are shown in Table I. The age at diagnosis, volume of postoperative residual disease, FIGO stage, presence of intraoperative ascites and patient outcome were obtained retrospectively from patient records as shown. The median follow-up time for the cohort was 15.5 months (range 3-72). The 66 patients received first-line platinum-based chemotherapy. Among them, 62 cases received taxane simultaneously as T-C chemotherapy following primary surgery (93.9%).

Table I. Clinical and pathological characteristics of the serous epithelial ovarian cancer patient cohort (n=66).

Clinicopathological parameters	No. of patients (%)
Age	
≤65 years	55 (83.3)
>65 years	11 (16.7)
FIGO stage	
III	52 (78.8)
IV	14 (21.2)
Residual disease	
≤2 cm	28 (42.4)
>2 cm	38 (57.6)
Ascites	
≤500 ml	25 (37.9)
>500 ml	41 (62.1)
Disease progression	
No	9 (13.6)
Yes	57 (86.4)

Immunohistochemistry. Immunostaining was performed on buffered formalin-fixed, paraffin-embedded tissue sections. The sections were deparaffinized, and standard immunohistochemical techniques were performed using Ventana XT system (BenchMark® XT; Ventana Medical Systems, Inc., Tuscon, AZ, USA) in accordance with the manufacturer's instructions. Antigen epitopes were retrieved using Ventana Benchmark CC1 standard program. The primary antibodies used in this study were: anti-cyclin D1 (rabbit monoclonal clone SP4; Ventana Medical Systems, Inc.), anti-pRb (mouse monoclonal clone 13A10; Novocastra Laboratories Ltd., UK; 1:100), anti-p16 (mouse monoclonal clone 16P04; Ventana Medical Systems, Inc.), anti-p53 (mouse monoclonal clone DO-7; Ventana Medical Systems, Inc.), anti-$p21^{Waf1}$ (mouse monoclonal clone EA10; Calbiochem, Darmstadt, Germany; 1:50), anti-$p27^{Kip1}$ (mouse monoclonal clone SX53G8; Dako, Glostrup, Denmark; 1:20) and anti-cyclin E (mouse monoclonal clone HE12; Medical and Biological Laboratories Co., Ltd., Japan; 1:500). Antibodies from Ventana Medical Systems, Inc. were pre-diluted. The antibodies were incubated at 37°C for 32 min (60 min for p21, Rb and cyclin E). The slides were counterstained with hematoxylin and mounted for microscopic examination. Positive and negative controls were tested in parallel for each staining.

Immunostaining evaluation. At least 500 tumor cells were evaluated for immunostaining, and the percentage of stained cells was calculated. The evaluation of immunostaining was conducted in a blinded manner by two independent screeners, without knowledge of the clinical and pathological characteristics of the cases. Standardization of scoring was achieved by comparison of scores between screeners, and discrepancies were resolved by consensus. The percentage of positive nuclear immunostaining in cells of the tumor sections was calculated. Scores are expressed as a percentage of positive nuclear

Table II. Immunohistochemical analysis of cell cycle gene expression in advanced serous epithelial ovarian cancer.

	Cyclin D1	pRb	$p16^{Ink4a}$	p53	$p27^{Kip1}$	$p21^{Waf1/Cip1}$	Cyclin E
Range of staining (% positive)	0-50	0-90	0-90	0-90	0-80	0-70	0-90
Median staining (%)	10	50	80	60	50	5	50
Cutoff value (%) (positive staining)	>20	>50	>50	>40	>40	>5	>70
Positive tumors (%)	11 (16.7)	26 (39.4)	42 (63.6)	37 (56.1)	37 (56.1)	20 (30.3)	11 (16.7)

Range of staining indicates the proportion of positive nuclear staining within representative areas of the tumor samples. Cut-off value is based on published reports. Number of specimens showing positive staining is provided in the bottom row.

staining within representative areas of the tumor sample. The percentage score above, whose staining is considered representative of overexpression, is based on published reports (11). The range and the median percentage of immunostaining, percentage values regarded as overexpression (cutoff value) and the numbers of specimens displaying positive staining are shown in Table II. Representative photomicrographs of tumor tissue showing positive and negative staining for the specific antigens are presented in Fig. 1.

Statistical analysis. The associations between clinicopathological parameters and the immunostaining scores were analyzed. The correlations between the expression of each gene and the clinicopathological parameters were analyzed using the Chi-square test. $p \leq 0.05$ was considered to be statistically significant. For survival analysis, event time distributions were evaluated using the Kaplan-Meier method, and differences in survival rates were compared using the log-rank test for univariate analysis and Cox proportional hazards regression model for multivariate analysis. PFS was calculated from the date of primary surgery to the date of disease progression. The duration of OS was defined from the date of primary surgery to the date the patient succumbed to the disease or to the date of last follow-up. The treatment-free interval (TFI) was defined as being from the last date of first-line chemotherapy to the date of recurrence or last follow-up without recurrence.

Results

Expression of G1-S phase-regulatory proteins and the association with clinicopathological parameters. The expression of G1-S phase-regulatory proteins was analyzed by immunohistochemistry in advanced serous EOC. Overexpression of cyclin D1, pRb, p16, p53, $p27^{Kip1}$, $p21^{Waf1/Cip1}$ and cyclin E was detected with incidences of 16.7, 39.4, 63.6, 56.1, 56.1, 30.3 and 16.7%, respectively. Associations of the expression of each protein and clinicopathological parameters are shown in Table III. The volume of postoperative residual disease and the presence of ascites were not correlated with the expression pattern of any of the studied proteins. The expression of p53 appeared to be positively correlated with that of $p16^{Ink4a}$ (Table III). No other significant association among the gene expressions was observed.

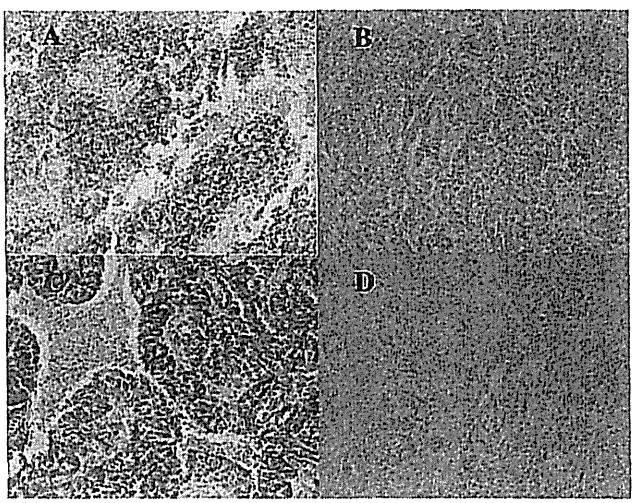

Figure 1. Immunohistochemistry on primary tumor tissue. Representative stainings for cyclin D1 and $p27^{Kip1}$ expression are shown. Magnification, x100. (A) Cyclin D1-positive staining. (B) Cyclin D1-negative staining. (C) $p27^{Kip1}$-positive staining. (D) $p27^{Kip1}$-negative staining.

