1. がん化学療法の外来移行

今日のがん化学療法は、有効性の高い新薬や支持療法の開発、治療の標準化、がん化学療法委員会によるレジメン審査や管理と共に患者の生活の質（Quality of Life：QOL）の維持・向上を目的[1]に、また診療報酬等の改定やがん対策基本法に位置付けられるがん治療の均てん化も手伝って入院治療から外来治療へ移行している。

1）薬剤科、通院治療室の関与が困難なレジメン

外来におけるがん化学療法を安全かつ確実に実施するためには、院内の業務連携はもとより院外の保険調剤薬局（以下、保険薬局）との情報共有が重要である。注射剤を含む化学療法であれば、院内の通院治療室における外来化学療法の加算条件である注射の必要性、副作用、用法・用量、その他の留意点等を文書により説明するので、治療内容に合わせて当該患者に院内職員が関わることとなる。しかしながら、医薬分業の推進に伴って院外処方箋の発行率が約95％となっている四国がんセンター（以下、当院）では、経口抗がん剤の場合には当該患者に対して情報管理が薬剤科や通院治療室としても十分に対処できない。

2）経口抗がん剤の台頭

経口の抗がん剤に関しては、従前はフッ化ピリミジン系の内服薬として「テガフールやフルオロウラシル、更にはウラシル配合のテガフール」が用いられていたが、プロドラックの開発・臨床試験の検証に伴いその有用性と安全性が確保され、表1に示す薬剤が日常診療でも汎用されるようになった。また、最近では経口分子標的薬のエルロチニブ、ソラフェニブ、スニチニブが市販化された。このような経口抗がん剤の初回投与が入院または院内処方される場合には、薬剤師による薬剤管理指導にて患者への適切な情報提供ができるものと考えるが、初回の院外処方における副作用のモニタリングに関しては注射薬の対応に比べ不足している。

2. 保険薬局における問題点

1）保険薬局との研修会・協議会

保険薬局は、平成18年の医療法の改正により「調剤を実施する薬局」として医療提供施設[2]と位置付けられた。保険薬局は外来がん化学療法において、注射剤を含むレジメンではその支持療法、経口抗がん剤及びその他の併用薬に至る薬剤指導を実施している。その際の情報源は、処方箋と患者又は家族からの聞き取り情報を基に対応しなければならない。保険薬局における薬剤指導や患者の状態管理は、原疾患の把握から治療内容の決定に始まり、薬剤の継続又は変更、中止又は終了といった一連の情報があってしかるべきと思われるが、その詳細を院外処方箋に明記することは到底困難なことである。また、その他には保険薬局の地域格差、個々の薬剤師の対応に起因する問題点もあると考える。

当院では地元薬剤師会を通して保険薬局の薬剤師に向け、当院の治療実態を紹介のうえ薬学的情報提供の要点を整理しつつ、患者及び家族にはいかなる薬剤指導が必要なのかを模索する研修会・協議会を2006年に3回開催した（表2）。第1回目と2回目の開催形式は研修会とした。内容については、当院の職員（医師、薬剤師、看護師）が講師となって、がんの病態、治療内容と薬剤指導において保険薬局で対応を望む要点とその効率的な手段について提案した。3回目は個別具体的な方策を検討するために小規模な協議検討会の形式とした。

表2　研修会・協議会

開催年月日	形式	参加人数(名)
2006年1月26日	研修会	120
2006年5月28日	研修会	約150
2006年11月21日	協議会	28

表1　内服抗がん剤に関する新規の効能・用法

一般名「商品名」	承認年月	新規の効能・用法
テガフール・ギメラシル・オテラシルカリウム「ティーエスワン®」	・2006年8月 ・2007年1月 ・2007年8月	・膵がん適応追加 ・胃がん術後補助療法適応追加 ・胆道がん適応追加
カペシタビン「ゼローダ®」	・2007年12月	・結腸がん術後補助療法適応追加 ・乳がんの新用法用量追加（2週投与1週休薬）
エルロチニブ「タルセバ®」	・2007年12月	・非小細胞肺がん
ソラフェニブ「ネクサバール®」	・2008年1月	・腎細胞がん
スニチニブ「スーテント®」	・2008年4月	・消化管間質腫瘍 ・腎細胞がん

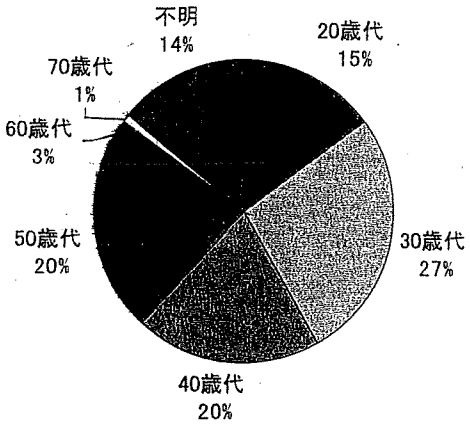

図1　研修会聴講者の年齢別グラフ

2）アンケート調査

1回目の研修会では、聴講者にアンケート調査を実施した。質問要旨は①抗がん剤の取り扱いの有無、②抗がん剤に関する薬剤指導の有無、③がんの告知状況の把握方法と説明時の対応、④医療機関に求める情報提供のあり方と今後の取組方針などである。第1回目の聴講者は120名であったが、アンケートの回答者は32名で回収率は27％と決して良いものではなかった。その要因は調査票の回収タイミングにも問題があると考えられたが、質問内容そのものに答えにくいものがあったと推測される。図1は聴講者の年齢別グラフを示す。

アンケートの結果からは、抗がん剤の取り扱いが有るとの回答は31名、抗がん剤に関する説明指導の経験が有

表3　抗がん剤の説明

Q：抗がん剤は何の薬と説明してますか？

A（複数回答可）；

<告知有り>	<告知無し>
抗がん剤	腫瘍の増殖を防ぐ
がんの薬	細胞の増殖を防ぐ
腫瘍の増殖を防ぐ	再発予防
細胞の増殖を防ぐ	悪い細胞を殺す
再発、転移を防ぐ	がんの進行を抑える
悪い細胞を殺す	組織の異常を改善する
がんの進行を抑える	肺の薬、胃の薬（罹患臓器に対する薬）
組織の異常を改善する	マーカーの値を下げる薬
腫瘍を小さくする	用法・用量のみ
聞かれない限り答えない	薬剤名のみ
あまり触れない	あまり触れない
	あいまいに

表4　指導方針

Q：どのような方法で、どの程度まで指導しているか？

A（複数回答可）；

指導の方法	指導の程度
患者から聞き取る	患者から質問があれば答える
高齢者の場合、家族に	医師の説明を確認し、補足程度
コンプライアンスのチェックのみ	Dr指示の遵守が必要な薬
口頭でまたは図表を用いて	有効血中濃度について
メーカーのパンフレット使用	口頭で効果、用法、副作用
治療方針がわかればそれに沿って	副作用の有無、説明
質問があったとき	副作用で重篤な症状にポイントを絞って
告知されていて医師から説明を受けているときは説明しない	自分のできる範囲で薬効薬理作用、副作用、飲み方、連絡先など
家族の来局が多く、患者に直接説明していない、副作用の有無を含めフォローしていくよう勤める	薬剤提供の紙を渡して、副作用までは踏み込まない
患者から、どの程度知っているか知らされているか聞き出しその程度	

るは26名であった。告知の状況次第では薬剤の情報提供が困難になる場合も見受けられた。また、今後の情報提供のあり方については、個々の薬剤師の資質向上も大切であるが、医療機関からの患者に係る情報提供が重要とのことであった。

3) 問題の検索

アンケートでは、抗がん剤を何の薬と説明しているかの設問では、告知の有無で回答枠を分けたが、告知有りでも「聞かれない限り答えない」「あまり触れない」と言った消極的な回答が見られた。更に、告知無しでは、「用法用量、薬剤名のみ」「あまり触れない」「あいまいに」となっていた（表3）。また、どのような方法で、どの程度まで指導しているかの設問では、患者の質問や聞き取り情報を基に製薬会社が用意するパンフレットを活用し

