ね感度75～80%，特異度90～97%，正診率90～95%程度とされ，高い正診率が示されている[5, 6]．これらの値はセンチネルリンパ節生検での一般的な一致率である，感度90～95%，特異度95～100%，正診率95～99%には及ばないが，触診あるいは超音波検査と比較し客観的な画像として捉えられ，また，とくに高い特異度という点からセンチネルリンパ節生検のときバックアップ郭清を行う症例の臨床判断材料の1つとしてその有用性が試みられている．

Ⅳ MRI/CTの今後の展望

①ハイリスク患者に対する検診

近年，遺伝子診断や乳がんリスク診断の研究の進歩に伴って，乳がん発症リスクの高い女性に対するフォローの重要性とその方法が議論されるようになってきた．現在，National Comprehensive Cancer Network (NCCN)による乳がん検診のガイドラインでは，遺伝子異常や強力な家族歴をもつハイリスク女性には25歳から年に1回のマンモグラフィ検査に加えて年1回のMRI検査が推奨されている．日本においては，現在遺伝子検査や検診目的でのMRIは保険上認められていないが，患者の意識の高まりとともに遺伝子外来を開設する施設は増加しており，ハイリスクの診断がついた場合，検診としてMRIを行うようになる可能性は高い．このような場合，手術を前提とした範囲診断ではなく，乳がんがあるかどうかの存在，質的診断を行う必要性がある．今後さらなる診断精度の向上や，標準化された撮影方法と広く使用可能な診断基準が求められる．また，検診で行う場合，従来の造影剤を使用する検査では侵襲が大きいため，造影剤に代わるMRI撮影法が開発され議論され始めている．

②MRI/CTで発見された病変に対する対応

MRIやCTが検診だけでなく，広く日常診療に使用されるようになったことでマンモグラフィや超音波では明瞭に描出できない乳腺病変や乳がんを疑う病変が増加してきている．確定診断のための病理検査である細胞診や組織診のために，近年MRI/CTの画像を超音波画像と同期させ前者で認められた異常の部位を後者で特定するreal-time virtual sonography (RVS)といった装置や，MRIガイド下に穿刺を行うシステムや器具が研究，開発され始めた．こういった装置が今後，MRIやCTによる検診の始まりとともに広く使用される可能性があり，乳がんの早期診断において大変有用なモダリティになると思われる．

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麻酔科医のための

3D 解剖学講座

7時限目

監修・ワンポイントメモ／
岡山大学 人体構成学　大塚 愛二
協力／パナソニック株式会社
写真撮影／武田 吉正・大塚 愛二

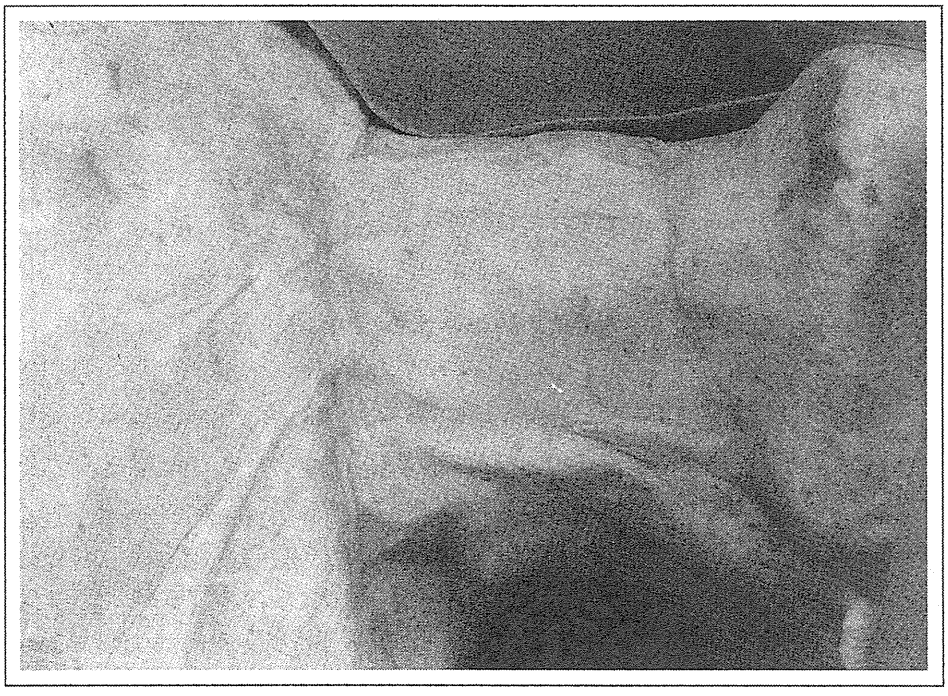
土井原 博義
岡山大学病院 乳腺・内分泌外科

内分泌外科手術

＜撮影方法＞
腕神経叢上神経幹が被写体の中心に来るように画角を設定した。カメラと上神経幹の距離を一定に保ちつつ5度ずつ異なる角度から撮影を行った。
便宜上，上神経幹を体幹左方水平位置から撮影したポイントを緯度0度，経度0度とした。真上からの撮影は北緯90度，頭側からの撮影は東経90度と表現した。

内分泌外科手術，主に甲状腺および副甲状腺の手術に必要な解剖について解説する。甲状腺切除術において特に重要なのは，反回神経，上喉頭神経外枝の走行であり，また頸部郭清術を伴う場合は，迷走神経，副神経，横隔神経，交感神経，胸管の走行である。いずれも損傷により術後の合併症やQOLに大きな影響を与えるので，注意が必要である。内分泌外科手術では，これらの神経と総頸動脈や内頸静脈などの大血管の関係を十分に理解することが重要である。

甲状腺，副甲状腺手術に必要な頸部の解剖



【写真1】
北緯50度，経度0度より撮影
体表からは胸鎖乳突筋，外頸静脈，鎖骨，甲状軟骨が観察できる。触診にて甲状腺の位置，総頸動脈，輪状軟骨を確認することができる。

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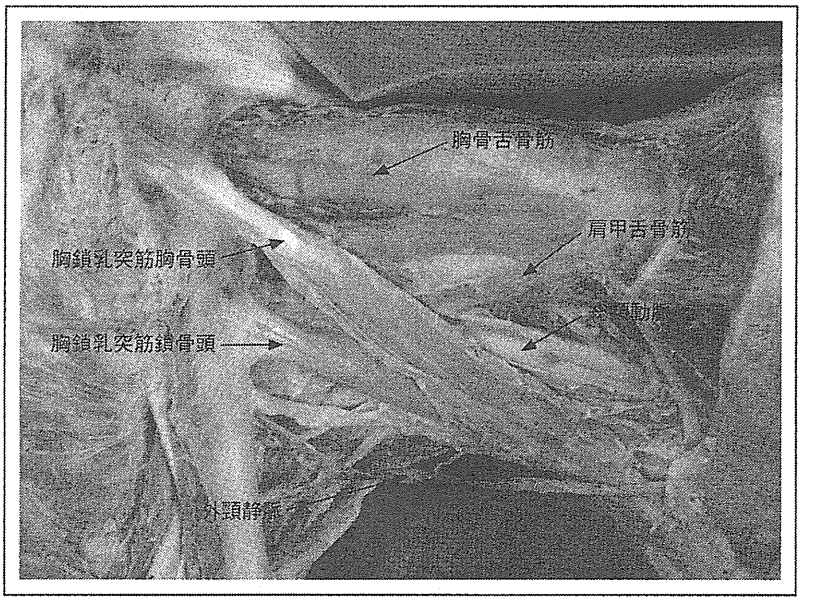
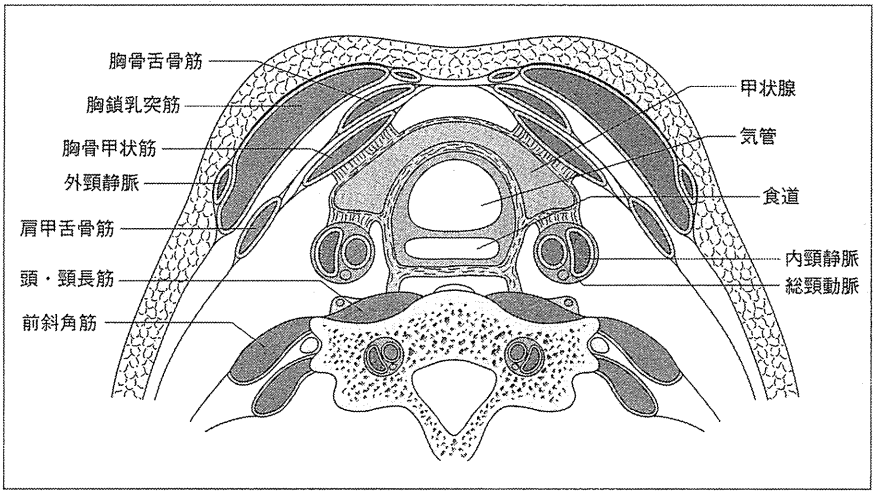
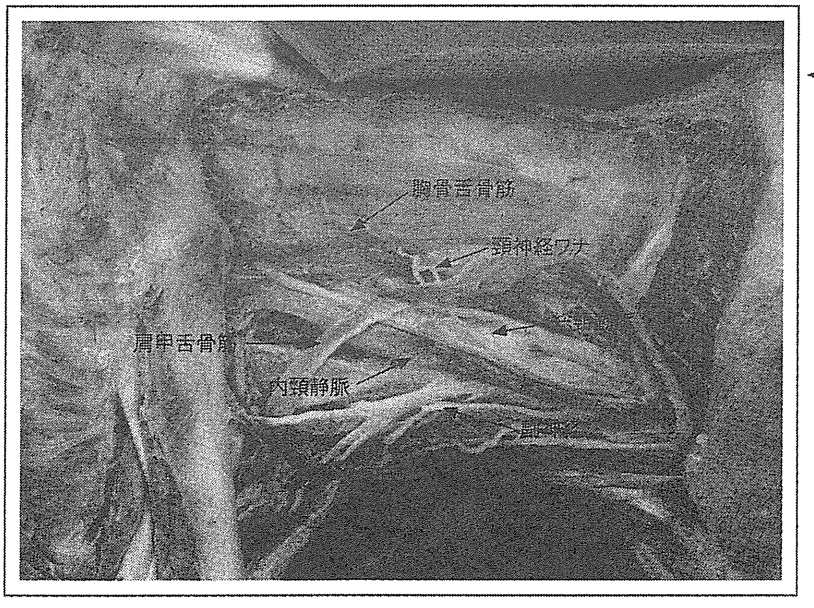


【写真2】北緯75度，経度0度より撮影
皮膚と広頸筋を除くと，胸鎖乳突筋の胸骨頭と鎖骨頭が見える。舌骨下筋群は甲状腺の前面を覆う縦走筋で，第1層は胸骨舌骨筋と肩甲舌骨筋からなる。