Correlation between G1-S phase-regulatory protein expression and patient outcome in advanced serous epithelial ovarian cancer. The relationship between gene expression and patient prognosis was assessed. Upon univariate analysis, the clinicopathological determinants of reduced OS included age and volume of residual disease >2 cm (Table IV). A molecular marker predictive of reduced OS upon univariate analysis was overexpression of cyclin D1 (p=0.00037, RR=0.28, 95% CI 0.044-0.40). Reduced expression of $p27^{Kip1}$ had a trend of association with a shorter OS (p=0.064, RR=1.88, 95% CI 0.97-4.21). Regarding PFS, overexpression of cyclin D1 (p=0.00063, RR=0.34, 95% CI 0.054-0.43) was significantly correlated with reduced PFS, but reduced expression of $p27^{Kip1}$ had no statistically significant correlation with PFS. The CA125 level, volume of intraoperative ascites, pRb, p16, p53, $p21^{Waf1/Cip1}$ and cyclin E expression exhibited no statistically significant correlation with either OS or PFS. Kaplan-Meier curves and log-rank p-values according to cyclin D1 expression, $p27^{Kip1}$ expression and residual tumor volume are shown in Fig. 2.

In the multivariate analysis using the Cox proportional hazards model, overexpression of cyclin D1 was identified

Table III. Association of gene expression and clinicopathological parameters in serous epithelial ovarian cancer.

	Cyclin D1	pRb	$p16^{Ink4a}$	p53	$p27^{Kip1}$	$p21^{Waf/Cip1}$	Cyclin E
Residual disease	0.15	0.99	0.14	0.7300	0.097	0.17	0.91
Ascites	0.82	0.10	0.12	0.3000	0.310	0.81	0.82
Cyclin D1		0.43	0.30	0.6600	0.820	0.19	0.77
pRb			0.45	0.0820	0.420	0.95	0.14
$p16^{Ink4a}$				**0.0049**	0.190	0.34	0.73
p53					0.380	0.51	0.37
$p27^{Kip1}$						0.67	0.82
$p21^{Waf/Cip1}$							0.90

Significant p-values are indicated in boldface type.

Table IV. Univariate analysis for the association of clinicopathological parameters and gene expression with clinical outcome in serous epithelial ovarian cancer.

Parameters	PFS			OS		
	RR	95% CI	p-value	RR	95% CI	p-value
Age (≤65 vs. >65 years)	0.71	0.300-1.46	0.32000	0.42	0.100-0.87	**0.02900**
Residual disease (≤2 vs. >2 cm)	0.62	0.360-1.04	0.07800	0.27	0.150-0.60	**0.00087**
CA125 (≤500 vs. >500)	0.83	0.440-1.54	0.56000	1.10	0.470-2.65	0.81000
Cyclin D1 (≤20 vs. >20%)	0.34	0.054-0.43	**0.00063**	0.28	0.044-0.40	**0.00037**
pRb (≤50 vs. >50%)	1.00	0.580-1.73	0.99000	0.90	0.440-1.81	0.76000
$p16^{Ink4a}$ (≤50 vs. >50%)	1.13	0.650-2.00	0.65000	0.97	0.480-1.96	0.93000
p53 (≤40 vs. >40%)	1.43	0.850-2.59	0.17000	1.38	0.690-2.83	0.35000
$p27^{Kip1}$ (≤40 vs. >40%)	1.08	0.630-1.87	0.78000	1.88	0.970-4.21	0.06400
$p21^{Waf1/Cip1}$ (≤5 vs. >5%)	0.82	0.440-1.45	0.48000	1.48	0.710-3.00	0.31000
Cyclin E (≤70 vs. >70%)	0.97	0.460-2.04	0.94000	0.86	0.330-2.19	0.75000
Ascites (≤500 vs. >500 ml)	0.81	0.470-1.37	0.43000	0.63	0.320-1.28	0.21000

Data census was at 75 months. Significant p-values are indicated in boldface type. PFS, progression-free survival; OS, overall survival; RR, relative risk; CI, confidence interval.

as the key determinant of OS (p=0.0019, RR=3.61, 95% CI 1.61-8.12) and PFS (p=0.0052, RR=2.70, 95% CI 1.35-5.41) (Table V). The volume of residual disease and reduced expression of $p27^{Kip1}$ were found to be independent predictors of OS (p=0.0092 and p=0.042, respectively), but not of PFS when incorporated into a multivariate model (Table V).

Association between chemosensitivity and G1-S phase-regulatory protein expression. In order to assess whether the clinicopathological parameters reflect the chemosensitivity, the cohort was divided into two groups: patients who relapsed within 6 months after the last date of first-line chemotherapy; and patients who had no disease progression within 6 months after the last date of first-line chemotherapy. Using the Chi-square test, overexpression of cyclin D1 (p=0.011) as well as residual tumor volume >2 cm (p=0.006) were found to be significantly associated with TFI, suggesting that these parameters are correlated with first-line chemosensitivity (Table VI). In contrast, expression of pRb, p16, p53, $p27^{Kip1}$, $p21^{Waf1/Cip1}$ and cyclin E had no statistical correlation with chemosensitivity.

Discussion

Various studies exist concerning the association between G1-S phase-related genes and EOC prognosis, however, the results are conflicting. Amplification of cyclin E in high-resolution oligonucleotide microarrays was previously found to be associated with poor response to primary treatment in serous ovarian cancer (10), but in the present study, cyclin E expression in immunohistochemical analysis revealed no significant correlation with patient outcome of advanced serous EOC. It is considered that the variety of histological types of EOC, different tumor stages, tumor heterogeneity, racial backgrounds of patients, research methodologies and sample sizes may contribute to inconsistent results. In this study, we focused on advanced serous cases (limited to stage