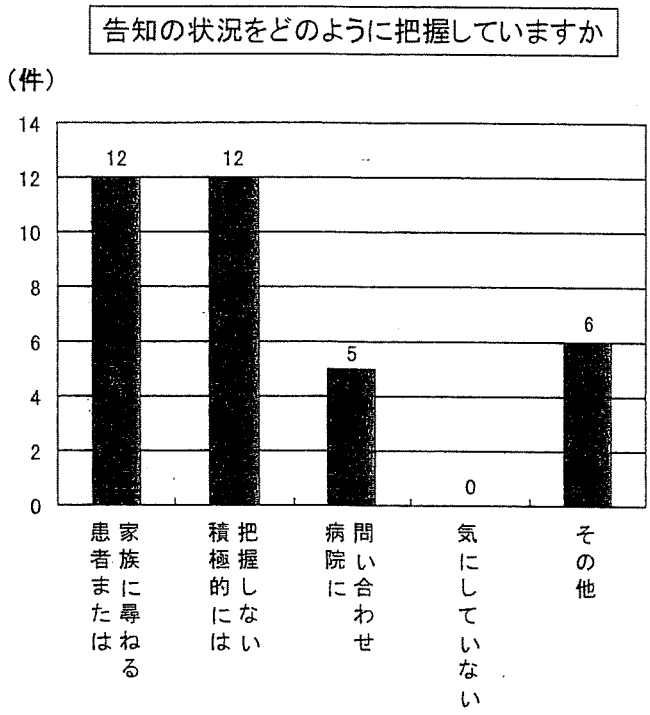

図2　告知の状況（複数回答可）

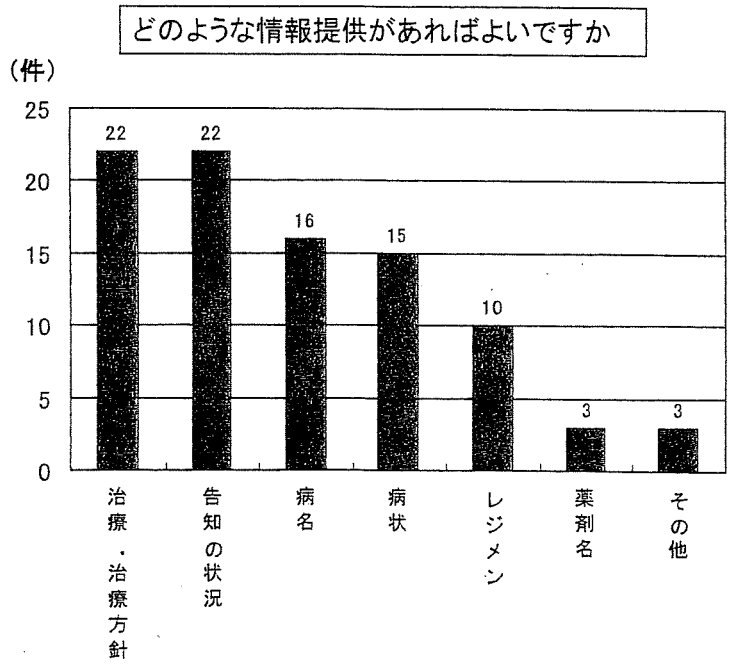

図3　病院からの情報提供のあり方（複数回答可）

て説明がなされていた。特に副作用の項目に関しては、その有無や事象及び重篤の度合いに応じた対応などが多く見受けられた（表4)。つまり、医薬品の添付文書より発生頻度の高い副作用や重篤な場合に関しては患者に伝達するべきものと判断していると考える。

保険薬局の薬剤師は、がん告知の状況把握においても幾つかの方法で確認を行っている（図2)。その取り扱いは繊細なものであり、指導する保険薬局の薬剤師は相応のストレスを感じているものと推測されるが、業務としては避けられないところである。保険薬局が病院からどのような情報提供を望むのかの質問では、「治療内容、方針」「告知の状況」「病名」「病状」「レジメン」などが挙げられた（図3)。

また、アンケートの最後に今後は抗がん剤の説明・指導をどのように行うかの設問に対し、「患者の理解を深めたい」「効果や副作用の対処を期待したい」「患者のために説明・指導を行うのは当然」等、がん患者への服薬指導の取り組みに係る意欲が見受けられた。

患者の医療情報に関して、病院側では個人情報の取り扱いに注意すべきであるが、業務上の重要な連絡、情報共有は欠かせないため病院と保険薬局との連携が求められる。実際の連絡窓口としては病院の薬局と保険薬局による薬薬連携がなされるべきである。

4）薬薬連携

2回目の研修会では、参加規模を若干拡大して約150名の参加者があった。具体的な内容は1回目のアンケート結果を公開して、より一層の連携を図るべく共通の認識を確認した。がん化学療法における情報提供では、患者にとってより安全で継続した治療を提供するために、医療機関と保険薬局の薬薬連携が望ましいと考えられた。図4は保険薬局を含めたチーム医療の概念図を示す。このように患者中心の医療を目指す上ではチーム医療の概念を医療機関内に留まらず保険薬局へも波及させるべきである。薬薬連携を効率的に行い、入院・外来・在宅を通じた医療の一貫性、継続性を図るためにはクリニカルパスが一つのツールとして有用であると考えた。なお、ツールの運用に際しては、過去の研修会に参加して当院の協力要請に呼応できる数カ所の保険薬局と協議会を設けて検討した。その参加人数は30名弱であった。

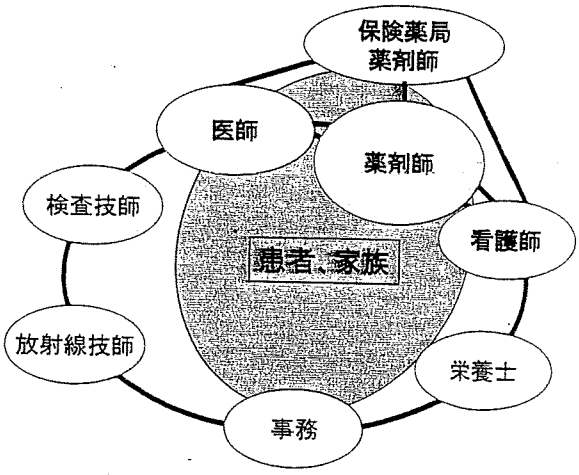

図4　保険薬局を含めたチーム医療の概念図

3. 支援ツール

1）患者日誌

保険薬局では患者情報、薬歴の記録を残して、患者には治療・薬剤に関する理解向上と服薬遵守を目的に「お薬手帳」を交付している。また、個別の治療レジメンによっては、製薬会社が提供する「使用薬剤のハンドブック」「服薬日誌」などを使用している。これらの利用は、治療に必要な情報伝達の手段の一つと考えるが、患者のセルフケアも含めて十分に活用されているかは疑問に感じることがある。何故ならば、新規の入院患者が持参薬と共に薬剤情報提供書を持ち込むことはあっても、お薬手帳を持参することは非常に少ないからである。

また、複数の医療機関を受診している患者が、かかりつけ薬局を1箇所に固定して薬剤の使用目的、副作用とその対策に係る理解、相互作用に関する知識などを熟知するには、医薬分業の制度が100％では無い現状からすると困難と考える。それならば、副作用の出現を否定できない抗がん剤治療を実施する当院としては、保険薬局と協力して患者及び家族への情報提供を標準化したいと考えた。

つまり、保険薬局では、病院からの患者情報（告知状況、副作用を含めた抗がん剤の説明方針、患者の検査値など）が不足している中で患者または家族からの聞き取りに苦慮しているものと思われる。その手段としては、レジメン毎の説明内容が院内の薬剤指導情報と同様に患者へ伝達されることは当然ながら、患者自らが服薬遵守を行うと共に生活目標における自己管理ができるツールの開発が必要である。

2）自己管理型ツールによる情報共有

患者日誌の使用は、経口抗がん剤を院外処方にて開始する患者を対象とした。レジメンは、臨床試験のACTS-GC[3]より胃がん術後補助療法として用いるティーエスワン®である。本剤は製薬会社のハンドブックも存在するが、患者の自己管理を目的に患者日誌を用意した（図5)。基本的には薬剤の使用目的、方法（本レジメンでは4週服薬、2週休薬といった治療スケジュール)、副作用とその対策、

あなたの症状が灰色の部分の状態のときは主治医へ連絡してください。

＜元気度＞

程度	状態
0	無症状。元気。
1	軽い症状があるが、日常生活はできる。
2	自分自身の世話はできるが、日常の生活はできない。
3	かなりの介助がしばしば必要。半日以上は寝てすごす。
4	寝たきり。すべてに介助が必要。

＜副作用について＞

程度	0	1	2	3
食事量	なし			
倦怠感	なし	疲れやすいが日常生活は可能である	だるくて肉体的な活動はできない	一日の半分以上は寝て過ごす
食欲不振	なし	食欲はないが食べられる	明らかに食事の量が減った	ほとんど食べられない
吐き気	なし	気持ち悪いが食べられる	気持ち悪いため明らかに食事の量が減った	ほとんど食べられない
嘔吐（回数）	なし	一日に1回	一日に2～5回	一日に6回以上
口内炎	なし	痛みはあるが普通に食べられる	痛みのため工夫したら食べられる	ほとんど食べられない
下痢	なし	便所に行った回数が普段の回数より1～3回多い	便所に行った回数が普段の回数より4～6回多い	便所に行った回数が普段の回数より7回以上
腹痛	なし	痛みはあるが普通に過ごせる	痛み止めで普通に過ごせる	痛み止めでも普通に過ごせない
手足のしびれ	なし	普通に動かせる	動かしにくい	生活に支障がある
発疹（手足の皮膚）	なし	痛みを伴わない皮膚の変化	痛みなどはあるが日常生活に支障はない	痛みなどがあり日常生活に支障がある
涙の量	なし	涙の量が多いが、見えにくいなどのことはない	見えにくいなどの機能障害はあるが生活に支障はない	見えにくいなどの機能障害があり、生活に支障がある

