特集 進行・再発婦人科がんの治療と管理

7. 転移性脊椎腫瘍と脊髄麻痺

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要旨

がん（悪性腫瘍）による脊髄麻痺の疫学，発生，症状出現様式，診断，薬物療法，放射線治療，手術療法の成績と治療選択に関する検討方法を解説した．がん終末期患者のQOLを大きく低下してしまう病態で，治療終了後5年過ぎても脊椎転移が起こることも念頭に，CT，MRI検査を実施し，早期治療に努めることが極めて重要である．

Key Words がん，脊髄圧迫症状，脊椎腫瘍

転移性脊椎腫瘍例の2～20%が脊髄圧迫症状（malignant spinal cord compression：MSCC）へと進行し，悪性腫瘍の死亡患者2.5%がMSCCによる1度以上の入院を経験し，がん患者の剖検では，脊椎転移巣は30～60%，硬膜圧迫が5%に確認される終末期の病態である．がん種でMSCCの頻度は異なり，骨髄腫，肺癌に高く7.9～13%，膵臓癌では極めて低く0.2%である．骨転移率の高い乳癌，肺癌，前立腺癌が，MSCC例の50～60%を占めるものの，骨外腫瘍や骨折を起こしやすい溶骨，混合型脊椎転移発生が多い骨髄腫，腎臓癌，甲状腺癌にMSCC発生例が多い．また，初診時進行骨転移例8～20%にMSCCが併発し，診断は遅れやすく，重篤な麻痺となりやすい．

豊富な毛細血管網と緩徐な血流をもつ造血骨髄は血行性転移の成立に適していて，骨の血流量は心拍量の10%程度にすぎないものの，転移発生頻度は肺肝に次いで骨，骨髄転移が3位，成人では造血骨髄が多い脊椎，骨盤，四肢近位部に集中する．脊椎内では小動脈が多く分布する椎体後半から椎弓根への移行部，血流が緩徐になる骨皮質直下にできやすい．また，骨盤や腹部内発生の結腸，婦人科がんでは逆流防止弁をもたないBatson傍脊椎静脈叢を介して肺転移成立前に脊椎転移を起こしやすい（図1e）．

臨床像と症状

1．脊椎転移の部位

転移性，浸潤性腫瘍の病態，神経症状を理解し治療方法を検討するために，転移病巣が発生した脊椎レベルと脊椎横断面の広がりで分類されることが多い．一般に脊椎転移好発脊椎レベ

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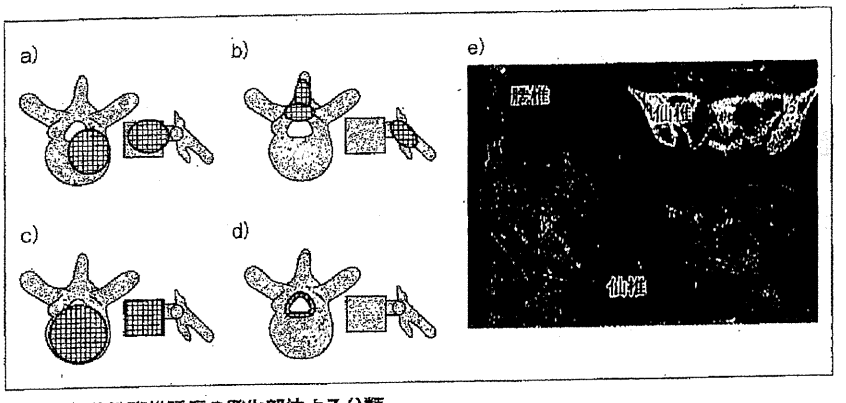

図1 転移性脊椎腫瘍の発生部位よる分類

a）前方要素（椎体）から硬膜外への増大，b）後方要素（椎弓，棘突起）からの硬膜外への増大，c）腫瘍は椎体全体を占め，圧迫骨折を契機に脊髄を圧迫して麻痺，d）骨転移を認めない硬膜外病変，その他傍脊柱発生の腫瘍からの浸潤，側方からの侵入，e）子宮体癌，6年後仙骨転移，強い下肢痛で発症．手術より粒子線治療が実施された．