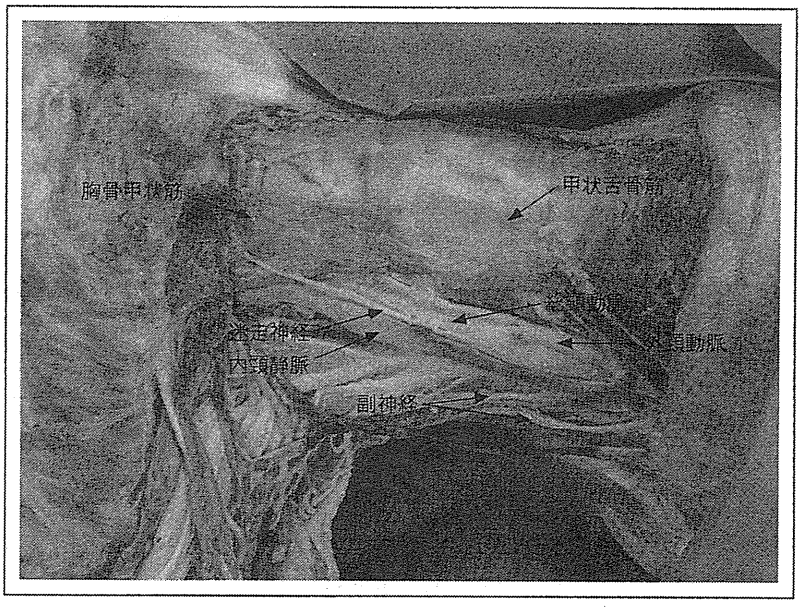
頸部は浅層筋（胸鎖乳突筋），中層筋（胸骨舌骨筋，肩甲舌骨筋，胸骨甲状筋），深層筋（頭・頸長筋と前斜角筋）の3層の筋群からなる。浅層筋と中層筋は前方に半円形の空間を作り，その中心部に気管があり，その両脇に大血管，神経が縦走している[1]（図1）。

▼図1　頸部の筋と筋膜（文献1より作成）





【写真3】北緯75度，経度0度より撮影
胸鎖乳突筋を切離すると，頸神経ワナが見える。



【写真 4】北緯 75 度，経度 0 度より撮影

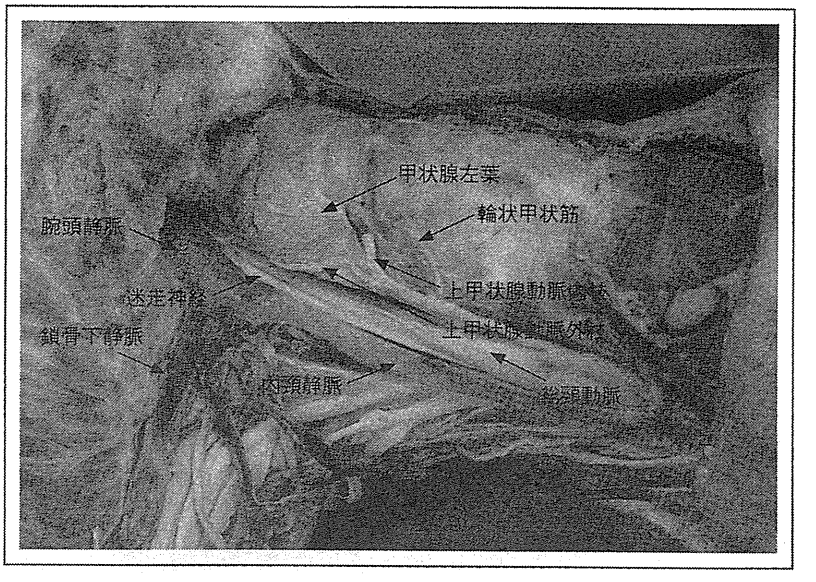
鎖骨と胸骨舌骨筋を切離すると，舌骨下筋の第 2 層の甲状舌骨筋と胸骨甲状筋が見える。舌骨下筋を支配するのが頸神経ワナで，舌下神経の枝のように見える上根は C_1 および C_2 に由来し，C_2 と C_3 に由来する下根と結合してワナをなしている。なお，万一この神経を切断しても，大きな障害はない[2]。

ワンポイントメモ 1

頸神経ワナを切断しても大きな障害が出ないのはなぜだろうか。次のような理由があげられよう。

・ワナの切断部位にもよるが，上根と下根の両方向からの神経線維がワナの中を走っているので，1 か所の切断では完全マヒを引き起こしにくい。

・舌骨下筋群の作用は舌骨を引き下げる（開口時に固定する）ことであるが，反対側にもあるので，まったくその機能がなくなることはない。



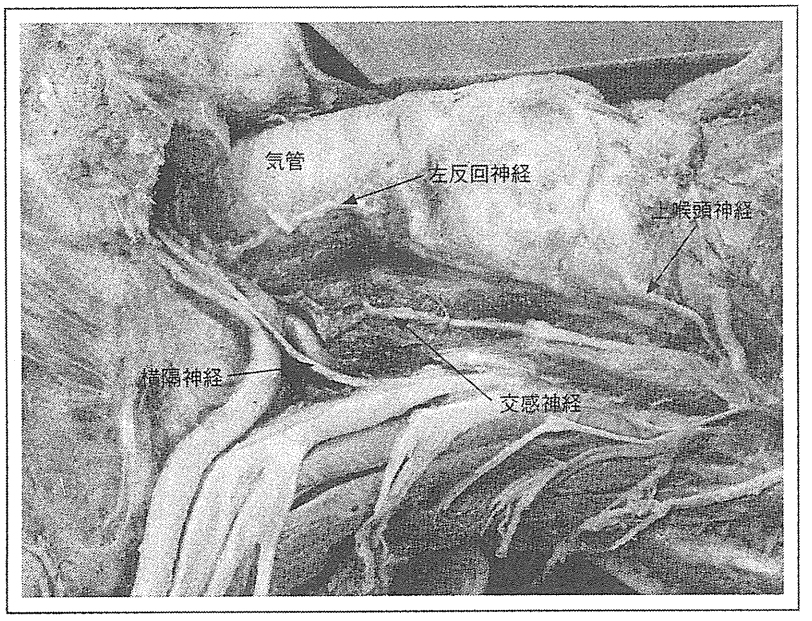
【写真 5】北緯 75 度，経度 0 度より撮影

甲状腺周囲の筋肉を除いた画像である。

外頸動脈から分枝する上甲状腺動脈は，甲状腺を栄養する最大の血管である。甲状腺上極から流入し，外枝，内枝，後枝に分かれる。上甲状腺動脈周囲には上喉頭神経（**写真 6 参照**）外枝が伴走しており，切断すると，長時間の発声ができない，低音になるといった症状が出るので注意が必要である。

下甲状腺動脈は，鎖骨下動脈から分岐した甲状頸動脈より分岐し，総頸動脈の裏面を横走し，甲状腺側面より流入する。下甲状腺動脈が分岐して甲状腺に流入する部では反回神経が交差することが多く，甲状腺切除の際に損傷しやすい部位である。

また，約 10％の頻度で腕頭動脈から分岐して甲状腺下縁に達する最下甲状腺動脈があり，気管前面を上行するので，気管切開時には注意が必要である。上甲状腺静脈および中甲状腺静脈は内頸静脈に流入するが，下甲状腺静脈は内頸静脈の下端部ないしは腕頭静脈に流入する。



ワンポイントメモ2

反回神経が欠損する場合がある。

通常，迷走神経の枝の反回神経は，右側は右鎖骨下動脈で，左側は大動脈弓でUターンし，上行して下喉頭神経となる。これは，胎生期の動脈弓と神経との関係により生じる現象である。まれではあるが，大動脈弓から，右総頸動脈，左総頸動脈，左鎖骨下動脈，右鎖骨下動脈の順に分岐し，しかも右鎖骨下動脈が食道の後ろを通るという破格例が知られている（0.2%：足立のG型）。この場合，Uターンで引っかかるはずの動脈弓が消失してしまっているので，反回神経と呼ぶべき神経が見当たらない。下喉頭神経は，迷走神経の枝として喉頭の高さで直接枝分かれする。術前の画像診断で大動脈弓から分岐する動脈の異常が確認できれば，このような破格の可能性をあらかじめ想定できるであろう。

【写真6】北緯45度，経度0度より撮影

リンパ節郭清の際に必要な側頸部の神経，血管の解剖である。迷走神経や副神経（**写真4，5参照**）に加え，横隔神経，交感神経などが重要である。

迷走神経は総頸動脈と内頸静脈の間を下行し，上喉頭神経を分岐する。上喉頭神経内枝は喉頭上半分を支配する知覚神経であるが，上喉頭神経外枝は輪状甲状筋や下咽頭収縮筋を支配する運動神経である。迷走神経はさらに下行して，右では鎖骨下動脈，左では大動脈弓の前面を通り，反回神経を分岐する。

反回神経は，気管と食道の間を上行し，食道枝を分岐した後に喉頭神経となり，喉頭諸筋に分布する。下甲状腺動脈，総頸動脈および気管壁で構成される三角の中を丁寧に剝離すると容易に同定できる。右は気管壁からやや離れた総頸動脈寄りで上行し，左は気管壁と食道の間を走行しており，左右で走行部位が異なるので注意を要する。損傷すると誤嚥や声帯の固定による嗄声をまねく。

前斜角筋の前面には交感神経幹と横隔神経が走行しており，損傷するとHorner症候群や横隔膜の挙上をきたす。

副神経は胸鎖乳突筋の上1/3を貫き，同筋を支配する胸鎖乳突筋枝を分岐した後に胸鎖乳突筋後縁に現れ，僧帽筋に至る。

甲状腺手術

甲状腺の手術術式は，腫瘍核出術，峡部切除術，葉部分切除術，葉切除術，亜全摘術（全体の2/3以上切除），準全摘術（残存組織が1g以下），全摘術からなり[3]，通常，良性疾患では，葉部分切除術または葉切除術，癌あるいはBasedow病では，亜全摘術，準全摘術，全摘術を行う。

■■■■ 甲状腺手術手順

◎体表からの確認

腫瘍の位置，可動性，硬度，リンパ節腫大などを触診で確認し，気管浸潤の有無や周囲血管への浸潤の有無を把握する（**写真1参照**）。

①皮膚切開および皮弁作成

鎖骨の上縁から1.5〜2横指頭側で，皮膚割線に沿って襟状切開を行う。広頸筋下の前頸筋群（胸骨舌骨筋，胸骨甲状筋）の前面で皮弁を作成する。頭側は甲状軟骨下縁，尾側は鎖骨上縁，側方は胸鎖乳突筋外縁まで行う（**写真2〜4参照**）。肩甲舌骨筋は外側に剝離，圧排しておく[4]。

②甲状腺の露出

前頸筋群と胸鎖乳突筋，甲状腺前面の間を剝離，前頸静脈を結紮切離後，前頸筋群を横切する。良性疾患の場合は前頸筋群を切離せず，外側へ圧排することもある。この操作により甲状腺の全貌が明らかになる（**写真5**）。