＊医療者のかたへ＊
検査値の記載または貼布、データのコピーをファイルしてください。

目標	検査値の目標（　月　日）検査値			
●医師の処方どおりに内服できる。	白血球 3000/mm³以上		肝機能：100IU/L以下	
●どんな副作用があるか知っている。	好中球 1500/mm³以上		GOT	
●副作用に合わせた生活ができる。	ヘモグロビン 8.0g/dl以上		GPT	
●内服、副作用の状況を記録できる。	血小板 7.5万/mm³以上		腎機能：1.2以下	
●緊急時の連絡先を知っている。	次回外来受診日	月　日	クレアチニン	

今回の内服期間	（　）週目：　月　日 ～　月　日
今回の内服量	

月日	／ 朝	夕	／ 朝	夕	／ 朝	夕	／ 朝	夕	／ 朝	夕	／ 朝	夕	／ 朝	夕
内服のチェック														

飲み忘れても、絶対に2回ぶんを一度に飲まないようにしてください。

元気度									
体温	熱								
体重	Kg								
食事	食事量								
副作用（あなたの状態を記入しましょう）	倦怠感								
	食欲								
	吐き気								
	口内炎								
	下痢								
	腹痛								
	風邪症状								
	手足のしびれ								
	手足の発疹								
	涙の量								
	その他								

図5　自己管理型の治療日誌

あなたの病気の名前は	
あなたの病気の状況は 【現病歴】	
ほかにかかっている病気 【既往症】	
治療のスケジュールは(　　)週投薬　(　　)週休み	

＊医療スタッフのかたへ＊
治療計画（レジメン）貼付をお願いします。

かかっている病院や診療所、開業の先生があれば教えてください。

かかっている病院などの名前	かかっている病気の名前

今、飲んでいるお薬（ほかの病院で処方されている）があれば教えてください。

＊医療スタッフのかたへ＊
既往歴と内服薬の確認をお願いします。
服薬指導箋をお持ちの場合はコピーをファイルしてください。
（または、用紙のファイルをお願いします。）

図6　患者の基本情報

注意事項、緊急時の連絡先などを説明する目的で日誌を活用するものである（図6）。日誌は患者の有害事象に関する情報が記録されるので、次回来院日にはその内容が主治医に伝わることになる。仮に伝達が不十分であるような場合には、保険薬局が患者支援を目的に当院の薬局や診療科、相談窓口（がん相談支援・情報センター）にその旨連絡を図り、主治医と協議できるように考えている。すなわち、日誌は、患者、主治医、薬剤科又は相談窓口、保険薬局の間の情報共有のツールと位置付けている。

日誌の中では、患者の身体上の問題となるような事象が生じた場合には、主治医へ連絡を取るよう記載している。

(a) 目標設定

日誌では患者の治療に関わる事項を理解してもらうため5つの目標設定を行っている。目標は、①医師の処方どおりに内服できる、②どんな副作用があるか知っている、③副作用に合わせた生活ができる、④内服、副作用の状況を記録できる、⑤緊急時の連絡先を知っているとした。

(b) 全身状態の指標（PS；Performance Status）

日常診療では全身状態の指標としてECOG（Eastern Cooperative Oncology Group）のPSを用いている。化学療法の際には、全身状態に問題が無いことを確認して治療を開始し、更には随時確認しながら継続している。抗がん剤の治療によって生じる有害事象が全身状態に影響を及ぼすことになる。日誌の中では、患者の自己管理を目的に「PS」のことを「元気度」という平易な用語で表現した。これにより、患者の「PS」に関するGradeの自己判断は容易になると考える。

(c) 検査結果

患者の来院時には必要な血液検査、生化学検査などが実施されるが、これらの結果は殆どの患者に提示しているので、検査結果から体調管理を行うことができる。そのためには検査結果の読み取り方、目標とする数値、感染症の危険性があればその予防策など治療上必要な情報を提供しておく必要がある。具体的な目標としては、白血球数を3,000/mm^{3}以上、ヘモグロビンを8.0g/dL以上、血小板を7.5万/mm^{3}以上などである。

3）保険薬局での指導要領

保険薬局における薬剤説明では、効能効果、用法用量及び想定される副作用の出現と対策である。

出現頻度の高い副作用は、消化器症状、骨髄抑制、皮膚障害などを説明している。特に、フッ化ピリミジン系薬剤では手足症候群、分子標的薬剤の場合には皮膚症状が頻発することを伝えている。その他には、検査結果から普段からの注意事項を指導することや緊急時の連絡先確認も欠かせない。

4）ツールの特徴

がん化学療法に伴う副作用については、医療従事者が用いるモニタリングシート[4]を活用する方法がある。日誌の副作用に関する記載方法では、有害事象共通用語基準Ver 3.0（日本語訳JCOG／JSCO版）を引用して、各事象のグレーディングを患者自らがチェックできるよう用語を簡素化している。また、副作用のグレードが2を超えるような場合には、病院へ連絡を促すようにしている。

試作した患者日誌は、患者の服薬遵守が行えると同時に、患者自らが生活目標をたてて副作用の把握と対策を図れることを目的とした。記載内容がアウトカム方式によるため、得られる情報がチーム医療の中で共有できることも利点である。

4. 運用と改善に向けて

日誌の運用に際しては、近隣の保険薬局を選択し相談協議の上で2008年1月より開始した。運用症例は20例弱である。保険薬局の薬剤師に日誌の問題点について、自由記載による意見・感想を求めたところ、日誌のシステム、患者教育、病院側及び保険薬局側の問題が幾つか挙げられた（表5）。改善策としては、院内の協力はもとより患者への継続的な教育や薬剤指導に関わる保険薬局の薬剤師の資質向上を考えている。

がんの治療は日進月歩であり、がん対策基本法の施行に伴い、現在では都道府県及び地域のがん診療連携拠点病院の整備[5]が進む中で、地域連携が挙げられるが、その中には保険薬局も重要な役割を担うものと考える。

考察

がん化学療法は入院治療から外来治療へと増加移行しており、中でも経口抗がん剤においては分子標的薬剤の新規開発が進んでいる。これらの新規薬剤では有害事象の捉え方も従来のものとは異なることが多いので、普段からの患者の自己管理が重要である。そのような状況においては、患者教育、情報共有の役割を保険薬局が担えるよう医療機関の薬剤部門は連携を十分に図る必要がある。医薬分業の進展に伴って患者が保険薬局で受ける薬剤指導は、当該医療機関の情報と相違のないものでなければならないと考える。当院では、がん専門病院として地域のがん診療連携拠点病院と共に標準化されたがん化学療法を実施する必要がある。また、薬剤に関しては、患者及びその家族に対する十分な情報提供を保険薬局と円滑に実施できるよう図りたい。

今回、試作した患者日誌はクリニカルパスに位置づけて実践してみたが、改善を要するものの有用であると考

表5　保険薬局からの意見・感想

システムとして ・日誌の用紙不足 ・その日の頁が直ぐ分かるような仕組みが便利 ・検査値のコピーを貼付してはどうか
患者教育として ・持参しない ・来局を重ねる間に日誌を忘れる ・日付が不明 ・日誌は問題なくても、口頭で問題症状を発言される
病院として ・医師の記入漏れ ・記入例については病院薬剤部との協議が必要
保険薬局として ・アプローチに戸惑う、本人が立ち入られているのではないかとの気遣い ・データ転記、コメント記入には抵抗がある

える。更に今後は、がん化学療法のレジメン毎に地域連携パスに組み入れることで、患者中心の医療を目的とした院内外の共有情報になると期待している。なお、治療日誌は従来のお薬手帳として情報提供のツールになるよう地元薬剤師会等と協議を図っていきたい。

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Surg Today (2009) 39:201–206
DOI 10.1007/s00595-008-3827-2

SURGERY TODAY

The Triangulating Stapling Technique for Cervical Esophagogastric Anastomosis After Esophagectomy

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Abstract

Purpose. To evaluate the safety and feasibility of the triangulating stapling technique (TST) for cervical esophagogastric anastomosis after esophagectomy (CEGA).

Methods. The subjects were 123 patients who underwent transthoracic esophagectomy with three-field lymph node dissection and reconstruction with a 3.5-cm wide gastric tube, for thoracic esophageal cancer. We performed the TST for CEGA in 33 patients operated on after December, 2006 (TST group) and hand-sewn anastomosis in 90 patients operated on between 2002 and 2006 (HSA group).

Results. In the TST group, CEGA was performed in an end-to-end fashion using three linear staplers. The first anastomosis was applied to the posterior walls of the remnant esophagus and gastric tube in an inverted fashion. The second and the third anastomoses were done in an everted fashion to make the anterior wall. The end-to-end HSA was performed with interrupted sutures using 4–0 absorbable material. Anastomotic leakage occurred in only 1 (3.0%) of the 33 TST patients, but in 13 (14.4%) of the 90 HSA patients ($P = 0.07$). The frequency of anastomotic stenosis was 9.1% and 25.6% in the TST and HSA groups, respectively ($P < 0.05$).