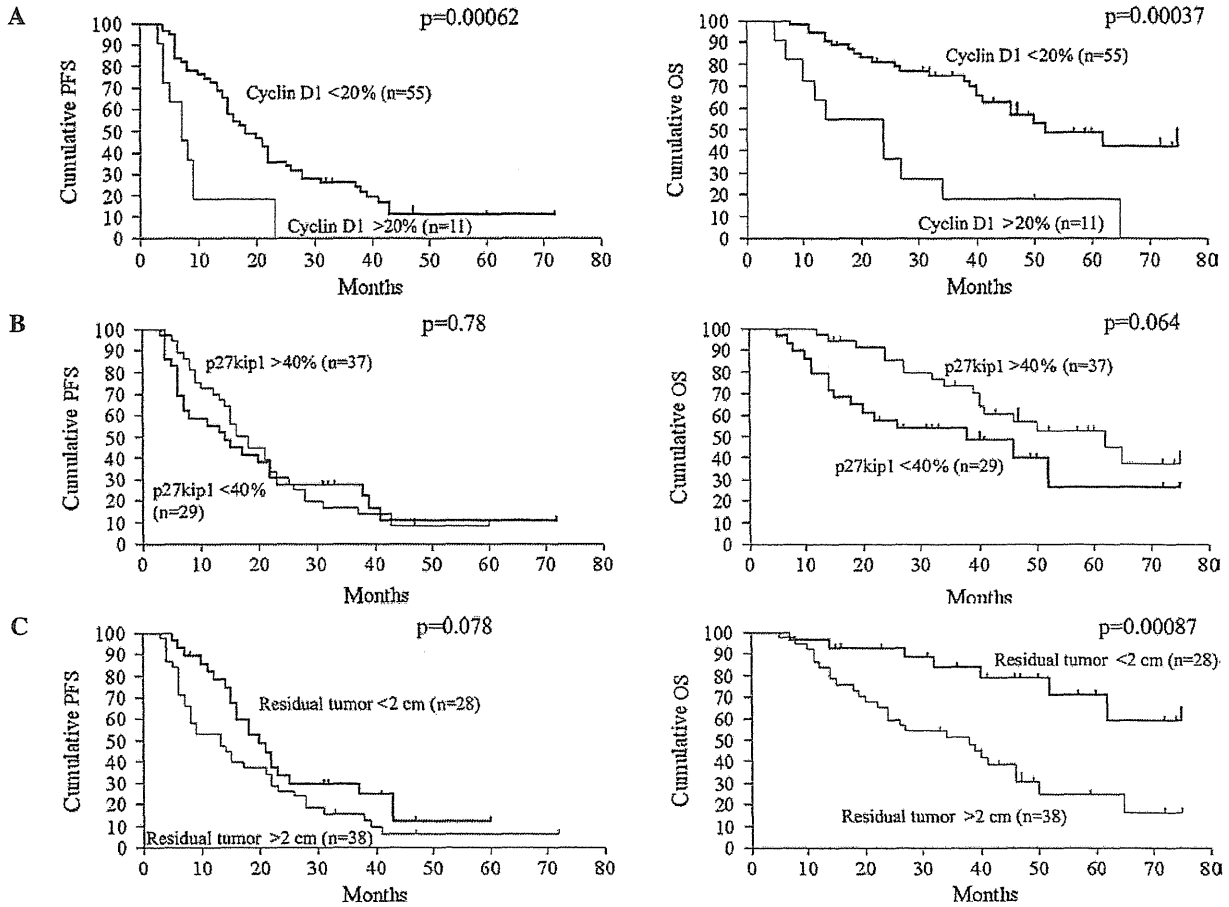

Figure 2. Kaplan-Meier curve. Kaplan-Meier curves and log-rank p-values for 5-year PFS and OS in the context of cyclin D1 expression (A), $p27^{Kip1}$ expression (B) and residual tumor volume (C) are shown. PFS, progression-free survival; OS, overall survival.

Table V. Multivariate Cox regression analysis of PFS and OS of patients with serous EOC.

Clinical parameter	PFS			OS		
	RR	95% CI	p-value	RR	95% CI	p-value
Residual disease (≤2 vs. >2 cm)	1.53	0.89-2.64	0.1200	3.06	1.32-7.12	**0.0093**
Cyclin D1 (≤20 vs. >20%)	2.70	1.35-5.41	**0.0052**	3.61	1.61-8.12	**0.0019**
$p27^{Kip1}$ (≤40 vs. >40%)	1.01	0.59-1.72	0.9700	2.15	1.03-4.51	**0.0420**

Significant p-values are indicated in boldface type. EOC, epithelial ovarian cancer; PFS, progression-free survival; OS, overall survival; RR, relative risk; CI, confidence interval.

III/IV cases) at a single institution with similar surgical and chemotherapeutic procedures administered in order to eliminate such bias.

Cyclin D1, a regulatory kinase subunit that is selectively associated with cyclin-dependent kinase 4 (CDK4), is a crucial modulator of G1 progression in the cell cycle (16). In our analysis, overexpression of cyclin D1 was detected in 11% of the cases (Table II). The overexpression of cyclin D1 was previously observed in 14-89% of EOC cases (11,17-19), but the underlying mechanism has yet to be elucidated. Amplification of cyclin D1 in ovarian tumors occurs infrequently (20). Furthermore, the mostly small cyclin D1 copy gains are not associated with an increase in detectable cyclin D1 protein by immunohistochemistry (21). These findings suggest that the post-transcriptional regulation of cyclin D1 protein production is complex. Recently, Jiang *et al* performed a systematic validation of the predicted microRNAs for cyclin D1 and revealed that microRNAs suppressed the endogenous cyclin D1 protein and mRNA levels *in vitro* (22). microRNAs may aid in determining the mechanism of cyclin D1 expression.

Barbieri *et al* reported that cyclin D1 overexpression significantly influenced the clinical outcome in advanced EOC

Table VI. Association of the TFI and clinicopathological parameters in serous EOC.

	TFI <6 months, n (%)	TFI ≥6 months, n (%)	p-value
Residual disease			
≤2 cm (n=28)	4 (6.0)	24 (36.4)	**0.006**
>2 cm (n=38)	19 (28.8)	19 (28.8)	
Cyclin D1			
≤20% (n=55)	15 (22.7)	40 (60.6)	**0.011**
>20% (n=11)	8 (12.1)	3 (4.6)	
pRB			
≤50% (n=40)	12 (18.2)	28 (42.4)	0.305
>50% (n=26)	11 (16.7)	15 (22.7)	
$p16^{Ink4a}$			
≤50% (n=24)	7 (10.6)	17 (25.8)	0.464
>50% (n=42)	16 (24.3)	26 (39.4)	
p53			
≤40% (n=29)	11 (16.7)	18 (27.3)	0.642
>40% (n=37)	12 (18.2)	25 (37.9)	
$p27^{Kip1}$			
≤40% (n=29)	13 (19.7)	16 (24.3)	0.132
>40% (n=37)	10 (15.2)	27 (40.9)	
$p21^{Waf/Cip1}$			
≤5% (n=46)	19 (28.8)	27 (40.9)	0.165
>5% (n=20)	4 (6.0)	16 (24.3)	
Cyclin E			
≤70% (n=55)	18 (27.3)	37 (56.1)	0.644
>70% (n=11)	5 (7.6)	6 (9.1)	

p-values are for TFI <6 months vs. TFI ≥6 months (Chi-square test, Yates correlation). Significant p-values are indicated in boldface type. EOC, epithelial ovarian cancer; TFI, treatment-free interval.

cases with residual disease greater than 2 cm. They identified cyclin D1 overexpression as an independent prognostic factor in multivariate analysis (5). Similarly, Bali *et al* identified cyclin D1 overexpression as an independent prognostic factor in the multivariate analysis of 134 serous EOC cases (11). In our study, overexpression of cyclin D1 was significantly correlated with reduced OS and PFS in both univariate and multivariate analyses, suggesting that overexpression of cyclin D1 actually contributes to the prognosis of advanced serous EOCs; therefore, its application to clinical practice is expected.

We found that both overexpression of cyclin D1 and residual tumor volume were significantly associated with TFI, suggesting that these parameters are correlated with first-line chemosensitivity (Table VI). Zhou *et al* showed that inhibition of cyclin D1 expression by siRNA in oral squamous cell carcinoma cells resulted in a decrease in cisplatin IC_{50} level. *In vivo* transplantation models also confirmed a cisplatin-sensitizing effect of cyclin D1 knockdown in these cell lines (23). In addition, it was reported that overexpression of cyclin D1 was associated with reduced chemosensitivity and a higher survival rate upon cisplatin administration in a pancreatic cancer model (24). Moreover, inhibition of cyclin D1 expression rendered cells more susceptible to cisplatin-mediated apoptosis in the same model (24). Taken together, these findings indicate that cyclin D1 expression may contribute to chemoresistance in a number of cancers, although further investigation is required. Therefore, we speculate that overexpression of cyclin D1 contributes to poor prognosis, which may in part be mediated by chemoresistance in ovarian cancer.