③血管処理

まず上甲状腺動静脈の切離から開始する。上喉頭神経外枝を傷つけないように，甲状腺上縁で動静脈が前内枝と後側枝に分岐してから切離する（**写真5**）。続いて中甲状腺静脈，下甲状腺動静脈を切離するが，下甲状腺動脈は反回神経と交差しており，傷つけないよう注意が必要である。

④反回神経の同定（**写真6**）

右反回神経は気管壁よりやや外側，左反回神経は気管食道溝を上行しており，いずれも下甲状腺動脈と交差している。したがって，甲状腺を内前方に脱転後，下甲状腺動脈が甲状腺に流入する直前で反回神経を同定することが可能である。右反回神経は動脈の前面を通ることが多く，左反回神経は逆に後面を通ることが多いが，分岐した動脈に挟まれていることも少なからずある。

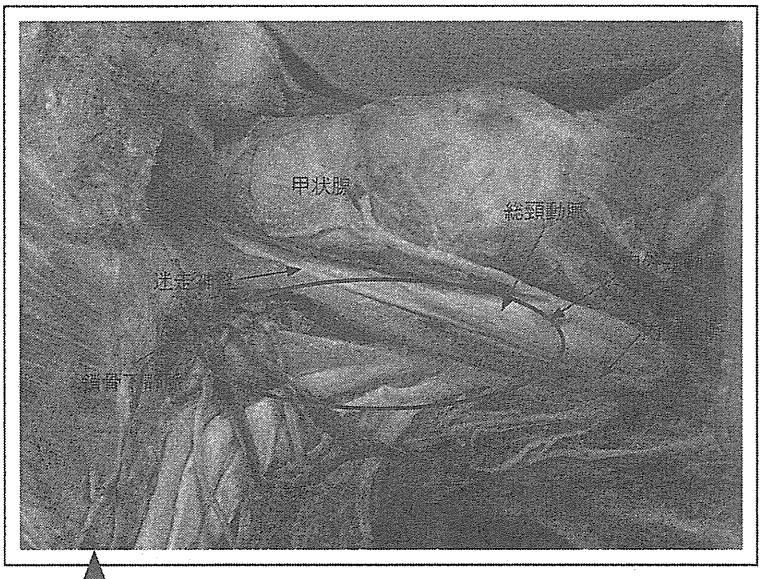
⑤甲状腺切除

甲状腺と気管壁は比較的疎になっており，気管壁に沿って甲状腺を剝離すれば出血はほとんどない。最後に，輪状軟骨の尾側で気管壁と甲状腺を固定しているBerry靭帯を結紮切離して，摘出を終了する。

⑥内深頸リンパ節郭清（**写真7**）

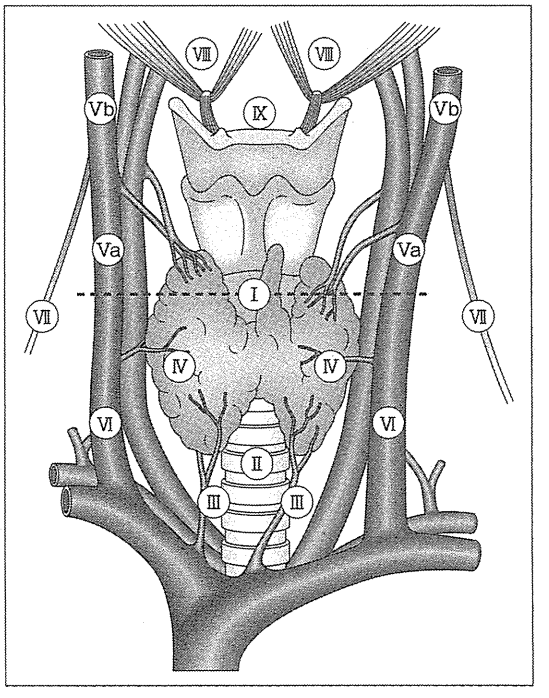
図2に甲状腺所属リンパ節の名称を示す。通常はⅠ～Ⅶまでのリンパ節と，前面は胸鎖乳突筋，後面は前斜角筋と後頸筋，内側は内頸静脈，頭側は内外頸動脈分岐部に囲まれたリンパ節を郭清する。

まず内頸静脈および迷走神経を全長にわたって剝離し，さらに尾側に向かって剝離を進め，静脈角を処理する。左側では胸管があるので，リンパ漏を引き起こさないように注意して剝離，結紮する。次に頭側に向かって剝離を進め，頸横動脈，横隔神経，副神経を確認しながら外側および頭側を結紮処理して郭清を終了する。



【写真7】北緯45度，経度0度　○はリンパ節郭清範囲

▼図2　甲状腺の所属リンパ節（文献3より，作成）
Ⅰ：喉頭前，Ⅱ：気管前，Ⅲ：気管傍，Ⅳ：甲状腺周囲，Ⅴ：上内深頸，Ⅵ：下内深頸，Ⅶ：外深頸，Ⅷ：顎下，Ⅸ：頤下，----：輪状軟骨下縁



副甲状腺について

副甲状腺は通常，甲状腺近傍に 4 腺存在する。しかし，その解剖学的定数と位置は一定でなく，過剰腺や異所性副甲状腺が多く存在する。上副甲状腺は第 4 鰓嚢（さいのう）より胸腺とともに下降し，甲状腺の比較的上部に定着するが，下副甲状腺は第 3 鰓嚢より胸腺とともに頸動脈鞘内を下降し，途中で胸腺と分かれて甲状腺下部に定着する。約 80％の症例では 4 腺であるが，3 腺のみや 5 腺以上の過剰腺もときにみられる。また，甲状腺近傍に 85％の症例で副甲状腺を確認することができるが，特に下副甲状腺は胸腺内，食道裏面や甲状腺内にも異所性甲状腺としてみられることがある[1]。

副甲状腺の手術

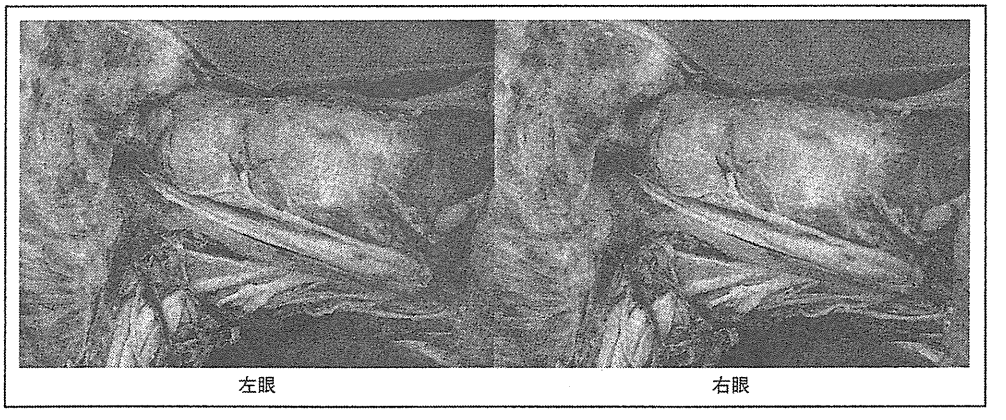
皮膚切開および皮弁作成は甲状腺手術と同様である。まず胸鎖乳突筋と前頸筋群の間を剝離（**写真 2**），甲状腺外縁を確認後，腫大した副甲状腺を検索する。上副甲状腺は甲状腺の背面で反回神経が下甲状腺動脈と交差し，輪状軟骨下縁で喉頭に入るあたりを中心に検索する。下副甲状腺は甲状腺下極から胸腺舌部と分布範囲は広く，時に縦隔内にも存在する（**写真 8**）。いずれも副甲状腺周囲を丁寧に剝離，被膜を破らないように摘出することが重要である。二次性副甲状腺機能亢進症のように，4 腺摘出した場合は，摘出した副甲状腺から 80 mg 程度を前腕筋肉内あるいは胸鎖乳突筋内に移植して，手術を終了する[5]。

【写真 8】北緯 5 度，経度 5 度　赤色部は下副甲状腺の位置

以上，甲状腺および副甲状腺手術において重要な頸部の解剖のポイントを解説した。本疾患の手術では反回神経，迷走神経，副神経など術後の QOL に影響する神経が多く見られ，慎重な手技が必要とされるが，何よりも血管との関係を踏まえた解剖を熟知することが重要である。

ステレオ画像

甲状腺および周囲の血管，神経を示す。



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症 例

エンテカビル投与によって乳癌化学療法時のHBV再活性化を予防した1例

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A Case of the Usage of Entecavir to Prevent Hepatitis B Virus Reactivation during Chemotherapy in Breast Cancer Patient: Takayuki Muraoka[*1], Naruto Taira[*1], Tadahiko Shien[*1], Hiroyoshi Doihara[*1], Akinobu Takaki[*2] and Shinichiro Miyoshi[*1] ([*1]*Dept. of Cancer and Thoracic Surgery, and* [*2]*Dept. of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences*)

Summary

Hepatitis B virus (HBV) reactivation is a serious clinical problem for HBV infected patients, and one of its possible causes is chemotherapy for malignant disease. At the onset of active hepatitis, planned chemotherapy should be discontinued and acute or fetal fulminant hepatitis must be induced in some cases. Therefore, it is desirable to prevent virus reactivation during chemotherapy in HBV-positive patients. We report a case in which adjuvant chemotherapy for a breast cancer patient was accomplished safely by using entecavir. The patient was a 48-year-old woman with breast cancer whose HBV infection had been pointed out when she was 20 years old. Breast reconstruction was performed, followed by mastectomy. Pathological findings were invasive ductal carcinoma, three positive nodes, estrogen and progesterone receptor-positive, and HER2-negative. An adjuvant chemotherapy with anthracycline followed by taxane was planned. Blood chemistry revealed the seroconversion of HBV and the quantity of HBV-DNA was 2.8 log copies/mL. Administration of the anti-virus agent, entecavir, was started three weeks before chemotherapy. The HBV-DNA was decreased under the titer of detection and no re-increase in HBV-DNA was found during chemotherapy. Planned chemotherapy was accomplished safely without HBV reactivation. **Key words**: Hepatitis B virus reactivation, Entecavir, HBV-DNA (*Received Feb. 28, 2011/Accepted Apr. 9, 2011*)