Conclusions. Cervical esophagogastric anastomosis using TST may reduce the frequency of anastomotic leakage and stenosis. This technique is a safe and reliable alternative for CEGA after esophagectomy.

Key words Linear stapler · Triangulating stapling technique · Esophagogastric anastomosis · Anastomotic leakage · Anastomotic stenosis

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Received: May 18, 2008 / Accepted: May 27, 2008

Introduction

The most frequent and serious postoperative complications of esophagectomy are respiratory failure and anastomotic leakage, both of which are associated with high mortality.[1–3] Anastomotic leakage is thought to be caused by ischemia of the gastric or colon conduit and by the methods of anastomosis and surgical techniques used.[2,4,5] The methods used for esophagogastric anastomosis after esophagectomy vary among institutes, although many reports have addressed this issue. In Japan, the standard surgical procedure for thoracic esophageal cancers is transthoracic esophagectomy with extended cervical, mediastinal, and abdominal lymph node dissection, known as the "three field lymph node dissection" (3FLND). This procedure includes cervical esophagogastric anastomosis (CEGA) and CEGA failure occurs more frequently than intrathoracic esophagogastric anastomosis failure.[6] The leakage of CEGA after transthoracic esophagectomy is associated with more serious sequelae, such as empyema and sepsis, than after transhiatal esophagectomy without thoracotomy.[7] Thus, a reliable and safe anastomotic method is necessary to prevent this life-threatening complication of esophagectomy.

Postoperative stenosis at the CEGA is also a critical postoperative complication, which compromises the patient's quality of life by causing anastomotic stenosis-related dysphagia.[4,8] These patients require repeated endoscopic dilation of the stenosis. Furthermore, anastomotic leakage often leads to anastomotic stenosis.[9,10] Therefore, we must develop an anastomotic method that reduces the risk of anastomotic stenosis and leakage.

The triangulating stapling technique (TST) was first used for a colorectal anastomosis by Venkatesh et al. in 1993[11] and has been utilized mainly for colo-colonic anastomoses.[12] The TST seems to be a safe, reliable, and easy technique for reconstruction of the alimentary

tract, which reduces the occurrence of both postoperative anastomotic leakage and stenosis.[11,12] However, to our knowledge only one study has been published on esophagogastric anastomosis after esophagectomy, based on the results of only 12 cases.[13] We conducted this study to evaluate the application of the TST for CEGA after transthoracic esophagectomy with 3FLND for thoracic esophageal cancers, in comparison with hand-sewn anastomosis (HSA).

Patients and Methods

Patients

Between January 2002 and April 2008, 175 patients underwent surgery for esophageal cancer in the National Kyushu Cancer Center, Japan. All cancers were pathologically diagnosed as squamous cell carcinoma. Among these patients, 122 with a thoracic esophageal cancer underwent transthoracic esophagectomy with 3FLND, performed via a right-side thoracotomy. The alimentary tract was reconstructed using a gastric tube made of the greater curvature of the stomach. CEGA was performed by hand sewing until September 2006 (HSA group; n = 90), and by the TST after October 2006 (TST group; n = 33) through a retrosternal or posterior mediastinal route. The TST group included one patient with CEGA performed after a bypass operation. All of the operations were performed by a single surgeon (Y.T.) or by other surgeons under the direction of Y.T.

Surgical Technique

A gastric tube, 3.5 cm wide, was made along the greater curvature of the stomach using linear staples (GIA Universal 60–3.5, Tyco Healthcare, Mansfield, MA, USA) with seromuscular sutures added along the staple line. This narrow gastric conduit was long enough to be pulled up to the cervix through a retrosternal or posterior mediastinal route and had adequate blood supply to its tip.[14] The width of 3.5 cm was considered suitable

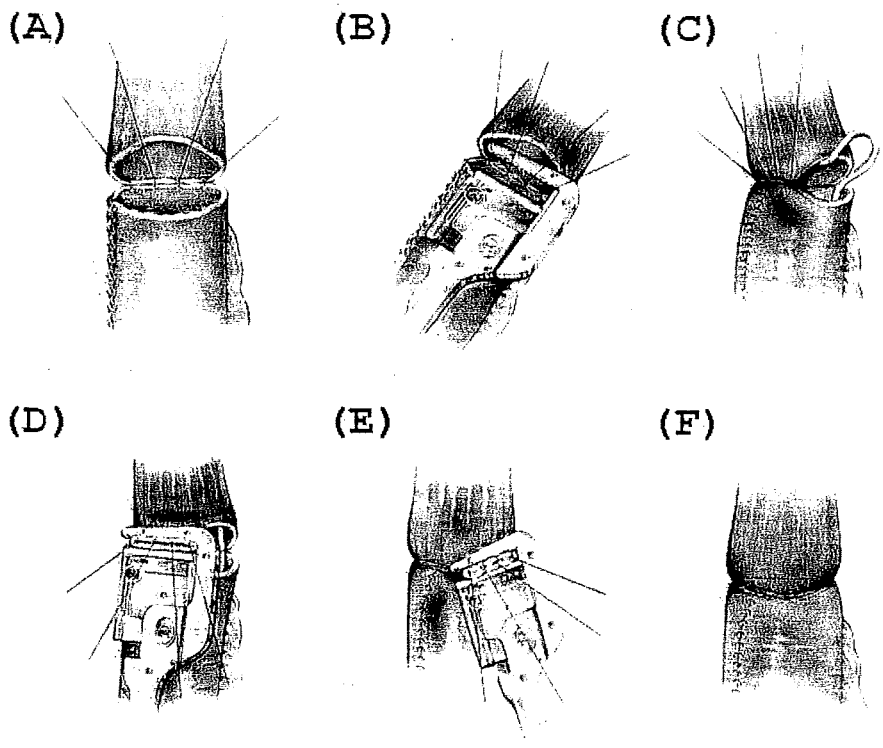

Fig. 1A–F. Triangulating stapling technique for cervical esophagogastric anastomosis using three linear staplers. **A** Three or four suspension sutures are placed through all layers of the posterior wall of the remnant esophagus and gastric tube. **B** The first anastomosis is done using a linear stapler, pulling up the suspension sutures to securely anastomose all layers of the posterior wall in an inverted fashion. **C** Before the second anastomosis of the anterior wall, a nasogastric tube is inserted and advanced downward into the gastric tube. **D** A linear stapler is applied to create the second side of the anastomosis on the anterior wall. It is important that the second suture intersects the first staple line of the posterior wall. **E** Note that the linear staple line of the gastric tube is positioned at the center of the second side of the triangle. The third anastomosis is done in the same manner, using suspension sutures. **F** The end-to-end anastomosis using triangulating stapling technique is completed. Note that the three staple lines are mutually intersected. The two sides of the anterior wall are stapled in an everted fashion

for end-to-end cervical anastomosis between the remnant esophagus and the gastric tube. For the TST group, an end-to-end CEGA was performed using three linear staplers (TA30–3.5, Tyco Healthcare; Fig. 1). The first instrumental anastomosis was applied to about two-thirds of the posterior wall of the remnant esophagus and the gastric tube in an inverted fashion. The first linear stapler was applied to the esophagus and gastric tube after three or four suspension sutures in the whole layer were added to secure the first anastomosis. Great care was taken to securely suture the whole layer by pulling up the suspension sutures. After firing the first linear stapler, the remaining walls of the esophagus and gastric tube were resected sharply and the stapler was released. Next, suspension sutures were secured to both ends of the staple line on the sutured posterior wall. To make the anterior wall of the reconstructed tract, the second and the third anastomosis were performed in the same manner using the second and third linear staples; however, these were done in an everted, rather than in an inverted fashion. When performing the TST, it is critical that the staple lines are securely intersected mutually and that the longitudinal staple line of the gastric conduit is positioned at the center of the suture line to the right of the anterior wall. Thus, the triangle-shaped end-to-end anastomosis was completed between the remnant esophagus and the gastric tube in the cervical region (Fig. 2). Before the third stapling, a 14-F or 16-F nasogastric tube was inserted by the anesthetist and advanced downward into the gastric tube for postoperative monitoring.

End-to-end HSA for CEGA was performed interruptedly using 4–0 absorbable monofilament sutures in a single layer for the anterior wall and in two layers for the posterior wall. In both the TST and HSA groups, a leakage test was done just after completion of the anastomosis, by inflating the reconstructed tract with dyed saline. A closed suction drain was placed in the anastomotic region.