$p27^{Kip1}$ is a cyclin-dependent kinase (cdk) inhibitor that regulates cell cycle progression from the G1 to S-phase. In non-cycling cells, $p27^{Kip1}$ binds to cyclin E-cdk2 complexes and inhibits their activation. In contrast, $p27^{Kip1}$ binding to catalytically active cyclin D-cdk4/6 complexes results in $p27^{Kip1}$ degradation and the subsequent release of cdk2 from inhibition in proliferating cells (12,25). Therefore, $p27^{Kip1}$ helps to coordinate a balance between proliferation and arrest (12,25). In the present study, reduced expression of $p27^{Kip1}$ was detected in 43.9% of the cases. In previous immunohistochemical studies of ovarian tumors, 36.2-100% exibited low expression of $p27^{Kip1}$ (26). We found that reduced expression of $p27^{Kip1}$ was associated with shorter OS (p=0.064), but had no statistically significant correlation with PFS (p=0.78). When incorporated into a multivariate model, reduced expression of $p27^{Kip1}$ was found to be an independent predictor of OS (p=0.042) (Table V). The relationship between $p27^{Kip1}$ expression levels and prognosis is controversial. Conflicting data regarding the possible prognostic role of $p27^{Kip1}$ status also exist for ovarian cancer. Psyrri *et al* evaluated subcellular

localization and protein levels of $p27^{Kip1}$ in 150 advanced EOCs and found that low nuclear $p27^{Kip1}$ expression was associated with improved prognosis, suggesting its potential as a strong predictor of outcome in advanced EOCs (12). On the other hand, Shigemasa *et al* reported that negative $p27^{Kip1}$ expression was significantly correlated with poor survival in serous EOC patients, suggesting that the underexpression of $p27^{Kip1}$ caused by a post-translational mechanism may contribute to development and progression and may result in poor prognosis of serous EOCs (27). It is hypothesized that different methodologies of immunohistochemical grading may account for these discrepancies. Our result suggests that $p27^{Kip1}$ is associated with the prognosis of this disease, as previously reported.

In conclusion, overexpression of cyclin D1 contributed markedly to poor prognosis in advanced serous EOC; this may in part be mediated by chemoresistance. Cyclin D1 may be a target for overcoming the refractory nature of advanced serous EOC.

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References

1. Takakura S, Takano M, Takahashi F, Saito T, Aoki D, Inaba N, Noda K, Sugiyama T, Ochiai K; Japanese Gynecologic Oncology Group: Randomized phase II trial of paclitaxel plus carboplatin therapy versus irinotecan plus cisplatin therapy as first-line chemotherapy for clear cell adenocarcinoma of the ovary: a JGOG study. Int J Gynecol Cancer 20: 240-247, 2010.
2. Shimada M, Kigawa J, Ohishi Y, Yasuda M, Suzuki M, Hiura M, Nishimura R, Tabata T, Sugiyama T and Kaku T: Clinicopathological characteristics of mucinous adenocarcinoma of the ovary. Gynecol Oncol 113: 331-334, 2009.
3. Katsumata N, Yasuda M, Takahashi F, Isonishi S, Jobo T, Aoki D, Tsuda H, Sugiyama T, Kodama S, Kimura E, Ochiai K, Noda K; Japanese Gynecologic Oncology Group: Dose-dense paclitaxel once a week in combination with carboplatin every 3 weeks for advanced ovarian cancer: a phase 3, open-label, randomised controlled trial. Lancet 374: 1331-1338, 2009.
4. Armstrong DK, Bundy B, Wenzel L, Huang HQ, Baergen R, Lele S, Copeland LJ, Walker JL and Burger RA: Intraperitoneal cisplatin and paclitaxel in ovarian cancer. N Engl J Med 354: 34-43, 2006.
5. Barbieri F, Lorenzi P, Ragni N, Schettini G, Bruzzo C, Pedullà F and Alama A: Overexpression of cyclin D1 is associated with poor survival in epithelial ovarian cancer. Oncology 66: 310-315, 2004.
6. NIH Consensus Conference: Ovarian cancer. Screening, treatment, and follow-up. JAMA 273: 491-497, 1995.
7. Terauchi F, Nishi H, Moritake T, *et al*: Prognostic factor on optimal debulking surgery by maximum effort for stage IIIC epithelial ovarian cancer. J Obstet Gynaecol Res 35: 315-319, 2009.
8. Du Bois A, Reuss A, Pujade-Lauraine E, Harter P, Ray-Coquard I and Pfisterer J: Role of surgical outcome as prognostic factor in advanced epithelial ovarian cancer: a combined exploratory analysis of 3 prospectively randomized phase 3 multicenter trials: by the Arbeitsgemeinschaft Gynaekologische Onkologie Studiengruppe Ovarialkarzinom (AGO-OVAR) and the Groupe d'Investigateurs Nationaux Pour les Etudes des Cancers de l'Ovaire (GINECO). Cancer 115: 1234-1244, 2009.
9. Chi DS, Eisenhauer EL, Zivanovic O, *et al*: Improved progression-free and overall survival in advanced ovarian cancer as a result of a change in surgical paradigm. Gynecol Oncol 114: 26-31, 2009.
10. Etemadmoghadam D, deFazio A, Beroukhim R, Mermel C, George J, Getz G, Tothill R, Okamoto A, Raeder MB, Harnett P, Lade S, Akslen LA, Tinker AV, Locandro B, Alsop K, Chiew YE, Traficante N, Fereday S, Johnson D, Fox S, Sellers W, Urashima M, Salvesen HB, Meyerson M, Bowtell D; AOCS Study Group: Integrated copy number and expression analysis of chemoresistant ovarian carcinomas. Clin Cancer Res 15: 1417-1427, 2009.
11. Bali A, O'Brien PM, Edwards LS, Sutherland RL, Hacker NF and Henshall SM: Cyclin D1, p53, and $p21^{Waf1/Cip1}$ expression is predictive of poor clinical outcome in serous epithelial ovarian cancer. Clin Cancer Res 10: 5168-5177, 2004.
12. Psyrri A, Bamias A, Yu Z, *et al*: Subcellular localization and protein levels of cyclin-dependent kinase inhibitor p27 independently predict for survival in epithelial ovarian cancer. Clin Cancer Res 11: 8384-8390, 2005.
13. Khouja MH, Baekelandt M, Nesland JM and Holm R: The clinical importance of Ki-67, p16, p14, and p57 expression in patients with advanced ovarian carcinoma. Int J Gynecol Pathol 26: 418-425, 2007.
14. Milde-Langosch K and Riethdorf S: Role of cell-cycle regulatory proteins in gynecological cancer. J Cell Physiol 196: 224-244, 2003.
15. Kommoss S, du Bois A, Ridder R, Trunk MJ, Schmidt D, Pfisterer J, Kommoss F; AGO-OVAR: Independent prognostic significance of cell cycle regulator proteins p16(INK4a) and pRb in advanced-stage ovarian carcinoma including optimally debulked patients: a translational research subprotocol of a randomised study of the Arbeitsgemeinschaft Gynaekologische Onkologie Ovarian Cancer Study Group. Br J Cancer 96: 306-313, 2007.
16. Sherr CJ: G1 phase progression: cycling on cue. Cell 79: 551-555, 1994.
17. Dhar KK, Branigan K, Parkes J, *et al*: Expression and subcellular localization of cyclin D1 protein in epithelial ovarian tumour cells. Br J Cancer 81: 1174-1181, 1999.
18. Worsley SD, Ponder BA and Davies BR: Overexpression of cyclin D1 in epithelial ovarian cancers. Gynecol Oncol 64: 189-195, 1997.
19. Barbieri F, Cagnoli M, Ragni N, Foglia G, Bruzzo C, Pedullà F and Alama A: Increased cyclin D1 expression is associated with features of malignancy and disease recurrence in ovarian tumors. Clin Cancer Res 5: 1837-1842, 1999.
20. Masciullo V, Scambia G, Marone M, *et al*: Altered expression of cyclin D1 and CDK4 genes in ovarian carcinomas. Int J Cancer 74: 390-395, 1997.
21. Diebold J, Mösinger K, Peiro G, *et al*: 20q13 and cyclin D1 in ovarian carcinomas. Analysis by fluorescence in situ hybridization. J Pathol 190: 564-571, 2000.
22. Jiang Q, Feng MG and Mo YY: Systematic validation of predicted microRNAs for cyclin D1. BMC Cancer 9: 194, 2009.
23. Zhou X, Zhang Z, Yang X, Chen W and Zhang P: Inhibition of cyclin D1 expression by cyclin D1 shRNAs in human oral squamous cell carcinoma cells is associated with increased cisplatin chemosensitivity. Int J Cancer 124: 483-489, 2009.
24. Biliran H Jr, Wang Y, Banerjee S, *et al*: Overexpression of cyclin D1 promotes tumor cell growth and confers resistance to cisplatin-mediated apoptosis in an elastase-myc transgene-expressing pancreatic tumor cell line. Clin Cancer Res 11: 6075-6086, 2005.
25. Blain SW, Scher HI, Cordon-Cardo C and Koff A: p27 as a target for cancer therapeutics. Cancer Cell 3: 111-115, 2003.
26. Nam EJ and Kim YT: Alteration of cell-cycle regulation in epithelial ovarian cancer. Int J Gynecol Cancer 18: 1169-1182, 2008.
27. Shigemasa K, Shiroyama Y, Sawasaki T, *et al*: Underexpression of cyclin-dependent kinase inhibitor p27 is associated with poor prognosis in serous ovarian carcinomas. Int J Oncol 18: 953-958, 2001.