要旨 B型肝炎ウイルス(HBV)感染者の抗癌化学療法施行時にはHBVの再活性化が問題となる。活動性肝炎を発症すると治療の変更を迫られるのみでなく,急性肝炎から劇症肝炎を起引する場合があるため,HBV再活性化の予防が求められる。われわれはエンテカビルの予防投与により,乳癌術後の補助療法を安全に完遂した症例を経験した。患者は20歳時にHBV感染を指摘されていた48歳,女性で,左乳癌のため乳房切除と広背筋皮弁による乳房再建術を受けた。最終診断はER陽性,PgR陽性,HER2陰性でT1N1M0,stage ⅡAの硬癌であった。アンスラサイクリンとタキサンによる化学療法とホルモン剤による補助療法を予定した。seroconversion状態で,HBV-DNA量は2.8 log copies/mLであった。HBV再活性化の予防のため化学療法開始前にエンテカビルを投与した。3週目にHBV-DNA量は検出感度以下となり,化学療法を開始した。以後DNA量は検出感度以下で推移し,肝炎の発症も認めず治療を完遂した。

はじめに

B型肝炎ウイルス(HBV)感染者の悪性腫瘍に対する化学療法の施行時には宿主免疫能の低下に伴い,HBVの再活性化が問題となる。HBVの再活性化には非活動性キャリアからのものと既往感染者からのものの二つがある。特に非活動性キャリアからの再活性化は既往感染者からの再活性化より高頻度で,化学療法施行時には再活性化予防の対策が求められる[1,2]。

以前より血液疾患などに対する化学療法施行時にはHBVの再活性化に関する対策がなされ,ラミブジン使用の有効性に関する報告が存在するが[3],エンテカビル

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など新規の核酸アナログ薬やHBV感染率の地域による相違などを含めて検討された十分なエビデンスはない[4]。

われわれは，HBVキャリアに対する乳癌術後補助療法に先行して日本肝臓学会の「免疫抑制・化学療法により発症するB型肝炎対策ガイドライン」に従いエンテカビルを予防的に使用することで，予定の治療を安全に完遂した症例を報告する。

Ⅰ．症　例

患者: 48歳，女性。

主訴: 自覚症状なし。乳癌検診で異常を指摘された。

既往歴: 20歳時にHBV感染を指摘されたが，その後は特別な検査や治療を受けていない。

現病歴: 2009年の乳癌検診で左乳癌と診断され，当院にて左乳房切除と広背筋皮弁による乳房再建術を施行した。最終病理診断は，浸潤性乳管癌，組織学的浸潤径は1.8 cm，ly2，v0，腋窩リンパ節は17個中3個に転移陽性，核grade 1，エストロゲン受容体陽性，プロゲステロン受容体陽性，HER2スコアは2+，HER2 FISHは陰性であった。術後補助化学療法として，アンスラサイクリンとタキサンの逐次投与を行う予定とした。

血液生化学検査: 外来での末梢血液，生化学および肝炎関連マーカーの一覧を（Table 1）に示す。トランスアミナーゼ値は正常範囲であり，活動性肝炎の所見を認めなかった。HBs-Ag（+）（COI 1,000），HBe-Ag（−），HBe-Ab（+）（100%），HBs-Ab（−），HBc-Ab（+）（100%）とseroconversion後のHBV非活動性キャリアの状態であった。HBV-DNA 2.8 log copies/mLであった。

術後経過: 化学療法の実施に先立ち，HBV再活性化予防目的にエンテカビル0.5 mg/dayの内服を開始した。内服開始18日目にHBV-DNAは検出感度以下となり，初回化学療法を実施した。HBs抗原値は変化しなかった。レジメンはAC（doxorubicin 60 mg/m^2，cyclophosphamide 600 mg/m^2）の3週毎の投与とした。通常，ACレジメンでは制吐剤としてステロイドホルモンを使用しているが，ステロイドホルモンの使用はHBV再活性化の危険因子とされているため，初回投与では使用しなかった。AC初回投与で認められた主な副作用は，悪心・嘔吐grade 2，脱毛grade 1であり，トランスアミナーゼ値の上昇を認めなかった。AC 2コース目からは悪心・嘔吐の対策として，コース前日夜のベンゾジアゼピンの投与と，前投薬としてのみdexamethasone 8 mg/bodyを併用した。悪心・嘔吐はgrade 1に軽減し，AC 4コースを完遂した。その後paclitaxel 80 mg/m^2，毎週の12回投与を行った。前投薬として，ヒスタミン1，2阻害剤，dexamethasone 8 mg/bodyを使用した。paclitaxel投与により認められた副作用は，しびれgrade 1，倦怠感grade 1と軽微なものであり，重篤な合併症なく完遂した。全経過中でトランスアミナーゼ値の上昇を認めず，4週毎にスクリーニングしたHBV-DNA量は検出感度以下を保ったまま推移した。化学療法終了後はtamoxifen内服を継続している。エンテカビル投与は化学療法終了後6か月，合計投与期間1年間で終了した。その後6か月を経過し，HBV-DNA量は検出感度以下のままで推移し，肝炎の出現も認めなかった。

Table 1 Laboratory data on admission before the operation

WBC	5,680/μL	γ-GTP	10 IU/L
RBC	4.47×10^6/μL	Ch-E	365 IU/L
Hb	10.4 g/dL	Na	141 mmol/L
Plt	205×10^3/μL	K	4.1 mmol/L
TP	7.2 g/dL	Cl	106 mmol/L
Alb	4.3 g/dL	BUN	11.7 mg/dL
T-Bil	0.67 mg/dL	Cr	0.81 mg/dL
GOT	13 IU/L	PT	11.0 sec
GPT	11 IU/L	APTT	32.8 sec

Ⅱ．考　察

HBV感染は東南アジアやアフリカなどの発展途上国に多く，全世界の80%がこれらの地域に集中しており，先進国では比較的まれである[5]。地域によっても肝細胞癌発生率や生存率は異なり[6]，その経過観察や治療方針に関する十分なエビデンスを備えたガイドラインは作成されていない[4]。本邦では成人の血中HBs抗原の測定によるHBV感染のスクリーニングが一般化しているが，欧米では流行地域での居住歴がある場合と悪性腫瘍に対する化学療法や免疫抑制療法を施行する場合にかぎってスクリーニング検査が行われている[1,4,7]。このため欧米での報告をそのまま本邦の臨床に応用することには慎重を要す。

慢性B型肝炎の治療開始を検討する基準はアメリカ[1]・ヨーロッパ[7]・日本，と各国の人種・異なるHBVジェノタイプ分布などの地域性を鑑みて定められている。2011年度の日本肝臓学会のガイドラインでは，35歳以上ではALT≧31 IU/LでHBe抗原陽性例はHBV-DNA量5 log copies/mL以上，HBe抗原陰性例では4 log copies/mL以上とされている。第一選択は基本的にはエンテカビルで，出産希望などがある場合にはインターフェロン療法のオプションもある。HBV-DNA量はウイルスの活動度と相関し，トランスアミナーゼ値よ

り鋭敏にウイルスの活動度を反映する[8]。また，seroconversion後の症例でもHBV-DNA量が高値の場合，肝生検を含めた精査が推奨されており，seronegative化とそれに引き続く肝炎発症をより早期に同定する目的でHBV-DNAに加えてHBe抗体ならびにHBe抗原も測定することが望ましい[7]。

現在，本邦で慢性B型肝炎に使用可能な核酸アナログ薬として，ラミブジン，アデフォビル，エンテカビルの3剤が保険適応となっている。2000年に最も早く保険承認されたラミブジンは使用開始から2年後の耐性ウイルスの出現率が46%と高率である[9]。一方，2006年保険承認されたエンテカビルは2年後に耐性ウイルスの出現を認めなかった[10]。また，ラミブジンに耐性を獲得したウイルスはエンテカビルに交叉耐性を示すものが含まれるため，現在では慢性B型肝炎初回治療時の第一選択薬はエンテカビルとなっている。1年間の使用におけるエンテカビルのラミブジンに対する優越性を検証した二重盲検無作為比較試験がある。HBe抗原陽性慢性B型肝炎715例の検討では主要評価項目である肝生検組織における組織学的改善のみならず，血液学的評価項目において，HBV-DNA量減少率，トランスアミナーゼ値正常化率がエンテカビル，ラミブジンにおいて各々67% vs 36%，68% vs 60%であった[11]。またHBe抗原陰性慢性B型肝炎症例648例の検討でも同90% vs 72%，78% vs 70%で[12]，やはりエンテカビルの優越性が示された。

化学療法施行時にはHBVキャリアもしくは既感染者におけるウイルスの再活性化が問題となる。キャリアに対する化学療法時には化学療法前に慢性B型肝炎の治療基準を満たさない群の約30%でHBVが再活性化する[13]。DNA量が上昇の傾向に転じた場合，予定の化学療法継続が困難となるばかりでなく，急性肝炎や劇症肝炎の発生によって死亡することもあるためウイルスの再活性化を予防することが大切である[4]。

Yeoらは化学療法を施行したHBVキャリア138症例を追跡調査し，HBV再活性化の危険因子について解析を行った結果，HBV-DNA量，ステロイドホルモンの使用，悪性リンパ腫，乳癌が独立した危険因子であると報告した[14]。このためHBVキャリアに対する化学療法時にはHBV-DNA量を減少させておく，ステロイドホルモンの使用を控えるなどの対応を行うことが望ましい。本症例では初回のAC療法時に通常前投薬として使用するステロイドホルモンを投与しなかったが，激しい嘔吐が出現し，以後の治療継続が困難であったため，慎重にステロイドホルモンを投与した。悪性腫瘍に対する化学療法の施行時において，HBV-DNA量が慢性B型肝炎の治療基準に合致しない患者にも抗ウイルス薬の投与によりDNA量を減少させておくことは有意義である。この場合にも核酸アナログ薬が中心的役割を果たす。Yeoらは乳癌化学療法時に，ラミブジンを使用することで，HBV再活性化を15%に減少させた[13]。日本肝臓学会の「免疫抑制・化学療法により発症するB型肝炎対策ガイドライン」においては先述の耐性ウイルスの問題よりエンテカビルの使用が推奨されており，本症例でもこのガイドラインに従って予防投与を行った。化学療法開始の18日前からエンテカビル投与を開始し，HBV-DNA量を測定することで，HBVの再活性化がないことを確認しながら治療を完遂できた。HuiらはHBs抗原陰性例のHBV再活性化症例においてHBV-DNA量はトランスアミナーゼ値に平均18週間先行して上昇することを報告している[8]。従来われわれは，肝炎の活動度を把握するために血中トランスアミナーゼ値を測定してきたが，今後はより鋭敏なマーカーであるHBV-DNA量を肝炎活動度の評価指標として測定する予定である。

化学療法に先立って予防投与した核酸アナログ薬の投与終了時期に関しても十分なエビデンスはないが，ガイドラインではHBs抗原陽性症例では一般的な肝炎治療に準じて1〜2年間の投与を推奨している[15]。化学療法終了後8か月でHBVが上昇した悪性リンパ腫のHBV再活性化症例が存在するため[16]，化学療法終了後も1年間程度は核酸アナログ薬の投与もしくは定期的なHBV-DNA量の測定が必要ではないかと考える。一方でDNA量の測定には時間と費用を要するため，今後費用対効果の検証などが求められる。今後，HBe抗原陽性ならびに陰性のHBVキャリア乳癌患者における化学療法施行前にエンテカビルを投与する群としない群による無作為比較試験が望まれるが，倫理上の問題もあり，今後の新たな試験組み立ては困難である。既存の研究から乳癌化学療法時にもHBV再活性化予防にはエンテカビルが有効であると考えられる。

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Original article

Does primary tumor resection improve outcomes for patients with incurable advanced breast cancer?