ルでは，腰椎，下部胸椎，上部胸椎，仙骨，頸椎の順に多く，さらに腫瘍の広がりを脊髄を中心に前方要素(腹側—椎体)，外側要素，後方要素（椎弓）(**図1**)へと分類する．最も多いタイプの前方要素の脊椎転移は，MSCC 原因の86%を占め，腫瘍が脊髄を前方から圧迫するので，脊髄前角神経路障害である下肢の運動障害，歩行障害を起こしやすい．椎体から後方要素の骨転移で，椎弓根の消失（ペディクルサイン，ウィンクサイン）などの初期単純X線所見で発見され，腫瘍は側方より脊髄を圧迫するので，対側の運動麻痺，同側知覚障害（Brawn Sequard 症候群），同側の神経根刺激症状が初期症状となる．一般に子宮原発悪性腫瘍の骨転移例はまれで，骨転移発生の約3%，腸骨，仙骨，腰椎へ転移しやすい．頸椎や胸椎に比較し運動麻痺は軽く，馬尾神経や腰部仙骨神経叢の症状である頑固な下肢の刺激痛や腰痛，臀部痛が先行する．

その他のまれな様式として，硬膜外腔の転移5%，椎体周辺の肺癌パンコースト腫瘍等が椎管孔から浸潤して起こるMSCCは10%，髄内転移はMSCCの1~2%，肺癌，乳癌，甲状腺癌等でまれに観察される．

2．MSCCの症状

MSCCの臨床症状は，疼痛と神経障害で，背部痛約9割，運動低下76%，膀胱障害50%，知覚障害50%の頻度で，錐体路障害と支配神経レベルの知覚障害等を神経学的診断方法を実施することで病巣レベルを推測できる．さらに，不全麻痺で発見されても，3割弱は24時間以内に完全麻痺へと増悪するので経過観察を怠ってはならない．

麻痺増悪に関わる因子として，歩行障害，深部反射亢進，レントゲン検査による圧迫変形，骨転移の存在，骨転移診断後1年以上経過した症例，60歳以下の症例の6つの因子が麻痺発生に強く関与し，因子をまったくもたない場合4%の麻痺発生率に比較して6因子をすべてもつ症例では，MSCC発生危険率は88%と極めて高い．また，発症から放射線治療開始まで2週間以内の症例では，2週間以後の症例に比較して機能温存率は高く，麻痺発生後の迅速な対応が不可欠である．治療が遅れる原因として，MSCC患者3分の2が，がんの再燃で麻痺が起こることを認識していないことが指摘さ

れている．患者や診断者の認識不足，病院連携の不備は大きな治療障害因子である．

検査と診断

脊椎転移は，単純X線撮影，骨シンチグラフィーでスクリーニングされるが，骨梁間型や高齢者の骨粗鬆症例では診断感度は低く，緊急造影CTやMRIによる精査が不可欠である．運動，知覚異常，膀胱直腸括約筋の障害についての問診と神経学的診察に基づいた麻痺レベル情報とともに，MRI画像，造影CT画像撮像を実施して病態診断を行う．有用性は定まっていないが，PETやPET-CT検査も参考にして，迅速な診断が重要である．

治　療

MCSSの治療は，放射線治療，手術療法，化学療法，緩和治療の組み合わせで行われるべきである．軽微な症状のうちに早期発見されると放射線治療でも治療成績は良好である一方，進行，重篤な麻痺症例では緊急的治療が必要で，麻痺高度，増悪例では手術も考慮される．

1．放射線療法

治療目的は，①除痛，②病的骨折の予防，③脊髄圧迫症状改善で，通常30Gy10回分割外照射で，除痛効果80%と2〜3カ月後に65〜85%の骨再生が観察される．麻痺改善率は治療開始時の運動，歩行能力に左右され，独歩可能症例90%，支持歩行症例65%，不全麻痺40%，完全麻痺15%の歩行機能改善率が報告されている．ステロイド併用し麻痺の進行を防止しつつ放射線治療等が行われると成績は向上する．緊急照射開始などの迅速な対応や，化学療法の奏効性が高い癌種では積極的に放射線化学療法を併用することが推奨される．予後6カ月以上，溶骨性病変で十分な骨形成が必要な症例では，より抗腫瘍効果の高い高線量治療が実施されることもある．

1）MSCCに対する副腎皮質ホルモンの有用性

麻痺症例に対するステロイド併用治療は，非投与群より明らかに機能改善効果は認められ併用することが推奨されているが，麻痺を認めない症例の予防的ステロイド投与は必要ない．具体的な治療方法として，多くのガイドラインで，dexamethasone 16 mgの投与と，連日投与16 mg追加が記載されている．麻痺発生初期に大量dexamethasone 100 mg静脈投与，中等量10 mg静脈投与との比較研究では，疼痛軽減効果では有意差が報告されているものの，麻痺増悪防止効果は認めず，約10%と毒性も高く，大量ステロイド投与方法の有用性は少ない[2]．