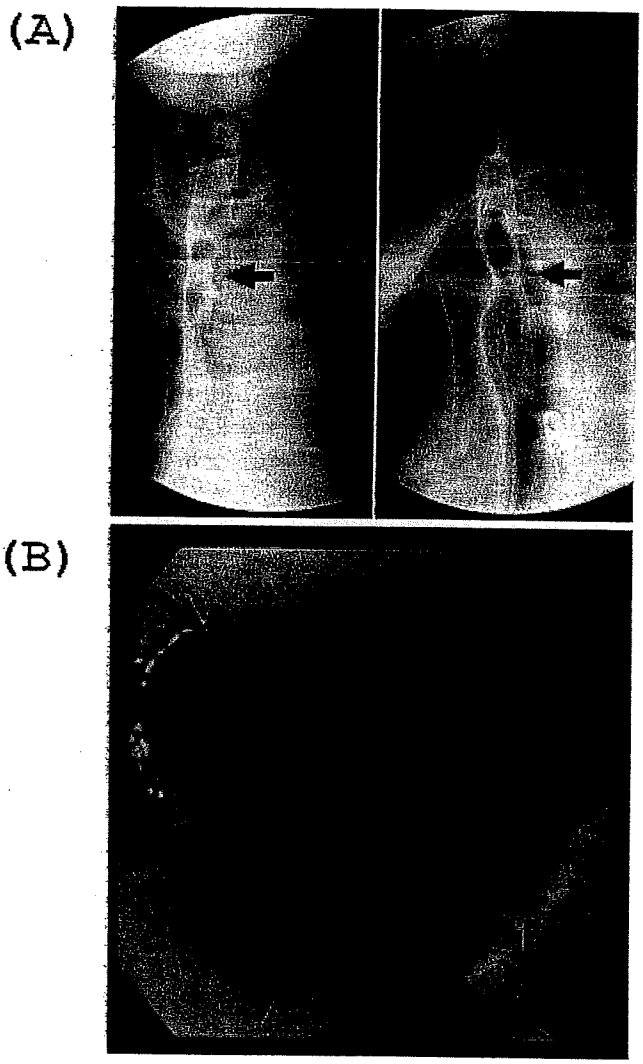

Fig. 2A,B. Demographic presentation of the triangulating stapling technique for cervical esophagogastric anastomosis. **A** Contrast-swallow on postoperative day 7 shows no anastomotic leakage. The passage of contrast medium is good and the anastomosis is sufficiently wide. **B** Endoscopic view of the anastomotic site on postoperative day 14. The triangle-shaped lumen is wide and three corners can be seen. Two of the three sides of the triangle are everted (*ev*) and there are no mucosal defects. One of the three sides is anastomosed in an inverted (*inv*) fashion. The three corners of the triangle are indicated by *arrows*

Definitions of Anastomotic Leakage and Stenosis

The condition of the draining fluid and cervical wound as well as the patients' general condition, determined by body temperature, pulse rate, C-reactive protein value, and white blood cell count, were monitored to detect any sign of anastomotic leakage. Anastomotic leakage was classified into the following three categories: minor leakage, defined as purulent and/or salivary discharge from a cervical drain without the need for surgical intervention, which resolved in fewer than 14 days; moderate leakage, defined as purulent and/or salivary discharge from a cervical drain without the need for surgical intervention, which took more than 15 days to heal; and severe leakage, defined as purulent and/or salivary discharge from a cervical drain, requiring surgical intervention for adequate drainage or repair. Anastomotic stenosis was defined as any stenosis in the anastomotic region, which required either endoscopic balloon dilation or bougienage at least once.

Statistical Analysis

Statistical analyses were performed using Student's *t*-test or the chi-square test, with the StatView software

program (SAS Institute, Cary, NC, USA). A P value of less than 0.05 was considered significant.

Results

The clinical characteristics of the patients in the HSA and TST groups are shown in Table 1. There were no significant differences in the patients' backgrounds between the two groups. There were no significant differences in the route of esophageal reconstruction, other operative procedures, operation time, or estimated blood loss between the groups. According to change in policy in our institute, after 2004, patients were extubated immediately after the operation. Thus, 40 patients from the HSA group and all of those from the TST group were managed without any mechanical ventilation.

The frequencies of anastomotic leakage and anastomotic stenosis are shown in Table 2. Anastomotic leakage occurred in only 1 (3.0%) of the 33 TST patients, but in 12 (13.3%) of the 90 HSA patients. Of the 13 cases of leakage in the HSA group, 2 (16.6%) and 8 (66.7%) were minor and moderate, respectively, and all resolved after drainage from a cervical drain inserted during the original surgery. However, 2 of these 13 patients required surgical intervention. In the TST group, there was only one minor leakage, which healed after 13 days of drainage. This occurred in the third TST group patient and the following 30 had no leakage. Anastomotic leakage tended to occur more frequently in the HSA group than in TST group, although the difference was not significant ($P = 0.09$). Anastomotic stenosis was observed in 24 (26.7%) patients from the HSA group and in 3 (9.1%) from the TST group, representing a significant difference ($P < 0.05$). The average number of dilation procedures was 3.1 in the HSA group and 3.6 in the TST group.

Table 1. Clinical characteristics of the patients who underwent hand-sewn or triangular stapling for cervical esophagogastric anastomosis

	HSA group ($n = 90$)	TST group ($n = 33$)	P value
Age (years), mean ± SD	62.9 ± 7.7	63.0 ± 7.9	NS
Sex Male:female	74:16	26:7	NS
Comorbidity			
Cardiac	18 (20.0%)	6 (18.2%)	NS
Pulmonary	4 (4.4%)	4 (12.1%)	NS
Renal	1 (1.1%)	1 (3.0%)	NS
Liver	4 (4.4%)	0	NS
Others	13 (14.4%)	4 (12.1%)	NS
p Stage[a] 0/1/2/3/4[a]	5/24/30/18/9	3/5/14/3/7[b]	NS
Neoadjuvant CRT –/+	64/26	26/7	NS

HSA, hand-sewn anastomosis; TST, triangular stapling technique; CRT, chemoradiotherapy; SD, standard deviation; NS, not significant
[a]The p Stage was based on the Japan Esophageal Society[23]
[b]One bypass operation was excluded

Table 2. Postoperative data after hand-sewn vs triangular stapling for cervical esophagogastric anastomosis

	HSA group ($n = 90$)	TST group ($n = 33$)	P value
Postop. complications			
Anastomotic leakage	12 (13.3%)	1 (3.0%)	NS
Minor[a]	2 (2.2%)	1 (3.0%)	
Moderate[a]	8 (8.9%)	0	
Severe[a]	2 (2.2%)	0	
Pulmonary	14 (15.6%)	4 (12.1%)	NS
Cardiac	3 (3.3%)	2 (6.1%)	NS
Renal/liver	5 (5.6%)	2 (6.1%)	NS
Others	10 (11.1%)	5 (15.2%)	NS
Anastomotic stenosis	24 (26.7%)	3 (9.1%)	<0.05
Operative mortality	1 (1.1%)	1 (3.0%)	NS

HSA, hand-sewn anastomosis; TST, triangular stapling technique; Postop., postoperative; NS, not significant
[a]See text for definition of leakage

Discussion

The risk of leakage at the anastomosis between the remaining esophagus and its replacement conduit after esophagectomy is highest among all types of gastrointestinal anastomoses.[3,7,15] Moreover, it is associated with high mortality, once it occurs.[1–3] Much effort has been devoted to reducing the occurrence of anastomotic leakage, with some good results.[10,16] However, the rate of anastomotic leakage after esophagectomy still exceeds 10% in the most series.[13,17–20] Anastomotic leakage is reported to be much higher after transthoracic esophagectomy with extended 3FLND, because of the excessive surgical stress.[15] Anastomotic leakage is attributed to ischemia of the gastric or colon conduit, and to technical problems, including the methods of anastomosis and surgeon's experience.[2,4,5] Many surgeons prefer to perform anastomosis using a circular stapler than to using a hand-sewn anastomosis, because the stapling technique is simpler and easier,[17] whereas many institutes use hand-sewn anastomosis for CEGA and achieve excellent results.[16] Beitler et al.[18] reviewed the results of four randomized and seven nonrandomized trials comparing circular stapled and hand-sewn esophagogastric anastomoses and concluded that both methods resulted in equivalent rates of anastomotic leakage.

The TST was originally designed for colorectal anastomosis and has been utilized mainly for colo-colonic anastomosis. It seems to be a safe, reliable, and easy technique in reconstruction of the alimentary tract, which reduces the occurrence of postoperative anastomotic leakage and stenosis.[11,12] Furukawa et al.[13] reported their experience of performing the TST for CEGA after esophagectomy in 12 patients. Their anastomotic leakage rate was greatly reduced to 8.3%, in comparison with 27% and 25% after hand-sewn anastomosis and circular stapled anastomosis, respectively. This rate is consistent with that in the current study. The one case of anastomotic leakage after TST in the current series involved the third among 33 patients, and this leakage resolved with conservative treatment within 1 week. Although the exact cause was unknown, the technique of TST might not have been established. No primary anastomotic leakage occurred in the subsequent 30 patients, although there was one case of leakage caused by direct spread of a mediastinal abscess and pyothorax to the anastomotic site in the retrosternal space.