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ABSTRACT

Background: Metastatic breast cancer (MBC) is considered incurable, and surgery has only limited benefit in the treatment of this disease. However, recent reports have indicated that primary tumor resection may improve patient outcomes. We retrospectively analyzed the surgical benefits and prognostic factors for patients with MBC who were treated at our center.

Methods: Ninety-two women, who had tumors of greater than 5 cm and distant metastasis at diagnosis, were included in this study. The effect of surgical treatment on survival was evaluated. Patient demographics and tumor characteristics were also investigated.

Results: Thirty-six patients had surgery for resection of primary tumors. There were no substantive differences between individuals, or between tumor characteristics, for patients who underwent surgery versus patients who did not. The median survival time for surgically treated patients was 25.0 months versus 24.8 months for patients who did not undergo surgical resection ($P = 0.352$). Only three patients relapsed within three months of surgery. For the remaining majority of patients, primary tumor resection gave some relief from the often severe symptoms that come from harboring a large tumor for an extended time. In univariate and subsequent multivariate analyses of predictive indicators, a diagnosis of triple-negative breast cancer and/or metastasis to more than three sites was significantly associated with a severe prognosis.

Conclusion: Primary tumor resection failed to prolong overall survival times in patients with incurable advanced breast cancer that was greater than 5 cm. However, surgery did improve the quality of life in patients who were expected to have a relatively long prognosis.

Introduction

MBC is considered incurable, and is therefore an unfavorable prognostic for survival. This remains true despite the fact that the survival rate of patients with MBC has slowly and steadily improved due to the development of diagnostic tools for earlier detection of metastatic lesions, and systemic therapies that include new biologics.[1,2] Because of the low cure rate, therapy for patients with MBC often seeks to prolong survival or to palliate symptoms, while preserving quality of life. Systemic chemotherapy is one of the most effective treatments for MBC, and has become increasingly central to the therapeutic strategy for this disease. Although this is traditionally done according to Hortobagyi's algorithm,[3] successful chemotherapy depends on the expression status of the estrogen receptor (ER), the progesterone receptor (PgR), and the human epidermal growth factor receptor 2 (HER2), or on the existence of life-threatening visceral metastases.

Surgical removal of primary tumors has not been established as a standard treatment for MBC because it is generally accepted that local therapy provides no survival advantage once metastases have occurred. Additionally, tumor excision may further stimulate metastasis.[4] Therefore, primary tumor resection in patients with MBC is usually only applied as a palliative treatment for symptomatic wound complications such as bleeding, ulcer formation, unpleasant smell, and purulent discharge. In recent years, however, some retrospective studies have reported advantages of debulking surgery in terms of improving patient outcome.[5–10]

The aim of this study was to determine through retrospective analyses if surgical removal of primary tumors affected the outcome of patients with incurable advance breast cancer. We also attempted to identify other predictive indicators for prognosis based on patient demographics and tumor characteristics.

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Patients and methods

Patients

We retrospectively reviewed individual medical records from the Hokkaido Cancer Center. In this study, incurable advanced breast cancer is defined as the presence of a tumor larger than 5 cm, and shows either invasion to chest wall or skin or is an inflammatory carcinoma and at least one metastatic site, including distant lymph nodes, bone, or visceral organs (lung, pleura, mediastinum, liver, and brain) at diagnosis. Between January 2000 and June 2010, 92 women were diagnosed with incurable advanced breast cancer at Hokkaido Cancer Center.

Evaluation of pathological factors

ER and PgR expression status was considered positive when ≧10% of cancer cell nuclei exhibited positive immunohistochemistry (IHC) staining, regardless of intensity. HER2-positive breast cancer was defined as a condition where tumors were immunohistochemically scored with a value of 3+, or 2+ with positive for fluorescein in situ hybridization.

Statistical analysis

Baseline characteristics were compared between women who received therapeutic breast surgery versus those who did not, using the chi-square test for independence. Overall survival time was plotted using a method based on the Kaplan–Meier estimator, and a log-rank test was applied for comparison. Prognostic factors predictive of survival were assessed through a univariate analysis using the Cox proportional hazards model, and hazard ratios (HR) are presented with their 95% confidence intervals (95% CI). Multivariate modeling was performed using the Cox proportional hazards model with forward selection. $P < 0.05$ was considered statistically significant.

Results

Surgical decision-making and complications

A total of 92 patients were included in this study. Median follow-up time for survivors was 27.4 months. Of 92 patients, 36 (39.1%) had surgery focused on a primary tumor. All of 36 patients had a total mastectomy, with or without axillary dissection, and three patients needed a skin graft. The mean time from diagnosis to surgery in the patients who received surgery was 6.5 months. Seven patients (19.4%) had emergency operations for uncontrollable bleeding from the tumor. The primary reason for elective surgery was to improve cancer-related local symptoms, such as pain or purulent discharge (22 patients, 61.1%). Seven patients undertook surgery based on their own desire to have the tumor removed. The average operation time was 118 ± 40 min, and blood loss was 289 ± 276 ml. Surgical complications were observed in six patients (16.7%), including: surgical site infection in four individuals, postoperative bleeding in one patient, and skin flap necrosis in one patient. No severe surgical morbidities or mortalities were observed.

Patient demographics and tumor characteristics

The patient demographics and tumor characteristics for surgically or non-surgically treated patients are listed in Table 1. There was no significant difference between patients who had primary tumor surgery and those who did not.

Patient outcomes

Overall survival was plotted using Kaplan–Meier curves based on the patient cohort according to surgery as shown in Fig. 1. Besides one patient who died from other disease 11 months after surgery, all patients died from primary breast cancer. Primary tumor resection did not significantly improve overall survival time in patients with incurable advanced breast cancer (surgery versus no surgery, median survival time (MST): 25.0 versus 24.8 months, $P = 0.352$). On the other hand, primary tumor resection allowed most patients some relief from symptoms relating to the skin-invasion tumor, such as odious smell, purulent discharge, and bleeding. Only 3 of 36 patients relapsed with local disease by three

Table 1
Comparison of characteristics of surgically versus non-surgically treated patients with incurable advanced breast cancer at diagnosis ($N = 92$).

Variable	Surgical removal of primary tumor		*P*-value
	Yes ($N = 36$)	No ($N = 56$)	
	No. of patient (%)	No. of patient (%)	
Age at diagnosis (years)			
<60	25 (69)	38 (68)	0.954
≥60	11 (31)	18 (32)	
Size (cm)			
<10	19 (53)	31 (55)	0.846
≥10	17 (47)	25 (45)	
Period of diagnosis			
2000–2005	18 (50)	19 (34)	0.125
2006–present	18 (50)	37 (66)	
Radiation therapy to the breast			
Yes	8 (22)	7 (13)	0.427
No	28 (78)	49 (88)	
Estrogen receptor (ER)			
Positive	19 (53)	38 (68)	0.074
Negative	17 (47)	17 (30)	
Unknown	0 (0)	1 (2)	
Progesterone receptor (PgR)			
Positive	9 (25)	22 (39)	0.101
Negative	27 (75)	33 (59)	
Unknown	0 (0)	1 (2)	
HER2 overexpression			
Yes	8 (22)	12 (21)	0.573
No	25 (69)	43 (77)	
Unknown	3 (8)	1 (2)	
Subtype			
ER+/HER2−	18 (50)	33 (59)	0.482
ER+/HER2+	1 (3)	5 (9)	
ER−/HER2+	7 (19)	7 (13)	
ER−/HER2− (Triple negative)	7 (19)	10 (18)	
Unknown	3 (8)	1 (2)	
Site(s) of metastasis at presentation			
Bone	17 (47)	44 (79)	
Lung	15 (42)	15 (27)	
Liver	13 (36)	11 (20)	
Brain	4 (11)	3 (5)	
Others	5 (14)	21 (38)	
Number of metastatic site			
1	21 (58)	29 (52)	0.568
2	12 (33)	17 (30)	
≥3	3 (8)	10 (18)	
Lines of chemotherapy			
0	7 (19)	14 (25)	0.595
1	10 (28)	19 (34)	
2	12 (33)	11 (20)	
≥3	7 (19)	12 (21)	

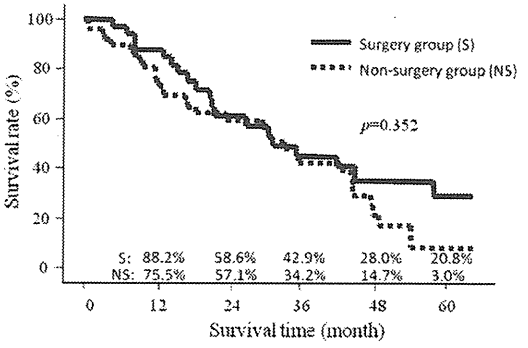

Fig. 1. Kaplan–Meier curves of overall survival for patients with incurable advanced breast cancer, with comparisons between the groups of patients who either had surgery (surgery group) or did not (non-surgery group).

months after surgery. This symptom-free condition in breast lasted for long-term: the median time of relapse-free local disease was 15.7 months. In patients who died from primary breast cancer, the median time of relapse-free local disease was 13.7 months after surgery. When analysis was limited to survivors, the median time of relapse-free local disease was 27.7 months after surgery.

Prognostic factors predictive of survival

Surgery did not provide a survival benefit for patients with incurable advanced breast cancer, although surgery was performed due to mainly not aim for cure but salvage mastectomy. Likewise, radiotherapy to the breast did not influence patient survival. Other prognostic factors predictive of survival are shown in Table 2. ER-negative, PgR-negative, or triple-negative breast cancers were associated significantly with decreased survival times. Survival rates for patients with triple-negative breast cancer were worse than for patients with any other type of breast cancer considered in this study (Fig. 2). Regarding metastatic profiles, the number of metastatic sites (more than three sites) was crucial as a predictive indicator, regardless of location. Age at diagnosis, tumor size, and period of diagnosis were not factors that impacted patient outcome in our study. Based on the results of univariate analysis, multivariable Cox regression analysis was subsequently done (Table 3). Both triple-negative breast cancer and the presence of more than three metastatic sites were independent negative predictive factors.

Table 2
Univariable Cox's regression analysis predicting overall survival in patients with incurable advanced breast cancer.