2）放射線治療スケジュール

前向き試験3，ケースコントロール2，3つの後ろ向きシステマチックレビュー研究によると，照射スケジュールの違いによる麻痺改善効果の差は認められていない[1]．患者の経済，身体的負担軽減を目的に1回線量の増強，短期間スケジュールも積極的に実施されている．

3）予防照射の意義と照射後のMSCC再燃に対する再治療

症状の軽微な時期の予防的放射線治療は機能面の成績で良好であるとの報告は多いが，脊椎転移がMSCCへと進展するリスクは最大2割，脊椎転移巣にすべてに予防的照射するほどの根拠は見出されていない．

一方，照射後の晩期再発例は放射線抵抗性であり，照射後脊髄炎，麻痺のリスクから一般に再照射は勧められていない．再照射に関する報告[1]によると，麻痺進行の再防止効果を認め，短い予後で放射線障害発生率は少ないとの反論もあるが，リスクの高い手術療法ともに標準的治療とは言い難い．再燃例は一般に予後不良で，全身状態や予後参考に，手術療法，再放射

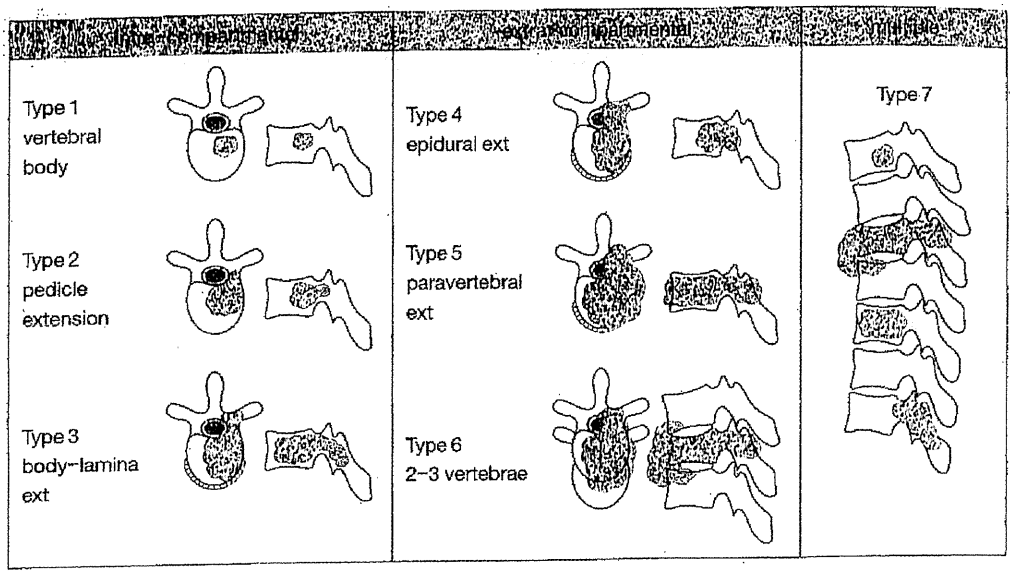

図2 富田の手術分類

脊椎コンパートメント内：type 1（椎体内），type 2（椎弓根進展），type 3（椎体，後方へ）

脊椎コンパートメント外：type 4（硬膜外進展），type 5（椎体近傍），type 6（上下椎体浸潤）

多発：type 7

単発脊椎転移で1年以上の予後予測される症例では，type 2−3 手術分類では脊椎全摘術，ないし前方後方から準根治的切除が行われ，type 5−6 でも，腫瘍削壊全摘術が選択される．予後予測が6カ月以上1年以内の場合，緩和的後方固定と放射線療法併用が選択されることが多い．当然，多発の type 7 でも緩和的後方固定±椎弓切除が行われることもあるが，内固定性の低さや予後不良で実施されることは少ない．

[文献5）より引用]

線治療，best support care の選択が慎重に検討されるべきである．

2．手術療法

MSCC に対する外科治療の目的は脊髄除圧と脊椎支持性を保つことであり，治療障害因子である骨破壊や圧迫変形が強い不安定な脊椎転移例には，脊椎外科医，放射線治療医ともに手術が必要だと考えている．一般に，転移性脊椎腫瘍の手術方法は3つに分類され，①脊椎椎弓切除と後方固定による脊椎支持性再建を行い，術後照射を併用する緩和的後方固定（椎弓切除・後方固定）法，②前方の単発腫瘍と脊体を切除し，欠損部に骨移植や人工椎体で置換し，前方固定機材で固定する椎体切除・前方固定法，③局所根治性を高めた脊椎腫瘍を一塊切除し，前方と後方ともにインスツルメントで再建する前方・後方固定方法，いわゆる脊椎全摘術がある．