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Research Article Open Access

Comparison of Two Sampling Procedures for Diagnosing Endometrial Carcinoma and Hyperplasia: Outpatient Tissue Biopsy Versus Cytologic Examination

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Abstract

Background: We compared the sensitivity of 2 diagnostic procedures—tissue biopsy and cytologic examination—for detecting endometrial carcinoma and hyperplasia in outpatients. The patients' degree of acceptance of these methods was also evaluated.

Methods: The study included 124 women who had been diagnosed with carcinoma and hyperplasia by histological examination in private clinics or were suspected to have endometrial carcinoma and hyperplasia—for example, those presenting with uterine bleeding and/or abnormal endometrial morphology on cytologic examination—at Jikei University Hospital, University of Yamanashi Hospital and National Hospital Organization Kure Medical Center from January 28, 1999, to August 28, 2006. Both cytologic examination (using Endocyte®) and tissue biopsy (using Suresample™) of the endometrium were performed before complete curettage and/or hysterectomy. The diagnosis made using these two outpatient procedures was compared to the final diagnosis made using curettage and/or hysterectomy. McNemar's chi-square test was used to evaluate the statistical significance.

Results: The sensitivity of tissue biopsy for detecting endometrial carcinoma and hyperplasia was 84% and 91%, respectively, and that of cytologic examination was 78% and 55%, respectively. There was a significant difference in the sensitivity of the 2 methods for detecting hyperplasia ($p = 0.045$). No patients complained of severe pain, and no other complication occurred during both methods. Both methods were well tolerated by the patients.

Conclusion: Our data indicate a certain diagnostic superiority of tissue biopsy over cytologic examination.

Keywords: Endocyte®; Suresample™; Endometrial carcinoma; Hyperplasia; Diagnostic procedure

Introduction

Each year, there are about 142,000 new cases of endometrial carcinoma worldwide, and an estimated 42,000 women die because of this type of cancer [1]. The surgical stage, determined according to the criteria of the International Federation of Gynecology and Obstetrics, reflects the 5-year survival, which is around 85% for stage I, 75% for stage II, 45% for stage III, and 25% for stage IV disease [1]. Endometrial cancer is often preceded by endometrial hyperplasia, which is a spectrum of morphologic and biologic alterations of the endometrial glands and stroma and is often secondary to hyperestrogenism. It has been shown that progression to carcinoma occurs in 1% of patients with simple hyperplasia, 3% of patients with complex hyperplasia, 8% of patients with simple hyperplasia with atypia, and 29% of patients with complex hyperplasia with atypia [2].

The Japanese Ministry of Health and Welfare investigated the effectiveness of mass endometrial carcinoma screening. During the 9-year study, 126 cases were detected by mass screening and 1,069 cases were diagnosed in outpatient clinics. Early-stage cases were significantly more frequent in the screening group ($p < 0.001$): 88.1% of the patients in the screening group had stage I disease, as compared to 65.3% of the patients in the outpatient group. The 5-year survival rate was also significantly higher in the screening group than in the outpatient group (94.7% vs 84.3%; $p = 0.041$) [3]. These statistics suggest that early detection of endometrial carcinoma and hyperplasia is necessary to improve the prognosis of these diseases.

Outpatient endometrial sampling is now replacing complete curettage as the method of choice for diagnosing endometrial disease. This procedure is easy to perform, associated with minimal patient discomfort, and reported to be highly sensitive in detecting endometrial carcinoma [4-14]. The Pipelle de Cornier® device (Laboratoire CCD, Paris, France) is an endometrial biopsy sampler that is seemingly better tolerated by patients than most other endometrial biopsy devices [15,16]. However, we cannot use this device because it is not available in Japan. Instead, we collect endometrial tissue by using the Suresample™ (Smith Medical International Ltd., Kent, UK) endometrial sampler, which is similar to the Pipelle® device. This endometrial sampler has an aperture not only on the side near the distal tip, similar to the Pipelle® device, but also at the distal tip and is expected to collect a larger sample. However, in Japan, cytologic examination is often used initially to detect endometrial carcinoma and its precursor stages, as stipulated by a 1987 health insurance law for the elderly. During this cytologic

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examination, endometrial cells are collected using the Endocyte® sampler (Laboratoire CCD, Paris, France). The cell processing technique is similar to that used for a cervical cytology smear and is thus relatively inexpensive.