Variable	No. of patients (%)	HR	95% CI	*P*-value
Age at diagnosis (years)				
<60	63 (68)	1.00	–	0.174
≥60	29 (32)	0.65	(0.35–1.21)	
Size (cm)				
<10	50 (54)	1.00	–	0.096
≥10	42 (46)	1.57	(0.92–2.68)	
Period of diagnosis				
2000–2005	37 (40)	1	–	0.6748
2006–	55 (60)	0.89	(0.52–1.53)	
Surgery				
Yes	36 (39)	1.00	–	0.213
No	56 (61)	1.42	(0.81–2.49)	
Radiation therapy to the breast				
Yes	15 (16)	1.00	–	0.525
No	77 (84)	0.81	(0.43–1.54)	
ER				
Positive	57 (62)	1.00	–	*0.001*
Negative	34 (37)	2.56	(1.48–4.43)	
PgR				
Positive	31 (34)	1.00	–	*0.042*
Negative	60 (65)	1.91	(1.03–3.57)	
HER2 overexpression				
Yes	20 (22)	1.00	–	0.993
No	68 (74)	1.00	(0.52–1.91)	
Triple-negative breast cancer				
No	17 (18)	1.00	–	*<0.001*
Yes	71 (77)	4.59	(2.38–8.85)	
Bone metastasis				
No	31 (34)	1.00	–	0.602
Yes	61 (66)	1.17	(0.65–2.09)	
Lung metastasis				
No	62 (67)	1.00	–	0.4638
Yes	30 (33)	1.24	(0.70–2.18)	
Liver metastasis				
No	40 (43)	1.00	–	0.2828
Yes	52 (57)	1.39	(0.76–2.52)	
Numbers of metastatic site				
1–2	79 (86)	1.00	–	*0.021*
≥3	13 (14)	2.36	(1.14–4.90)	
Lines of CTx				
0–2	70 (76)	1.00	–	0.812
≥3	22 (24)	0.93	(0.51–1.70)	

Discussion

Several recent retrospective reviews have indicated that primary tumor resection does contribute to a survival advantage for patients with stage IV breast cancer, compared to patients who did not receive surgery.[5–10] In contrast to these reports, the current study has shown that primary tumor resection did not improve the overall survival times in patients with incurable advanced breast cancer. One possible explanation for this discrepancy is the fact that other studies have included a large proportion of T1 or T2 breast cancer at time of diagnosis. Resection of a primary tumor less than 5 cm is less invasive and might be simpler than procedures needed to remove 5 cm tumors. In such cases, surgery may contribute to the improvement of survival. As a second possibility, variables between previous studies and our work have included diagnosis period differences. Therapeutic strategies for breast cancer have shifted increasingly toward an emphasis on systemic approaches that use new therapeutics, such as trastuzumab,[11] that have been developed as continuous approaches. In our analysis, the proportion of patients

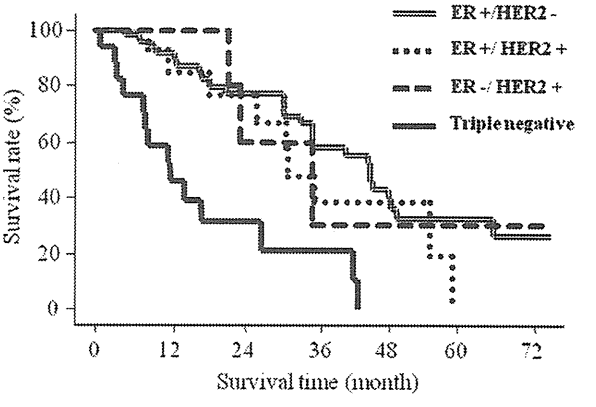

Fig. 2. Overall survival rates in each subtype of incurable advanced breast cancer.

Table 3
Multivariable Cox's regression analysis predicting overall survival in patients with incurable advanced breast cancer.

Variable	HR	95% CI	*P*-value
ER			
Positive	1.00	–	
Negative	1.45	(0.64–3.29)	0.371
PgR			
Positive	1.00	–	
Negative	1.10	(0.53–2.29)	0.791
Triple-negative breast cancer			
No	1.00	–	
Yes	3.52	(1.51–8.20)	*0.004*
Numbers of metastatic site			
1–2	1.00	–	–
≥3	2.89	(1.37–6.13)	*0.006*

who had surgery tended to decrease, although not significantly, in the second-half period of diagnosis compared to the first-half period. We considered that improvement by systemic therapies may affect any advantage of surgery on survival outcomes.

On the other hand, a reduction in tumor burden can improve patient survival. For other types of metastatic cancers, such as renal cell cancer,[12] ovary,[13] colorectal cancer,[14] and gastric cancer,[15] the beneficial impacts of primary tumor debulking surgery on patient survival have been well documented. It is presumed that removal of necrotic or avascular tumor areas, and a resulting reduction in tumor volume through debulking surgery, should allow therapeutic agents to more readily access target cancer cells,[16] although the detailed biological mechanisms underlying such a response remain unclear. For MBC, previous retrospective reports also have shown the potential advantages of debulking surgery for MBC.[5–10] Prospective and well-designed randomized studies in this area should shed light on new combinations of surgery and systemic therapies.

Surgery puts the patient at a disadvantage occasionally, with one disadvantage being loss of target lesion. In cases where no measurable or assessable disease feature other than the primary tumor exists, the monitoring of therapeutic efficacy is difficult after primary tumor resection. It is also possible that surgical stress negates its benefit, but a mastectomy usually takes about 2 h, and most patients can return to a pre-surgery status that is reflective of a normal life within a few days. Operation time and blood loss observed in this study seemed acceptable in the context of surgical burden or safety. Tamer et al. reported that the operation-related mortality rate is 0.24%, and that the morbidity rate of the most prevalent operative complication (surgical site infection) is low, at 4.34%.[17] The occurrence of surgical site infection was 11.1% in our study, but extensive morbidity was not observed. Moreover, admission duration was almost equal to other operative cases. We deduce that surgical resection of primary tumors is important for the regulation of local disease, since 33 of 36 patients who received primary tumor resection were allowed relief from symptoms relating to the local disease for more than a year post-surgery. However, we did not measure the quality of life with appropriate instruments. Further examination is necessary to show objective assessments except for local disease free time.

Analyses of other factors relating to prognosis for patients with incurable advanced breast cancer indicated that triple-negative breast cancer and metastasis to more than three sites were poor prognostic indicators. In previous studies, ER-positive status,[8,18] HER2 overexpression,[18] age at diagnosis (>50),[9] lack of visceral metastasis,[6,8,10] and existence of a single metastatic site[9] were indicated as favorable predictive factors. Our study has indicated that factors such as the number of metastatic sites and the status of ER, PgR, and HER2 expression are very important for determining a course of treatment for patients with incurable advanced breast cancer. Local advancement of breast cancer causes physical and psychological distress, and leads to markedly impaired quality of life for the remainder of the affected patient's life. We believe that primary tumor resection become a beneficial option for patients with favorable prognosis, even though life-prolongation is not expected from surgery alone, particularly if synergistic therapies can empower the potential post-surgical effects of resection.

Conclusion

Our retrospective study showed that local disease control by primary tumor resection failed to prolong overall survival in patients with incurable advanced breast cancer. Primary tumor resection can, however, lead to improved quality of life in cases where prognosis supports such surgery.

Conflict of interest statement

None declared.

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Inhibition of nuclear factor-kappaB suppresses peritoneal dissemination of gastric cancer by blocking cancer cell adhesion

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Currently, patients with peritoneal dissemination of gastric cancer must accept a poor prognosis because there is no standard effective therapy. To inhibit peritoneal dissemination it is important to inhibit interactions between extracellular matrices (ECM) and cell surface integrins, which are important for cancer cell adhesion. Although nuclear factor-kappa B (NF-κB) is involved in various processes in cancer progression, its involvement in the expression of integrins has not been elucidated. We used a novel NF-κB inhibitor, dehydroxymethylepoxyquinomicin (DHMEQ), to study whether NF-κB blocks cancer cell adhesion via integrins in a gastric cancer dissemination model in mice and found that DHMEQ is a potent suppressor of cancer cell dissemination. Dehydroxymethylepoxyquinomicin suppressed the NF-κB activity of human gastric cancer cells NUGC-4 and 44As3Luc and blocked the adhesion of cancer cells to ECM when compared with the control. Dehydroxymethylepoxyquinomicin also inhibited expression of integrin (α2, α3, β1) in *in vitro* studies. In the *in vivo* model, we injected 44As3Luc cells pretreated with DHMEQ into the peritoneal cavity of mice and performed peritoneal lavage after the injection of cancer cells. Viable cancer cells in the peritoneal cavities were evaluated sequentially by *in vivo* imaging. In mice injected with DHMEQ-pretreated cells and lavaged, live cancer cells in the peritoneum were significantly reduced compared with the control, and these mice survived longer. These results indicate that DHMEQ could inhibit cancer cell adhesion to the peritoneum possibly by suppressing integrin expression. Nuclear factor-kappa B inhibition may be a new therapeutic option for suppressing postoperative cancer dissemination. (*Cancer Sci* 2011; 102: 1052–1058)

Peritoneal dissemination is the most frequent process through which gastric cancer recurs,[1] and patients with this condition must currently accept a very poor prognosis.[2,3] Standard chemotherapy is currently not sufficiently effective for improving the survival of patients with peritoneal dissemination of gastric cancer. To inhibit peritoneal dissemination, it may be important to control the adhesion of cancer cells to the peritoneum. During cancer cell dissemination in the abdominal cavity, cancer cells make contact with the basement membrane through gaps between mesothelial cells.[4,5] The basement membrane beneath mesothelial cells comprises extracellular matrices (ECM) consisting of type 1 and 4 collagen, fibronectin or laminin,[6] and mesothelial cells also produce ECM.[7] The interactions between these ECM and cell surface integrins play very important roles in cancer cell adhesion and, therefore, cancer progression.[8]

Integrins are membrane-bound proteins that form heterodimers of α- and β-subunits at the cell surface. The α-subunits vary between 120 and 180 kD, and are non-covalently associated with β-subunits (90–110 kD). To date, 14 α subunits and eight β subunits have been identified, and after mutual dimerization, these subunits contribute to cell adhesion or regulation of signal transduction required for cell survival by making contact with appropriate ECM.[9,10] It has been reported that integrins α2, α3 and β1 play important roles in the peritoneal dissemination of gastric cancer,[11] and that antibodies to these integrins suppress peritoneal dissemination of gastric cancer in a mouse model.[12]

Nuclear factor-kappaB (NF-κB) was first identified and reported in 1986[13] and studied in the context of immune and inflammatory responses.[14] Nuclear factor-kappaB is a generic term for dimers of NF-κB1 (p50/p105), NF-κB2 (p52/p100), c-Rel, RelA (p65/NF-κB3) and RelB.[15] To date, involvement of NF-κB in cancer-related molecules such as cyclin D1,[16] intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1),[17] the Bcl family,[18] inhibitor of apoptosis (IAP), X-linked inhibitor of apoptosis protein (XIAP),[19] p53,[20] vascular endothelial growth factor (VEGF), interleukin (IL)-8,[21] MMP[22] and multidrug resistance protein 1 (MDR1),[23] has been elucidated. However, NF-κB has not been reported to be involved in cancer cell adhesion to the peritoneum via integrins.