The TST is also a time-saving modality. Furukawa et al.[13] reported reducing the time to perform CEGA by using the TST (24 min vs 54 min for hand-sewn anastomosis and 35 min for the circular stapler method). Although we did not record the exact time required for CEGA by the TST, it is known that the TST for CEGA can be completed within 30 min: approximately half the time required for hand-sewn anastomosis. This advantage may be an important contributor to the safety of esophagectomy because CEGA is the final step in all esophagectomies with lymph node dissection, which require extended surgical time and much concentration.

An additional advantage of the TST is that the CEGA can be made end-to-end in a safe, simple, and stable manner, using instruments. An end-to-end anastomosis of the alimentary tract is better than an end-to-side or side-to-side anastomosis. Although an end-to-end anastomosis can also be achieved with the hand-sewn method, the technical stability and the time it takes are problematic, and it requires extremely skilled surgeons. The circular stapling method is also a time-saving and stable technique for this anastomosis. However, CEGA using a circular stapler results in an end-to-side anastomosis, which may be associated with serious blood perfusion problems at the tip of the gastric conduit because the vascular net of the gastric tube wall is blocked by the stapling itself,[21] leading to anastomotic failure. Triangulating stapling does not disturb the blood supply to the tip of gastric tube.

Anastomotic stenosis is a critical postoperative complication of esophageal surgery because it can result in life-threatening aspiration pneumonia and at the very least, nutritional disorders. This complication occurs in 16% and 27%–31% of patients who undergo hand-sewn and circular stapled CEGA, respectively.[18] Even higher rates of 32% and 45%, respectively, have been reported by others.[13] Therefore, anastomotic stenosis at the CEGA is more common in stapled anastomoses than in hand-sewn anastomoses.[18,19] This is because the mucosa is not directly approximated in a circular stapled anastomosis, leaving a circular mucosal defect, which can lead to stenosis formation.[9,13,22] The TST approximates the mucosa in two of the three sides of the triangle because two of the three anastomoses are made in an everted fashion, resulting in accurate mucosa-to-mucosa apposition, which is considered good for anastomotic healing. This may explain the lower rates of anastomotic stenosis in this (9.1%) and other (8.3%) series.[13]

In conclusion, the TST is a good alternative method for CEGA for the following reasons: the anastomotic leakage rate is low; the anastomotic stenosis rate is low; an end-to-end anastomosis is possible; the mucosal defect is less than with circular stapling, causing less circular ulcer formation; and the technique is simple, even for inexperienced surgeons. Finally, the medical costs for three linear staplers are equivalent to, or less than, those for a circular stapler.

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Clin Exp Metastasis (2009) 26:215–227
DOI 10.1007/s10585-008-9233-8

REVIEW

The role of the *MTA* family and their encoded proteins in human cancers: molecular functions and clinical implications

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Received: 3 October 2008 / Accepted: 12 December 2008 / Published online: 31 December 2008

Abstract *MTA* (metastasis-associated gene) is a newly discovered family of cancer progression-related genes and their encoded products. *MTA1*, the first gene found in this family, has been repeatedly reported to be overexpressed along with its protein product MTA1 in a wide range of human cancers. In addition, the expression of *MTA1*/MTA1 correlates with the clinicopathological properties (malignant properties) of human cancers. MTA proteins are transcriptional co-repressors that function in histone deacetylation and are involved in the NuRD complex, which contains nucleosome remodeling and histone deacetylating molecules. MTA1 expression correlates with tumor formation in the mammary gland. In addition, MTA1 converts breast cancer cells to a more aggressive phenotype by repression of the estrogen receptor (ER) α trans-activation function through deacetylation of the chromatin in the ER-responsive element of ER-responsive genes. Furthermore, MTA1 plays an essential role in c-MYC-mediated cell transformation. Another member of this family, MTA3, is induced by estrogen and represses the expression of the transcriptional repressor Snail, a master regulator of "epithelial to mesenchymal transitions", resulting in the expression of the cell adhesion molecule E-cadherin and maintenance of a differentiated, normal epithelial phenotype in breast cells. In addition, tumor suppressor p53 protein is deacetylated and inactivated by both MTA1 and MTA2, leading to inhibition of growth arrest and apoptosis. Moreover, a hypoxia-inducible factor-1α (HIF-1α) is also deacetylated and stabilized by MTA1, resulting in angiogenesis. Thus, MTA proteins, especially MTA1, represent a possible set of master co-regulatory molecules involved in the carcinogenesis and progression of various malignant tumors. MTA proteins are proposed to be important new tools for clinical application in cancer diagnosis and treatment.

Keywords Metastasis-associated gene 1(MTA1) · Chromatin remodeling · Histone deacetylation · Gene expression · Protein modification · Cancer progression · Metastasis

Abbreviations

MTA	Metastasis-associated gene/protein
HDAC	Histone deacetylase
NuRD	Nucleosome remodeling and histone deacetylation
ER	Estrogen receptor
HIF	Hypoxia-inducible factor

Introduction

Recent advances in molecular biology have resulted in the discovery of a wide variety of new molecules involved in carcinogenesis and cancer progression. Although additional molecules related to cancer will be identified in the future, the existing and new molecules must fulfill two major requirements in order to be clinically useful as molecular targets for the diagnosis and treatment of human cancers. The first is that abnormalities in expression or structure of molecules of interest and their clinical relevance must be definitely demonstrated in human cancers by

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independent studies. The second is the underlying molecular mechanisms necessary for the molecules to exert their functions in carcinogenesis or cancer progression must be determined.

Among a number of cancer-related genes and molecules that have been discovered in the last few years, we identified a candidate metastasis-associated gene by use of a differential cDNA screening method. Thus, we identified a gene that was abundantly overexpressed in highly metastatic rat mammary adenocarcinoma cell lines compared to poorly metastatic cell lines [1, 2]. When this gene was sequenced, it was revealed as a completely novel gene without any homologous or related genes in the database. The rat gene was named *mta1* (metastasis-associated gene 1). A homologous gene was also expressed in human cancer cell lines [1], and its human cDNA counterpart, *MTA1*, was cloned by our group in 2000 [3]. Using surgically resected human tissues, we showed that high levels of *MTA1* mRNA expression were clinicopathologically correlated to the invasive and growth properties of gastrointestinal cancers, including esophageal, gastric and colorectal cancers [4, 5]. Subsequently, several reports from independent research groups followed our observations and showed similar correlations between *MTA1* expression and the malignant potentials of human cancers.

Several genes related to *MTA1* have now been identified, indicating *MTA1* consists of a gene family, which we now call the "*MTA* family". Further studies on molecular biological and biochemical properties of the MTA family have shown that the gene products of the main members of the family (MTA1, MTA2, and MTA3) are tightly associated in a protein complex called NuRD (nucleosome remodeling and histone deacetylation), which has transcriptional regulatory function via histone deacetylation and chromatin remodeling. At the moment, the MTA family has attracted widespread attention as one of the key molecules that plays an indispensable role in the genesis and progression of a wide variety of cancers [6–8]. In this brief review, we will examine the significance of the expression of MTA family members in human cancers and the important molecular mechanisms that are currently known by which MTA proteins exert their functions. Finally, future directions for clinical applications of this protein family for the diagnosis and treatment of human cancers will be discussed.

Members of the MTA family and their protein structures

At present, the MTA proteins represent a family of gene products encoded by three distinct genes (MTA1, MTA2, and MTA3) and six reported isoforms (MTA1, MTA1s, MTA1-ZG29p, MTA2, MTA3, and MTA3L). The molecular masses of the gene products of MTA1, MTA2, and MTA3 are approximately 80, 70, and 65 kDa, respectively. The nucleotide and protein alignment homologies and the phylogenetic comparative analyses are discussed elsewhere [8, 9].

Except for ZG-29p, the MTA family sequences contain several common domain structures [10]. One of these, the BAH (bromo-adjacent homology) domain is involved in protein–protein interactions. Another, the SANT (SWI, ADA2, N-CoR, TFIIIB-B) domain shares a high degree of homology with the DNA-binding domain of the Myb-related proteins, suggesting that this domain may be involved in DNA-binding. The ELM (egl-27 and MTA1 homology) domain has an unknown function [11]. MTA family members also contain a highly conserved GATA-type zinc finger motif, which indicates a direct interaction with DNA [3]. MTA1 has two src-homology (SH)-binding motifs at its C-terminal region, which are known to be important in signal transduction involving many kinase, adaptor and scaffolding proteins [1, 10]. Similar SH2- and SH3-binding domains are also found in MTA2 and MTA3. These common domain structures clearly show that the MTA family is involved in protein–protein and DNA-binding interactions, indicating possible functions in signal transduction and transcriptional regulation.

MTA proteins contain basic nuclear localization signals [1, 10]. They also localize in the nucleus in many cancer cells [4, 8]. However, MTA1 localizes to both the cytoplasm and nucleus in some tumors [12–14]. MTA3 also localizes to the nucleus, but it has no apparent nuclear localization signal [15]. MTA1s, a short splice-variant of MTA1, is predominantly localized in the cytoplasm [16].