A few studies have compared the 2 above-mentioned sampling procedures. However, in these studies, the diagnostic sensitivity was not sufficiently evaluated because of the small number of carcinoma and hyperplasia cases used for the investigation [13,17]. In the present study, in order to determine the optimal technique for detection of endometrial carcinoma and hyperplasia, we compared the diagnostic sensitivity of cytologic examination using Endocyte® and tissue biopsy using Suresample™; we also estimated the degree of patient acceptance for both these procedures.

Materials and Methods

This study included 124 patients who had been diagnosed with carcinoma and hyperplasia by histological examination in private clinics or were suspected of having carcinoma and hyperplasia—for example, those presenting with uterine bleeding and/or abnormal endometrial morphology on cytologic examination—at Jikei University Hospital, University of Yamanashi Hospital and National Hospital Organization Kure Medical Center from January 28, 1999, to August 28, 2006. Patients with complications such as pregnancy, acute pelvic infection, infection of the uterine cervix, and coagulation disorder were excluded. Both cytologic examination and tissue biopsy were performed for all the patients; the former was performed before the latter. This study was approved by the hospital ethics committees, and informed consent was obtained from all the patients.

Cytologic materials were obtained using Endocyte®. The Endocyte® sampler is composed of flexible plastic, is presterilized, and measures 21 cm in length; its greatest external diameter is 2.6 mm. Along its length are graduation marks that guide the operator in introducing the device into the endometrial cavity, as described by Byrne [8]. The collected cellular components were placed on a glass slide, crushed, and smeared using the regular pull-apart method. After fixation in 95% alcohol, Papanicolaou staining was performed. The cytologic findings were divided into different classes on the basis of structural abnormalities, such as papillary clusters, type A stroma, arborescent clusters, and back-to-back structures (Table 1) [18,19]. Outpatient tissue biopsy was performed using Suresample™. Suresample™ is a flexible, clear polypropylene suction curette containing an internal piston and measures 24 cm in length and 3.1 mm in external diameter. It has a round aperture with a diameter of 1.5 mm at the distal tip of its sheath and 2 oval apertures each measuring 5.9 × 1.5 mm at 3.2mm from the distal tip. In order to obtain a specimen, the device is inserted into the uterine cavity and negative pressure is then created within the sheath by withdrawing the piston. The device is rotated while also being moved back and forth several times within the uterine cavity. Suresample™ is then withdrawn, and the tissue sample is ejected into 10% buffered formalin by using the piston. The entire sample is histologically examined.

After collecting the sample for both procedures, the patient was asked to comment on the intensity of any pain experienced during the procedure. Pain or discomfort was subjectively graded as mild, moderate, or severe. Thereafter, in 93 patients, complete curettage and/or hysterectomy was performed. In the remaining 31 patients, these procedures were not performed because of the attending physician's decision or the patient's refusal. The final diagnosis was made on the basis of the histological findings of the samples obtained during complete curettage and/or hysterectomy. The diagnosis made using both outpatient procedures was then compared with the final diagnosis.

We estimated sensitivity for detecting endometrial carcinoma, sensitivity for detecting endometrial hyperplasia, and specificity of each procedure separately and reported them with 95% confidence intervals. Patients who were not diagnosed histologically were excluded from this analysis. McNemar's chi-square test was used to compare each measure of diagnostic accuracy. All reported p values for statistical tests are two-tailed, and $p < 0.05$ was taken to indicate statistical significance. Data management and statistical analysis were conducted at an independent academic data center, Translational Research Center, Kyoto University Hospital, using SAS version 9.2 (SAS Institute, Cary, NC).

Results

The median age of the patients was 54 years (range: 23-85 years). Of the 124 patients, 68 (55%) were postmenopausal, and 88 (71%) showed abnormal uterine bleeding.

Of the 93 patients who underwent complete curettage and/or hysterectomy, 69 were finally diagnosed with endometrial carcinoma, 11 with endometrial hyperplasia, 6 with other tumor, and 7 with normal endometrium. Of the 69 patients with endometrial carcinoma, 50 had endometrioid adenocarcinoma; 12, adenoacanthoma; 2, serous papillary adenocarcinoma; 2, clear cell adenocarcinoma; 1, mucinous adenocarcinoma; and 2, mixed carcinoma. Of the 50 endometrioid adenocarcinoma tumors, 33 were well differentiated, 12 were moderately differentiated, and 5 were poorly differentiated. Of the 11 patients with endometrial hyperplasia, 5 had complex hyperplasia with atypia, 3 had complex hyperplasia without atypia, and 3 had simple hyperplasia without atypia.

Of the 69 patients with endometrial carcinoma, cytological examination using Endocyte® revealed carcinoma (class V) in 54 patients, hyperplasia (class III or IV) in 7, and a normal endometrium (class II) in 5; in 3 patients, adequate samples could not be obtained.

Class	Findings
I	No abnormal findings.
II	Inflammatory findings or reactive changes because of an intrauterine device(IUD).
IIb	Papillary clusters with few structural abnormalities. Complex hyperplasia not fully suspected but follow-up necessary.
III	Papillary clusters accompanied by structural abnormalities. Complex hyperplasia suspected.
IV	Small number of arborescent clusters. Complex hyperplasia with atypia or worse suspected.
V	Clear glandular cavity with back-to-back structures and arborescent clusters. Endometrial cancer diagnosed.

Table 1: Different classes of cytologic findings of the endometrium.

	Final histological diagnosis					
Cytology (class)	Normal endometrium	EH (Atypical)	EMCA	Other tumor	Not performed	Total
I	3	0	0	0	6	9
II	4	5 (1)	5	1	13	28
III	0	5 (3)	6	1	4	16
IV	0	1 (1)	1	0	3	5
V	0	0	54	4	2	60
Inadequate	0	0	3	0	3	6
Total	7	11 (5)	69	6	31	124

EH: endometrial hyperplasia; EMCA: endometrial carcinoma

Table 2: Comparison between cytologic examination and the final histological study.

	Final histological diagnosis					
Biopsy	Normal endometrium	EH (Atypical)	EMCA	Other tumor	Not performed	Total
Normal endometrium	6	0	0	3	17	26
EH	1	10 (5)	6	0	4	21
EMCA	0	0	58	1	1	60
Other tumor	0	0	0	1	2	3
Inadequate	0	1	5	1	7	14
Total	7	11 (5)	69	6	31	124

EH: endometrial hyperplasia; EMCA: endometrial carcinoma

Table 3: Comparison between outpatient biopsy and the final histological study.

	Cytologic examination (95% Confidence interval) (%)		Tissue biopsy (95% Confidence interval) (%)		p*[1]
Sensitivity for detecting endometrial carcinoma	78.3	(66.7–87.3)	84.1	(73.3–91.8)	0.157
Sensitivity for detecting endometrial hyperplasia	54.5	(23.4–83.3)	90.9	(58.7–99.8)	0.045
Specificity	100.0	(59.0–100.0)	85.7	(42.1–99.6)	*[2]

*[1]McNemar's chi-square test
*[2]Not calculable due to specificity of 100%

Table 4: Comparison of diagnostic accuracy between cytologic examination and tissue biopsy.