A low-molecular-weight NF-κB inhibitor, dehydroxymethylepoxyquinomicin (DHMEQ), was newly developed by Umezawa.[24] Dehydroxymethylepoxyquinomicin specifically inhibits the nuclear translocation of p65 and prevents it binding to DNA[25]; it also has various anti-cancer effects in mouse models without obvious side-effects. Thus far, the following anti-cancer effects of DHMEQ have been reported: G1 arrest by inhibition of cyclin D1 expression;[26] and induction of apoptosis by inhibition of cIAP and XIAP,[27] or Bcl-2 and Bcl-xL.[28] Antitumor effects of DHMEQ have also been reported in *in vivo* models such as those for thyroid cancer,[27] prostate cancer,[29] hepatic cancer,[30] breast cancer,[31] pancreas cancer,[32] multiple myeloma,[28] malignant lymphoma[33] and leukemia.[26]

In the present study, we showed that NF-κB is associated with integrin expression in gastric cancer cell lines and that NF-κB inhibition by DHMEQ suppresses cancer progression by inhibiting the adhesion of gastric cancer cells to the peritoneum in a mouse model of peritoneal dissemination of gastric cancer.

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Materials and Methods

Cell cultures. The human gastric cancer cell line NUGC4 was obtained from the Japanese Cancer Research Resources Bank (JCRB, Osaka, Japan), and 44As3Luc with luciferase activity was constructed by one of the authors (K.Y.).[34] The 44As3Luc cells were derived from 44As3 cells, which is a highly peritoneal metastatic cell line, and were stably transfected with a pEGF-PLuc plasmid with CMV promoter (Clontech, Palo Alto, CA, USA). Human breast cancer cell lines MCF7 with constitutively low NF-κB activity and MDA-MB231 with constitutively high NF-κB activity were obtained from the American Type Culture Collection (Rockville, MD, USA).[31] The NUGC4 cells were cultured at 37°C in RPMI1640 (Sigma, St Louis, MO, USA) along with 10% fetal bovine serum (FBS); the 44As3Luc cells were cultured at the same temperature with RPMI1640 containing 100 μg/mL geneticin (Sigma); and the MCF7 and MDA-MB231 cells were also cultured at 37°C in 95% air and 5% CO_2 in DMEM (Sigma) along with 10% FBS.

Dehydroxymethylepoxyquinomycin (DHMEQ). We have originally designed and developed DHMEQ (molecular weight (MW): 261), a derivative of the natural antibiotic epoxyquinomycin C, to specifically target NF-κB.[24]

DNA-binding activity of NF-κB. To evaluate the DNA-binding activity of NF-κB in the steady state, 70% confluent cultures of NUGC4, 44As3Luc, MCF7 and MDA-MB231 in 10-cm dishes were stored at −80°C. To evaluate the effect of DHMEQ, the medium in the 70% confluent cultures of NUGC4 and 44As3Luc was replaced with 10 μg/mL DHMEQ solution, incubated for an appropriate time and stored at −80°C. The following day, nuclear proteins were extracted and examined using a p65 TransAM kit (ActiveMotif, Carlsbad, CA, USA). The absorbance was determined using a plate reader (Varioskan Flash, Thermo Fisher Scientific, Waltham, MA, USA). Each experiment was performed in triplicate.

NF-κB reporter gene assay. A GFP reporter gene construct was transfected using Cignal Reporter Assay kits (SA Biosciences, Frederick, MD, USA). Cultured cells were trypsinized and resuspended in Opti-MEM (Invitrogen, Carlsbad, CA, USA) with non-essential amino acids (Invitrogen) without antibiotics at a concentration of 2×10^5 cells in a 96-well plate. Cells were transfected with the reporter by culturing for 16 h with Surefect (SA Biosciences). After the medium was replaced with Opti-MEM with penicillin/streptomycin, the cells were incubated for an additional 8 h. The medium was then replaced with Opti-MEM containing 10 μg/mL of DHMEQ (or 0.024% of DMSO for the controls). The intensity of fluorescence was measured at appropriate times in triplicate using Varioskan Flash (excitation, 470 nm; emission, 515 nm).

mRNA expression of integrins in DHMEQ-treated cells. Real-time PCR was used to examine mRNA expression. The 44As3Luc cells were cultured in triplicate in 0.024% DMSO solution (controls) or in 10 μg/mL DHMEQ for the appropriate times. Total RNA was isolated using an RNeasy mini kit (Qiagen, Valencia, CA, USA) in accordance with the manufacturer's instructions. For cDNA synthesis, ReverTra Ace qPCR RT kit (Toyobo, Osaka, Japan) with Oligo(dT) 20 primer (Toyobo) was used in accordance with the manufacturer's instructions. For relative quantification by PCR, each cDNA product was analyzed in a LightCycler (version 1.4) using a QuantiTect SYBR Green PCR kit (Qiagen).

Flow cytometric analysis of integrin expression. p65 silencing was performed using p65 siRNA2 (BD Biosciences, Bedford, MA, USA). Next, 50% confluent cells were incubated for 24 h in medium without antibiotics in 10-cm dishes. Then, 33 nM p65 siRNA was added to each dish and transfected for 48 h. p65 silencing was confirmed by western blot analysis using primary antibodies against ×500 α-tubulin and ×1000 p65 protein (Cell Signaling, Beverly, MA, USA) and ×5000 goat anti-mouse IgG for tubulin or anti-rabbit IgG for the p65 protein. With regard to DHMEQ treatment, the medium in 70% confluent cell cultures in 10-cm dishes was replaced with 10 μg/mL DHMEQ solution (0.024% DMSO for the controls) and cultured for the appropriate times. These cells were trypsinized and analyzed using flow cytometry (FACS Caliber; Becton Dickinson, Franklin Lakes, NJ, USA). The antibodies used for these assays were integrin α2, integrin α3, integrin β1 and isotype controls for these integrins. All antibodies were obtained from R&D Systems (Minneapolis, MN, USA).

Adhesion assay. We evaluated the anti-adhesive effect of DHMEQ by using a plate pre-coated with ECM constituting the peritoneal basement membrane. The medium in 70% confluent cell cultures in 10-cm dishes was replaced with 10 μg/mL DHMEQ solution (or 0.024% DMSO for the controls), and the dishes were incubated for 24 h. These cells were trypsinized, assembled, adjusted to a concentration of 1×10^6 cells/mL with RPMI and distributed on the pre-coated plates (80 μL per plate). Next, the cells were incubated at 37°C for 1 h. Except for the non-treated plate, all plates were washed three times with 100 μL of FBS-free RPMI. After washing, 10 μL of ×50 diluted Cell Counting kit F (CCKF; Dojindo, Osaka, Japan) was added to each well, and the fluorescence intensity of the remaining live cells (adhesive cells) was evaluated using Varioskan Flash at 30 min after CCKF administration (excitation, 490 nm; emission, 515 nm). Pre-coated plates were manufactured by BD Biosciences and the ECM coated on the plates were types 1 and 4 collagen, fibronectin and laminin.

DHMEQ cytotoxicity assay. The cells were seeded into 96-well plates at 5×10^3 cells/well in 10% FBS-containing medium. Twenty-four hours later, the medium in the wells was replaced with different concentrations of DHMEQ solution or 0.048% DMSO solution, and the cells were then incubated again for 24 h. Lactate dehydrogenase (LDH) activity of the supernatant was measured using an LDH cytotoxicity detection kit (Takara Bio, Shiga, Japan).

Animal experiments. Six-week-old male BALB/c-nu/nu mice, each weighing approximately 20 g, were obtained from CLEA Japan, Inc. (Tokyo, Japan). The mice were grouped as follows: (i), implantation of DMSO-treated cells; (ii) implantation of DHMEQ-treated cells; (iii) implantation of DMSO-treated cells with peritoneal lavage; and (iv) implantation of DHMEQ-treated cells with peritoneal lavage. Each group comprised four mice. Then, 2×10^6 44As3Luc cells, which had been treated with 10 μg/mL DHMEQ (or 0.024% DMSO for the controls) for 24 h, were injected intraperitoneally into the above mentioned mice. One hour after injection, laparotomy and peritoneal lavage were performed using phosphate-buffered saline (PBS). Peritoneal lavage was performed through a 1-cm incision through which 5 mL of PBS was slowly injected. Bio-imaging was performed before and after the peritoneal lavage, and on days 2, 5, 10, 15 and 20 in order to evaluate cancer progression. Luminescence was evaluated at approximately 7 min after intraperitoneal injection of 1500 μg/mouse D-luciferin potassium salt (Synchem OHG, Altenburg, Germany). *In vivo* imaging was performed using Photon Imager Hu (Biospace Lab, Paris, France) with the mice under isoflurane anesthesia (Abbott Japan, Tokyo, Japan). Images were captured using Photo Acquisition 2.6 (Biospace Lab) with 0.5 min exposure and processed using Photo Vision Plus. Signal intensity was quantified as the sum of all detected photon counts (count per minute [CPM]) within the region of interest (ROI). All procedures involving animals and their care were approved by the Ethics Committee of Hokkaido University in accordance with institutional and Japanese governmental guidelines for animal experiments.

Scanning electron microscopy (SEM) of the peritoneal wall. The peritoneal walls of mice injected with cancer cells

were fixed with 10% formaldehyde for 180 min and then overnight at 4°C with 1.25% glutaraldehyde solution. The fixed samples were dehydrated in a 30–100% graded ethanol series and immersed in tert-butyl alcohol overnight at −20°C. These samples were dried using ES-2000 (Hitachi High-Technologies Co., Tokyo, Japan) for 3 h and ion-sputtered using E-1030 (Hitachi) for 120 s. The peritoneal surface was observed under a scanning electron microscope (S-3500N; Hitachi).

Statistics. The mean and SD were calculated for all variables, except the data from the flow cytometry. Between-group statistical significance was determined using the Student's t test. $P < 0.05$ was considered as statistically significant.

Results

DHMEQ effectively suppresses p65-DNA binding activity in gastric cancer cells. In the steady state, the p65-DNA binding activities in NUGC4 and 44As3Luc cells were as high as that in MDA-MB231 cells, a positive control cell with high binding activity. The activity in MCF7 cells is constitutively low as previously reported [31], and hence these cells were used as the negative control (Fig. 1A). The binding activities in both cells reached their lowest levels 2 h after the addition of 10 μg/mL DHMEQ (as a final concentration) and returned to initial conditions within 24 h (Fig. 1B). A GFP reporter assay showed that DHMEQ significantly suppresses transcriptional activity in both cells (Fig. 1C). On the basis of these results, we considered that DHMEQ had a similar effect in NUGC4 and 44As3Luc cells. Therefore, we used 44As3Luc cells in the following experiments. We planned to evaluate cancer progression using bioimaging.