The expression of MTA proteins in various cancers and its clinicopathological and biological relevance

Clinicopathological relevance of the increased MTA1 expression in human cancer tissues

Since the first report by us showing that the up-regulation of *MTA1* expression was significantly correlated to the malignant properties of human gastric and colorectal cancers [4], many researchers have been investigating the expression levels of MTA family members, especially MTA1, in various human cancers. This has revealed that the expression levels of MTA family members have clinicopathological significance (The data are summarized in Table 1).

Breast cancer

MTA1 was identified as a candidate progression molecule that was associated with breast cancer metastasis [1, 2] and

growth (the antisense RNA of MTA1 inhibited the growth of highly metastatic breast cancer cell lines [3]). The involvement of MTA1 in the carcinogenesis or progression of human breast cancer was also shown by other data using clinical samples. For example, Martin et al. [17] mapped the chromosomal locus 14q that might be responsible for axillary lymph node metastasis in human breast cancers by comparing the rate of loss of heterozygosity between node-positive and -negative breast cancers. They found that the *MTA1* gene was contained in that gene locus, suggesting that

Table 1 Clinicopathological implications of the increased MTA1 expression in various human cancer tissues

Type of cancer	Method	Clinicopathological implications	Reference
Breast cancer	LOH	Higher LN meta.	[17]
	IHC	Earlier recurrence	[18]
	IHC	Higher tumor grade	[19]
		Higher MVD (angiogenesis)	
Gastrointestinal cancer			
Esophageal	RT-PCR	Deeper adventitial invasion	[5]
		Higher LN meta.	
	IHC	Deeper adventitial invasion	[21]
		Higher LN meta.	
		More advanced stage	
		Poorer prognosis	
Gastric	RT-PCR	Deeper serosal invasion	[4]
		Higher LN meta.	
Colorectal	RT-PCR	Deeper wall invasion	[4]
		Higher LN meta.	
	RT-PCR	Higher expression in cancer tissue	[20]
Carcinoid			
Gastric	RT-PCR	Deeper tumor invasion	[25]
Small intestine	RT-PCR	Malignant carcinoid	[22]
		More liver and LN meta.	
Appendiceal	RT-PCR	Significant increase in malignant tumors	[26]
Pancreatic	IHC	Poorer prognosis (in combination with HDAC1)	[28]
Hepatocellular	RT-PCR	Shorter disease-free survival	[29]
	IHC	Larger tumor size	[12]
		More vascular invasion	
	IHC	More microvascular invasion	[30]
		Higher recurrence rate	
		Poorer survival	
Other cancers			
NSCLC	RT-PCR	Larger tumor size	[31]
		Higher LN meta.	
Thymoma	RT-PCR	More advanced stage	[33]
Ovarian	RT-PCR	Higher LN meta.	[32]
	IHC	More advanced stage	[34]
		Higher FIGO staging	
Prostate	IHC	Metastatic prostate ca.	[35]
Lymphoma	Microarray	Highest expression in diffuse B-cell lymphoma	[36]
HNSCC	Microarray	Higher LN meta.	[37]
	IHC	Higher LN meta.	[38]
		More advanced stage	
		Deeper wall invasion	

NSCLC non-small cell lung cancer, *HNSCC* head and neck squamous cell carcinoma, *IHC* immunohistochemistry, *LOH* loss of heterozygosity, *RT-PCR* reverse transcription-polymerase chain reaction, *MVD* microvessel density, *LN meta.* lymph node metastasis

MTA1 is a strong candidate for a breast cancer metastasis-promoting gene. Furthermore, using immunohistochemistry they examined the MTA1 protein expression in primary human breast cancer samples and demonstrated that node-negative breast cancers with overexpression of MTA1 protein had a higher risk of disease relapse similar to node-positive tumors. Thus, the overexpression of MTA1 may be a useful predictor of early disease relapse [18].

Jang et al. [19] also showed that MTA1 overexpression was closely associated with higher tumor grade and high intratumoral microvessel density in surgically resected human breast cancers, suggesting that MTA1 may be a useful predictor of an aggressive phenotype and a possible angiogenesis-promoting molecule in breast cancer.

Gastrointestinal cancer

By using a reverse-transcription polymerase chain reaction (RT-PCR) method, we demonstrated that the higher expression of *MTA1* mRNA in surgically resected human gastric and colorectal cancer specimens compared to the paired normal counterpart tissues was significantly correlated to the depth of cancer invasion and lymph node metastasis [4]. This study was the first to demonstrate the clinical relevance of MTA1 expression to the malignant potentials of human cancers. Higher expression of *MTA1* mRNA was also shown in colorectal cancers compared to the normal counterpart tissue by another group [20].

Using a RT-PCR method, we found that human esophageal squamous cell cancers overexpressed *MTA1* mRNA. The overexpressing cancer cells showed significantly higher frequencies of adventitial invasion and lymph node metastasis and tended to have a higher rate of lymphatic involvement [5]. Using immunohistochemistry, we further examined the protein expression level of MTA1 in human esophageal squamous cell cancers and reconfirmed the results obtained by RT-PCR [21]. In this study, we also demonstrated that MTA1 was a predictor of poor prognosis after surgery [21].

In another observation, Kidd et al. [22] showed that it was useful to examine the expression of *MTA1* mRNA and MTA1 protein in order to determine the malignant potential and the propensity to metastasize of small intestinal carcinoid (enterochromaffin cell) tumors. When compared to nonmetastatic primary tumors, the expression of MTA1 was increased in malignant small intestinal carcinoids and in metastases to liver and lymph nodes [22–24]. This same group further reported that MTA1 was a good candidate genetic molecular marker to discriminate gastric carcinoids from other gastric neoplasms [25] as well as malignant appendiceal carcinoids from benign tissue [26]. In these studies, MTA1 was thought to be a good marker to define the malignancy of carcinoid tumors.

In addition to cancers of the gastrointestinal tract, the involvement of MTA1 overexpression in carcinogenesis and cancer progression was shown in other gastrointestinal tumors, such as pancreatic cancers and hepatocellular carcinomas. Iguchi et al. [27] examined *MTA1* mRNA expression in pancreatic cancer cell lines and resected pancreatic cancer tissues and found that increased levels of *MTA1* mRNA expression might be involved in the progression of pancreatic cancer. Recently, Miyake et al. [28] showed the expression level of MTA1 protein correlated with poorer prognosis of pancreatic cancer patients.

The possible association of *MTA1* expression with the malignant properties of hepatocellular carcinomas (HCC) was first reported by Hamatsu et al. [29]. In this study, *MTA1* mRNA level was assessed by RT-PCR in resected human HCC tissues, and its high expression predicted a lower disease-free survival rate after curative hepatectomy for HCC. Using immunohistochemistry, Moon et al. [12] examined MTA1 protein expression in resected human HCC specimens. They showed that overexpression of MTA1 was associated with HCC growth and vascular invasion and that nuclear localization of estrogen receptor (ER) α inversely correlated with MTA1 expression, suggesting that MTA1 was involved in negative regulatory mechanisms. Ryu et al. [30] reported that MTA1 was closely associated with microvascular invasion, frequent postoperative recurrence, and poor prognosis in patients with HCC, especially in those with hepatitis B virus (HBV)-associated HCC.

Other cancers

The relationship between MTA1 expression and malignant properties, such as invasion and metastasis, has been investigated in many other carcinomas and sarcomas. High expression of *MTA1* mRNA was correlated clinicopathologically with lymph node metastasis of human non-small cell lung cancers [31] and ovarian cancers [32], and to the advanced stage and invasiveness of thymomas [33]. Dannennmann et al. [34] reported that overexpression of MTA1 protein in ovarian cancer was significantly correlated to more advanced stage and higher FIGO staging. The potential role of MTA1 protein expression has also been suggested in the progression of human endometrial carcinomas [14]. In prostate cancers, Hofer et al. [35] showed that metastatic prostate tumors demonstrated significantly higher intensities of MTA1 protein expression and higher percentages of tissue cores staining positive for MTA1 than in clinically localized prostate cancers or benign prostate tissues. The high expression of MTA1 in diffuse B-cell lymphomas was also reported in human cases [36].

Using DNA microarray analysis, Roepman et al. [37] investigated gene expression patterns in lymph node

metastases of head and neck squamous cell carcinomas. They showed that the *MTA1* gene was the only single gene that showed consistently changed expression between numbers of matched pairs of primary tumor and lymph node metastases. Recently, further evidence was reported showing that overexpression of MTA1 protein in oral squamous cell carcinoma correlated to higher lymph node metastasis, deeper wall invasion and more advanced stage [38].

Biological relevance of MTA proteins to carcinogenesis and cancer progression

In addition to the clinicopathological evidences mentioned above, the biological relevance of MTA proteins to carcinogenesis and cancer progression has been made much clearer by the following important experiments.