Tissue biopsy using Suresample™ identified 58 cases of endometrial carcinoma, while 6 were misdiagnosed as endometrial hyperplasia. In 5 patients, adequate samples could not be obtained. Of the 11 patients with endometrial hyperplasia, cytologic examination using Endocyte® revealed hyperplasia (class III or IV) in 6 patients and a normal endometrium (class II) in 5. Tissue biopsy using Suresample™ helped to identify 10 cases of hyperplasia; in 1 patient, an adequate sample could not be obtained (Table 2 and Table 3). The sensitivity of Endocyte® and Suresample™ for detecting endometrial carcinoma was 78% and 84%, whereas the sensitivity for detecting endometrial hyperplasia was 55% and 91%, respectively. The specificity of Endocyte® and Suresample™ for detecting endometrial disease was 100% and 86%, respectively. These data suggest that as compared to cytologic examination using Endocyte®, outpatient endometrial tissue biopsy using Suresample™ has a significantly higher sensitivity for detecting endometrial hyperplasia ($p = 0.045$; Table 4).

Pain was reported to be nil by 42 (34%) and 49 (40%) patients, mild by 69 (56%) and 67 (54%) patients, and moderate by 13 (10%) and 8 (6%) patients during the insertion of Endocyte® and Suresample™, respectively. None of the patients complained of severe pain. Pain was reported to be nil by 30 (24%) and 42 (34%) patients, mild by 77 (62%) and 70 (56%) patients, and moderate by 17 (14%) and 12 (10%) patients during the collection of samples using Endocyte® and Suresample™, respectively. No patient complained of severe pain. In all patients, bloody discharge from the cervix after cell collection was either absent or minimal.

Discussion

To the best of our knowledge, this is the first study to investigate the diagnostic accuracy of outpatient endometrial tissue biopsy using Suresample™. However, there are several studies on the use of the Pipelle® device, which is similar to Suresample™ [4-7,13,15,16]. A meta-analysis revealed that the Pipelle® device has a sensitivity of 99.6% and 91% in postmenopausal and premenopausal patients, respectively [5]. Another review showed that the sensitivity of the Pipelle® device varies between 86% and 100% [4]. We found the sensitivity of Suresample™ to be 84%, which is lower than that of the Pipelle® device, as mentioned above. In the present study, the inadequate sample (no specimen obtained or insufficient specimen for adequate assessment for histological or cytological diagnosis) at the outpatient examination was regarded as 'negative' for the calculation of the sensitivity, in contrast to some previous studies where inadequate diagnoses were excluded from the calculations [4,8,10-12]. Had we calculated sensitivity by excluding inadequate samples, the sensitivity of Suresample™ for detecting endometrial carcinoma would be 91%, which is similar to the values reported in previous studies. Moreover, no patient with carcinoma was falsely diagnosed as having a normal endometrium by outpatient tissue biopsy. Other reports have showed that the sensitivity of cytologic examination for diagnosing endometrial carcinoma is 74.1–100% [8-14]. One study evaluated and compared the accuracy of sampling using Endopap® and Pipelle® for diagnosing postmenopausal disease. The sensitivity of Endopap® and Pipelle® for detecting endometrial disease was 56% and 51% and the specificity was 94% and 100%, respectively. The sensitivity for endometrial carcinoma was 80% for Endopap® and 100% for Pipelle®. The authors therefore favored Pipelle® for diagnosing endometrial disease in symptomatic postmenopausal women [13]. In the present study too, the sensitivity of cytologic examination for detecting carcinoma tended to be lower than that of outpatient tissue biopsy. Furthermore, 5 patients with carcinoma were falsely diagnosed as having a normal endometrium by cytologic examination. Such false negatives pose a grave risk for patients when screened for endometrial carcinoma.

Outpatient endometrial sampling also aims to detect endometrial hyperplasia, because of the supposed role of the latter as a precursor of endometrial carcinoma. However, detecting endometrial hyperplasia in the smears of endometrial samples is very difficult. Therefore, the diagnostic rate is not always high because of the lack of cellular atypia. In fact, it has been reported that hyperplasia can be detected in only 32.3–80.5% of cases [8-12,14]. A meta-analysis shows that the sensitivity of the Pipelle® device in detecting atypical hyperplasia is 81% [5]. In the present study, the sensitivity of cytologic examination for detecting hyperplasia was 55%, whereas that of outpatient tissue biopsy was 91%. Thus, the difference between the sensitivity of these 2 methods is significant ($p = 0.045$).

At the time of sampling, adverse effects such as severe pain and bloody discharge decrease the patient's acceptance of the collection method. The adverse effects of the Pipelle® device, used without anesthesia, have been evaluated by surveying 40 patients. Although 2 patients (5%) complained of severe pain, none of the biopsy attempts were prematurely terminated as a result of pain and no complications related to endometrial sampling occurred [6]. The incidence and intensity of pain during and after a cytologic procedure using Endocyte® have also been examined. The present pain intensity index developed by Melzack assesses the overall discomfort or pain experienced on a scale of 0-5. Pain was reported as 0 (no pain) by 60% patients, as 1 (mild) by 30%, and as 2 (discomfort) by 10% [17,20]. In the present study, the intensity of pain tend to be stronger during cytologic examination using Endocyte® than tissue biopsy using Suresample™, despite the larger diameter of the Suresample™ probe. As the cytologic examination performed before the tissue biopsy made the insertion of the Suresample™ probe easier, we cannot provide definitive conclusions on the superiority of Suresample™ with regard to patient acceptance. However, during both procedures, none of the patients complained of severe pain and no complications occurred. This suggests that both outpatient sampling procedures were well tolerated, which is a finding consistent with those of previous reports [6,17,20].

Our data indicated a certain diagnostic superiority of outpatient tissue biopsy to cytologic examination. Because of the small number of patients with a normal endometrium, we could not sufficiently evaluate the specificity for detecting endometrial disease. Furthermore, this study did not compare the two methods in terms of cost effectiveness. Therefore, cytologic examination for detecting endometrial carcinoma and hyperplasia cannot be completely disregarded. Yet, our data suggest that the use of tissue biopsy in an endometrial carcinoma screening program might improve the detection rate of endometrial carcinoma and hyperplasia. For example, in the cases of patients with normal endometrial morphology on cytologic examination, who show strongly suspicious symptoms such as abnormal endometrium thickness on the ultrasonography and/or continuous genital bleeding, reexamination using tissue biopsy should be considered. Diagnostic superiority of outpatient tissue biopsy to cytologic examination is most likely because cytologic examination cannot provide the architectural detail. Recently, liquid-based cytology and cell block preparation were reported as the methods that had more excellent architectural preservation than conventional cytologic examination. Several reports suggest that those methods are useful for diagnosing endometrial disease [21-23]. Further studies focusing on the effectiveness of various methods including liquid-based cytology and cell block preparation are necessary.