Effect of NF-κB inhibition on integrin expression. In 44As3Luc cells, the mRNA of all integrins – α2, α3 and β1 – were significantly suppressed 2 h after the addition of 10 μg/mL DHMEQ (as a final concentration) compared with the control to which DMSO was added (Fig. 2A). The percentage reduction in the expressions of integrins α2, α3 and β1 was 27%, 31% and 8%, respectively. Flow cytometric analysis revealed that the expressions of all cell surface integrins on 44As3Luc cells were gradually suppressed after the addition of DHMEQ (Fig. 2B). Reductions in integrin expression (α2, α3 and β1) following DHMEQ addition was 68%, 83% and 45% at 24 h, respectively. Similarly, flow cytometric analysis of integrins α2, α3 and β1 revealed that the expressions of cell surface integrins in p65-deleted cells were suppressed to the same degree as in DHMEQ-treated cells (Fig. 2C). Reductions in integrin expression after p65 deletion were 34% (α2), 76% (α3) and 41% (β1). p65 silencing was confirmed by western blotting for nuclear and cytoplasmic p65 proteins (Fig. 2D).

Anti-adhesive effect of DHMEQ-treated cells in an *in vitro* assay. Significantly fewer 44As3Luc cells treated with 10 μg/mL DHMEQ (final concentration) remained alive on plates pre-coated with ECM after they were washed (ECM-adhesive cells) than 44As3Luc cells treated with DMSO (Fig. 3A). Reductions in the numbers of adhesive cells following DHMEQ addition were 18.3% (laminin), 34.8% (fibronectin), 38.2% (type 1 collagen) and 43.5% (type 4 collagen). The LDH value, which represents the cytotoxic effect, was significantly elevated in the supernatant of cells treated with DHMEQ at concentrations >17.5 μg/mL (Fig. 3B).

Effect of peritoneal lavage on implantation of DHMEQ-treated cancer cells on the abdominal wall. The number of cancer cells decreased in mice injected with DHMEQ-pretreated cells and subjected to peritoneal lavage (Fig. 4A). The intensity of bioluminescence after lavage was significantly reduced (reduction rate, 39%) in mice that were injected with DHMEQ-pretreated cells and subjected to peritoneal lavage compared with in mice injected with DMSO-pretreated control cells and subjected to

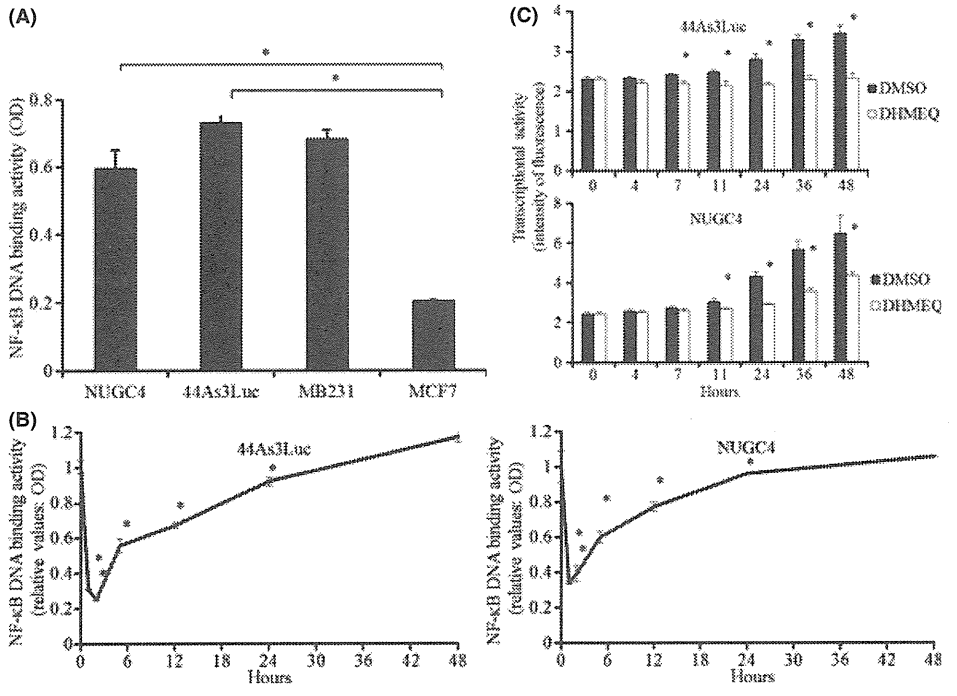

Fig. 1. Dehydroxymethylepoxyquinomicin (DHMEQ) effectively suppressed p65-DNA binding activity in gastric cancer cells. (A) Nuclear p65 protein binding activity to DNA in a steady state. MDA-MB231 cells were used as a positive control, and MCF7 cells were used as a negative one. *$P < 0.05$. (B) Time course of binding activity of nuclear p65 proteins to DNA in DHMEQ-treated cells. The binding activities of both cells were assessed at 2, 6, 12, 24 and 48 h after DHMEQ administration. *Significantly <0 h ($P < 0.05$). (C) Nuclear factor-kappa B (NF-κB) GFP reporter assay. The black bars show cells treated with DMSO, and white bars show those with DHMEQ. *Significantly more than controls ($P < 0.05$). OD, optical density.

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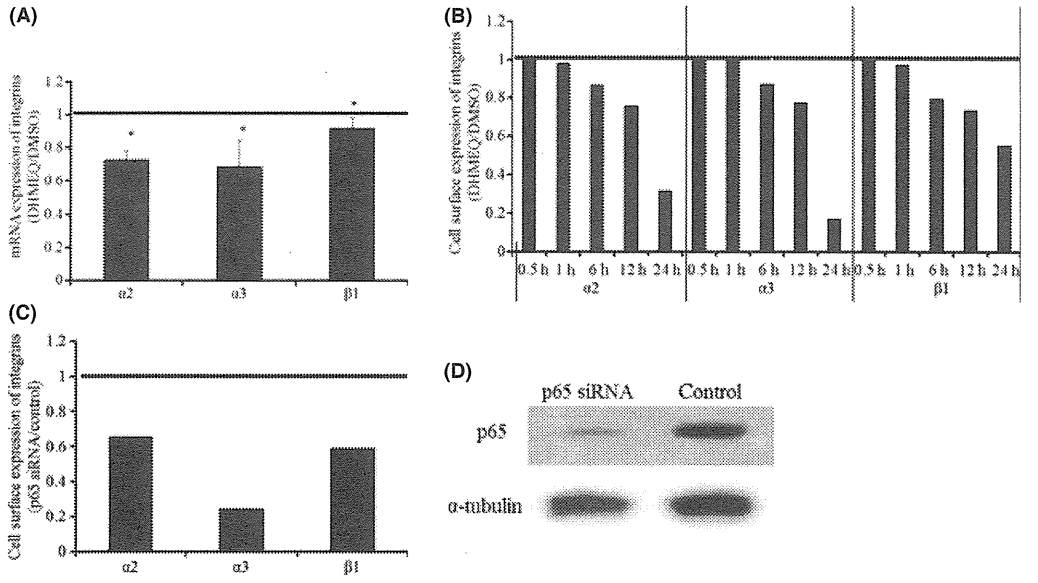

Fig. 2. Effect of nuclear factor-kappa B (NF-κB) inhibition on expression of adhesion molecules. (A) Quantitative evaluation of mRNA of integrins by real-time PCR. The graph shows the average of the ratio of copies of dehydroxymethylepoxyquinomicin (DHMEQ)-treated 44As3Luc cells to DMSO-treated cells at 2 h after DHMEQ administration. When the longitudinal value is below 1 (bold line), the integrin expression of DHMEQ-treated cells is lower than that of DMSO-treated cells. *Significantly less than controls ($P < 0.05$). (B) Expression of cell surface integrins of DHMEQ-treated cells. The graph shows the expression rate of cell surface adhesion molecules of 44As3Luc cells treated with DHMEQ compared with that of DMSO-treated cells for each time point. The bold line is as described above. (C) Expression of cell surface adhesion molecules of cells knocked down by p65 siRNA. The graph shows the rate of cell surface integrins of 44As3Luc cells knocked down by p65 siRNA. The bold line is as described above. (D) p65 deletion. The p65 deletion was confirmed by western blotting.

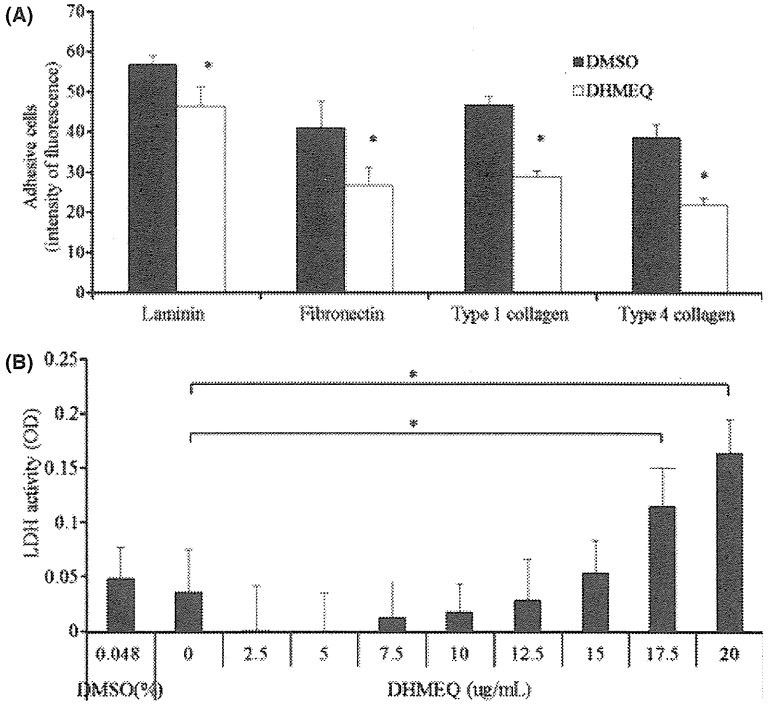

Fig. 3. Anti-adhesive effect of dehydroxymethylepoxyquinomicin (DHMEQ) pretreated cells in the *in vitro* study. (A) Adhesion assay. The bars show the fluorescence intensity of the remaining live cells on the plates. The black bars show cells pretreated with DHMEQ, and white bars show those with DMSO. *Significantly less than controls ($P < 0.05$). (B) Evaluation of cytotoxicity of DHMEQ. The graph shows lactate dehydrogenase (LDH) activity of the supernatant of the 44As3Luc cells treated with DHMEQ or DMSO. *$P < 0.05$. OD, optical density.

peritoneal lavage (Fig. 4B). The SEM revealed that cancer cells adhered less to the basement membrane of the peritoneum in mice injected with DHMEQ-pretreated cells than in those injected with DMSO-pretreated control cells (Fig. 4C).

Follow up of gastric cancer dissemination by *in vivo* imaging. The DHMEQ-pretreated 44As3Luc cells injected in mice grew slowly compared with the DMSO-pretreated cells (Fig. 5A). The increase in the CPM/mm^2 value of the