The direct evidence to show the association of MTA1 expression with breast cancer malignant properties was first obtained by Mazumdar et al. in 2001 [39]. They demonstrated that forced expression of the MTA1 protein in breast cancer cell line MCF-7 was accompanied by enhancement of the ability of cells to invade an artificial matrix and to grow in an anchorage-independent manner. They also showed that the enhancement was associated with the interaction between MTA1 protein and histone deacetylase, resulting in a repression of ERα-mediated transcription (This will be discussed in more detail later).

The above study was extended by further experiments by the same group where they showed direct in vivo evidence of the involvement of MTA1 in the carcinogenesis of breast cancer in an animal model [10, 40]. This group established transgenic mice that overexpressed MTA1 protein. The MTA1-transgenic mice showed an inappropriate development of mammary glands, and the mice eventually developed hyperplastic nodules and mammary tumors, including adenocarcinomas. Most interestingly, MTA1-transgenic mice were accompanied by high incidence of spontaneous B cell lymphomas, including diffuse large B cell lymphomas [13, 41].

The clinicopathological correlation of MTA1 overexpression with squamous cell carcinomas was reinforced by the experimental results of Mahoney et al. [42]. They transfected *MTA1* cDNA into immortalized human keratinocytes and clearly showed that forced expression of *MTA1* contributed to several aspects of enhanced metastatic behavior, including increased migration, invasion and survival in the anchorage independent state of the immortalized keratinocytes. Furthermore, Qian et al. [43] inhibited *MTA1* expression by RNA interference (RNAi) in a human esophageal squamous cell carcinoma cell line and showed the significant inhibition of in vitro invasion and migration properties of the cancer cells.

Direct evidence showing the role of MTA1 in the progression of pancreatic cancer was provided by Hofer et al. [44]. They transfected *MTA1* cDNA into the pancreatic cell line PANC-1 and demonstrated that enhanced expression of MTA1 promoted the acquisition of an invasive and metastatic phenotype and that it enhanced the malignant potentials of pancreatic adenocarcinomas by modulation of the cytoskeleton via IQGAP1.

Molecular mechanisms of the MTA family, especially MTA1, in carcinogenesis and cancer progression

As mentioned above, it was demonstrated by different approaches and by different laboratories that MTA1 overexpression was closely correlated with carcinogenesis and cancer progression of a wide range of cancers originating in disparate organs and tissues. This strongly indicates that MTA1 may be one of the important key molecules in the cancer progression field. Thus, it will be absolutely necessary to clarify the molecular mechanisms in which MTA family members exert their functions for the clinical utilization of MTA proteins for diagnosis or treatment of human cancers. Here, we introduce the several important functions of MTA proteins that have been clarified, especially those that are concerned with carcinogenesis and cancer progression.

Nucleosome remodeling and histone deacetylation complex and transcriptional regulation

The first notion about the molecular and biochemical functions of MTA1 was accidentally obtained by four independent groups in 1998–1999 [9, 45–48]. In these studies, two disparate chromatin modifying activities, ATP-dependent nucleosome remodeling activity and histone deacetylation, were functionally and physically linked in the same protein complex. This complex has been named the NuRD (Nucleosome remodeling and histone deacetylation), and it contains histone deacetylase (HDAC) 1, HDAC2, the histone binding proteins RbAp46/48 and the dermatomyositis-specific autoantigen Mi-2, which has been shown to have transcription repressing activity. Xue et al. [46] reported that MTA1 protein was found in the NuRD complex, and it had strong transcription repressing activity. Subsequently, Zhang et al. [47] reported that a protein similar to MTA1 (named MTA2) was also a component of the NuRD complex and that MTA2 is highly expressed in rapidly dividing cells. Later, MTA3 was identified as an estrogen-inducible gene product that forms a distinct NuRD complex [15]. We also reported the physical interaction between MTA1 and HDAC1 [49] (Fig. 1).

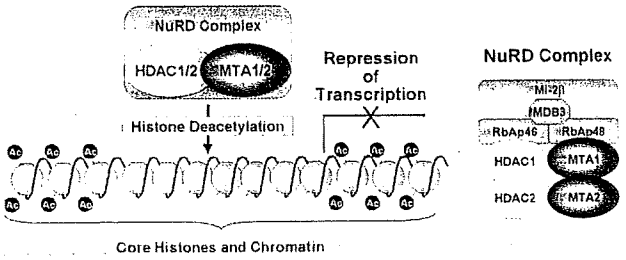

Fig. 1 The fundamental function of MTA proteins. The fundamental function of MTA proteins is chromatin remodeling and histone deacetylation, resulting in repression of transcription. MTA proteins are included in the protein complex named NuRD, which also contains histone deacetylases (HDAC1 and 2), major DNA binding protein 3 (MDB3), histone binding proteins RbAp46/48 and the dermatomyositis-specific autoantigen Mi-2, and has strong transcription repressing activities

Thus, the fundamental functions of the MTA family members appear to be exerted through a NuRD complex that has chromatin remodeling and histone deacetylating properties (There is also deacetylating property of non-histone proteins in the NuRD complex). In addition, the MTA-NuRD complex shows transcriptional repression activities [6–8, 10, 50]. Although all MTA family proteins are found in NuRD complexes, these proteins form distinct NuRD complexes that are thought to target different sets of promoters [9].

Repression of the transactivating function of ERα by MTA proteins

Although the involvement of MTA proteins in NuRD complexes suggested that such complexes might function in chromatin remodeling and histone deacetylation, a direct target of MTA proteins was first identified by Mazumdar et al. in 2000 [39]. MTA1 was identified as a molecule induced by a growth factor, heregulin-beta1 (HRG), which is a natural ligand of the human epidermal growth factor receptors HER3 and HER4 that can also transactivate HER2 (c-erbB-2) in human breast cancer cell lines. They showed that MTA1 directly interacted with the ligand-binding domain of ERα and that HRG stimulated the association of MTA1 and HDAC2 on the chromatin of an ER-responsive element (ERE) in the promoters of the estrogen responsive genes, such as pS2 and c-myc. This explains the phenomenon that activation of HRG/HER2 pathway in ER-positive breast cancers results in the suppression of ERα functions, resulting in more invasive and aggressive phenotypes observed in ER-negative breast cancers [51]. The repressive function of MTA1 on ERα is mediated through histone deacetylation by HDAC1 and HDAC2, suggesting that MTA1 has a potent corepressor function on the transactivation function of ERα through histone deacetylation (Fig. 2a). MTA2 has also been shown to physically interact with ERα and to repress its transactivating function. Furthermore, overexpression of MTA2 rendered cells unresponsive to estrogen and suppressed estrogen-induced colony formation in breast cancer cells [52] (Fig. 2a, b).

Recently, Khaleque et al. [53] showed that MTA1 binds to a heat shock factor 1 (HSF1), the transcriptional activator of the heat shock genes, in vitro and in human breast carcinoma samples. They demonstrated that HSF1-MTA1 complex formation was strongly induced by HRG and that the complex was incorporated into the NuRD complex and participated in repression of estrogen-dependent transcription in breast cancer cell treated with HRG.

Following the report by Mazumdar et al. [39], the same research group reported that several molecules, such as ménages a trios 1 (MAT1), MTA1-interacting coactivator (MICoA) and nuclear receptor interacting factor 3 (NRIF3), all interact with MTA1 and repress the transactivation function of ERα [8]. These three MTA1-binding proteins themselves have coactivator properties upon ERα transactivation. Talukder et al. [54] identified MAT1, an assembly and targeting ring finger factor for cyclin-dependent kinase-activating kinase (CAK), as a MTA1-binding protein. The interactions between CAK and MTA1 apparently regulate the transactivation activity of ERα in a CAK-dependent manner in breast cancer cells. In contrast, MICoA-mediated ERα transactivation functions are opposed by MTA1 through the recruitment of HDACs [55]. Furthermore, the interactions between MTA1 and NRIF3, an estrogen-inducible gene, may be important in modulating the sensitivity of breast cancer cells to estrogen [56]. Singh et al. [57] identified another MTA1-binding partner, Lim-only protein 4 (LMO4). LMO4 was found to be a component of the MTA1 corepressor complex, and its overexpression repressed ERα transactivation functions in a HDAC-dependent manner, proposed to result in the acquisition of the ERα-negative phenotype and increased aggressiveness in breast cancer cells.

A short form of MTA1 protein was subsequently identified and named MTA1s (Fig. 2a) [16]. MTA1s is a splice-variant of MTA1 and contains an ER-binding motif (nuclear binding motif) without any nuclear localization signals at the C-terminus. This protein localizes in the cytoplasm where it sequesters ERα, resulting in the prevention of ligand-induced nuclear translocation of ERα and of stimulation of the malignant phenotype of breast cancer cells. This suggests that the regulation of the cellular localization of ERα by MTA1s may represent a mechanism for redirecting nuclear receptor signaling by nuclear exclusion. MTA1s has also been shown to associate with casein kinase I-gamma2, which is an estrogen-responsive kinase [58].

MTA3 is the latest addition to the MTA family. It was identified as an estrogen-dependent component of the