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References

1. Amant F, Moerman P, Neven P, Timmerman D, Van Limbergen E, et al. (2005) Endometrial cancer. Lancet 366: 491-505.
2. Kurman RJ, Kaminski PF, Norris HJ (1985) The behavior of endometrial hyperplasia. A long-term study of "untreated" hyperplasia in 170 patients. Cancer 56: 403-412.
3. Nakagawa-Okamura C, Sato S, Tsuji I, Kuramoto H, Tsubono Y, et al. (2002) Effectiveness of mass screening for endometrial cancer. Acta Cytol 46: 277-283.
4. Clark TJ, Mann CH, Shah N, Khan KS, Song F, et al. (2002) Accuracy of outpatient endometrial biopsy in the diagnosis of endometrial cancer: a systematic quantitative review. BJOG 109: 313-321.
5. Dijkhuizen FP, Mol BW, Brölmann HA, Heintz AP (2000) The accuracy of endometrial sampling in the diagnosis of patients with endometrial carcinoma and hyperplasia: a meta-analysis. Cancer 89: 1765-1772.
6. Stovall TG, Photopulos GJ, Poston WM, Ling FW, Sandles LG (1991) Pipelle endometrial sampling in patients with known endometrial carcinoma. Obstet Gynecol 77: 954-956.
7. Huang GS, Gebb JS, Einstein MH, Shahabi S, Novetsky AP, et al. (2007) Accuracy of preoperative endometrial sampling for the detection of high-grade endometrial tumors. Am J Obstet Gynecol 196: 243.e1-5.
8. Byrne AJ (1990) Endocyte endometrial smears in the cytodiagnosis of endometrial carcinoma. Acta Cytol 34: 373-381.
9. Suprun HZ, Taendler-Stolero R, Schwartz J, Ettinger M (1994) Experience with Endopap endometrial sampling in the cytodiagnosis of endometrial carcinoma and its precursor lesions. I. A correlative cytologic-histologic-hysteroscopic diagnostic pilot study. Acta Cytol 38: 319-323.
10. Meisels A, Jolicoeur C (1985) Criteria for the cytologic assessment of hyperplasias in endometrial samples obtained by the Endopap endometrial sampler. Acta Cytol 29: 297-302.
11. LaPolla JP, Nicosia S, McCurdy C, Songster C, Ruffolo E, et al. (1990) Experience with the EndoPap device for the cytologic detection of uterine cancer and its precursors: acomparison of the EndoPap with fractional curettage or hysterectomy. Am J Obstet Gynecol 163: 1055-1059; discussion 1059-1060.
12. Palermo VG, Blythe JG, Kaufman RH (1985) Cytologic diagnosis of endometrial adenocarcinoma using the Endo-pap sampler. Obstet Gynecol 65: 271-275.
13. Van den Bosch T, Vandendael A, Wranz PA, Lombard CJ (1996) Endopap- versus Pipelle- sampling in the diagnosis of postmenopausal endometrial disease. Eur J Obstet Gynecol Reprod Biol 64: 91-94.
14. Tsuda H, Kawabata M, Yamamoto K, Inoue T, Umesaki N (1997) Prospective study to compare endometrial cytology and transvaginal ultrasonography for identification of endometrial malignancies. Gynecol Oncol 65: 383-386.
15. MacKenzie IZ (1992) Endometrial biopsy. Current Obstet Gynaecol 2:162-167.
16. Stovall TG, Ling FW, Morgan PL (1991) A prospective, randomized comparison of the Pipelle endometrial sampling device with the Novak curette. Am J Obstet Gynecol 165: 1287-1290.
17. Ferenczy A, Gelfand MM (1984) Outpatient endometrial sampling with Endocyte: comparative study of its effectiveness with endometrial biopsy. Obstet Gynecol 63: 295-302.
18. Oda M, Ishii Y, Omura M, Ishida N, Takeda T, et al (2000) Cytological findings of endometrial cancer. With emphasis on structural abnormalities. J Japan Soc Clin Cytol 39: 374-380. [in Japanese]
19. Ishii Y, Fujii M (1997) Criteria for differential diagnosis of complex hyperplasia or beyond in endometrial cytology. Acta Cytol 41: 1095-1102.
20. Melzack R (1975) The McGill Pain Questionnaire: major properties and scoring methods. Pain 1: 277-299.
21. Kipp BR, Medeiros F, Campion MB, Distad TJ, Peterson LM, et al. (2008) Direct uterine sampling with the Tao brush sampler using a liquid-based preparation method for the detection of endometrial cancer and atypical hyperplasia: a feasibility study. Cancer 114: 228-235.
22. Norimatsu Y, Kouda H, Kobayashi TK, Shimizu K, Yanoh K, et al. (2009) Utility of liquid-based cytology in endometrial pathology: diagnosis of endometrial carcinoma. Cytopathology 20: 395-402.
23. Kyroudi A, Paefthimiou M, Symiakaki H, Mentzelopoulou P, Voulgaris Z, et al. (2006) Increasing diagnostic accuracy with a cell block preparation from thin-layer endometrial cytology: a feasibility study. Acta Cytol 50: 63-69.

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Expression of the Wild Type Rearranged during Transfection Protooncogene in Ovarian Cancer

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ABSTRACT

Background : The rearranged during transfection (RET) protooncogene is expressed in a variety of cancers. The pathogenesis of ovarian cancer is poorly understood. The aim of this study was to determine whether the RET protooncogene is expressed in ovarian cancer.

Materials and Methods : The reverse transcriptase-polymerase chain reaction (RT-PCR) and immunohistochemical methods were used to confirm the expression of the RET protooncogene in two ovarian cancer cell lines and ovarian tumor samples.

Results : The PCR products of the RET protooncogene were 300 bp in both ovarian cancer cell lines. On immunohistochemical analysis using an anti-RET polyclonal antibody, positive signals were observed in 59 of 82 cases of ovarian cancer (72.0%). The rates of RET expression in ovarian cystadenomas and ovarian cystadenomas with borderline malignancy were 20.7% and 53.3%, respectively.

Conclusion : The wild type RET protooncogene is expressed in ovarian cancer. This result suggests that the RET protooncogene is involved in the pathogenesis of ovarian cancer.

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Key words : RET protooncogene, ovarian cancer, immunohistochemistry

INTRODUCTION

Ovarian cancer is a leading cause of death among gynecologic malignancies. Although survival rates have increased somewhat owing to adjuvant chemotherapy with paclitaxel and carboplatin, the overall survival rate in patients with ovarian cancer remains poor[1], because ovarian cancer is diagnosed at an advanced stage in most patients and because effective therapies are not available to prevent recurrence in patients who have shown a complete response to chemotherapy. Recently, new therapeutic approaches, such as targeted therapy, have been explored to improve the prognosis of patients with ovarian cancer. To develop a targeted therapy for ovarian cancer, a tumor-specific antigen must be identified.

The rearranged during transfection (RET) protooncogene encodes a receptor tyrosine kinase. The receptor tyrosine kinase controls cell growth and differentiation. It is also known to be activated as oncogenes in human tumors. In addition, RET is a characteristic protooncogene found in several hereditary and nonhereditary diseases, such as multiple endocrine neoplasia (MEN) 2A, 2B,

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