緩和的後方固定術は，脊椎腫瘍を処理せずに，脊髄圧迫除圧，麻痺の不良因子である脊椎の変形や不安定性を修復する治療方法で，術後に放射線治療併用して腫瘍を制御する．低侵襲（合併症発生率は10%）の後方固定術は，予後予測で6カ月から1年以内，多発転移の症例にも行えるなど応用，適応は広い．一方，椎体の転移性腫瘍全切除は，前方側方からのアプローチが必要で，腫瘍切除操作中の大量出血や長時間手術など手術侵襲は大きく（合併症発生率は25〜35%），1年から数年の予後予測可能な単発転移例の放射線治療抵抗性の甲状腺癌，腎臓

表1 治療成績別の改善率，悪化率，合併症発生率，死亡率

治療法	症例数	改善率（改善%）	症状悪化率（%）	合併症（%）	治療関連死（%）
放治療単独	1,485（'75-97）	39	16		
椎弓切除，放治	1,959（'73-95）	42	14.5		6.5
椎弓切除，固定	510（'75-95）	72（83）		11	6
椎体切除，固定	450（'82-97）	71（85）		23	6
前方後方固定	335（'93-98）	81（85）		37	2.6

[Siegal T, Siegal T, Brada M：Spinal metastatic disease. pp979-991. Oxford textbook of oncology. 2nd ed. Edited by Souhami RL, et al]

癌等で発症したMSCCに対する手術方法である．麻痺改善率，局所制御性ともに優れ，再発も少ない．さらに，椎体腫瘍を一塊として切除する全椎体切除術も開発され，2～3年内の再発率は10～20%と局所制御性は高いものの[5]，高度な手術技術が必要で実施可能な施設は限定される．

予後予測[5]，罹患椎体数，骨外病変進展状況（図2）を参考に，リスクベネフィットを考慮した手術適応や最適手術方法が実施される昨今，約10%であった死亡率は，2.5%以下にまで低下した．

手術治療と放射線治療との優劣は，前向き研究2報，比較試験1報，12報の後ろ向き研究をもとにした系統的レビューによると，手術療法の麻痺改善率は70%，疼痛改善率85%と成績は安定し，放射線治療単独と比較した場合，歩行機能維持率は手術群85%と放射線治療群64%，麻痺例の治療改善率は手術治療群58%，放射線治療群26%と手術療法群がともに優れ[3]，無作為比較試験でも手術併用群が歩行能力維持期間125日（放射線治療単独群35日），疼痛コントロール率ともに優れ，麻痺改善率は38%（放射線治療19%）と，機能温存をエンドポイントとした場合には，手術療法は不可欠である[4]．また，照射中に麻痺進行しても，追加手術治療で30%が救済される．一方，合併症の発生率は，術後照射群12%に比較して術前照射群39%と非常に高く，追加手術はより慎重な実施が望まれる．

国内でも脊椎骨転移，脊髄圧迫症候群に対して，緩和的固定，腫瘍切除，前方後方固定など，予後予測スコアリングに基づいた手術方法の選択が行われ，より安全で効果の高い手術療法が提供されており，欧米の成績に劣ることはないが，手術療法は補助的救済的治療である．

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一口メモ

婦人科がんによる骨転移はさほど多くありません。しかし，骨盤内神経叢や下肢の浮腫をはじめ，神経・運動系のトラブルでQOLが非常に低下した末期患者さんや，車いすで通院されている方も多いのも骨盤内臓器発生のがんの特徴です。骨転移や転移性脊椎腫瘍の最大の問題は，頑固な背部痛と対麻痺が発症しているのに，麻痺完成まで診断が遅れ，骨盤の症状として放置されていた症例も時々みかけることもあります。簡単な病歴を聴き，神経学的診察を実施して，MRI検査を実施していれば，予防可能な病態であることを思い出して下さい。

（川嶋広之）

次号予告

特集/胎児死亡と胎盤病理学

Published OnlineFirst February 16, 2011; DOI:10.1158/1078-0432.CCR-10-2066

Cancer Therapy: Preclinical

Preclinical Evaluation of Telomerase-Specific Oncolytic Virotherapy for Human Bone and Soft Tissue Sarcomas

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Abstract

Purpose: Tumor-specific replication-selective oncolytic virotherapy is a promising antitumor therapy for induction of cell death in tumor cells but not of normal cells. We previously developed an oncolytic adenovirus, OBP-301, that kills human epithelial malignant cells in a telomerase-dependent manner. Recent evidence suggests that nonepithelial malignant cells, which have low telomerase activity, maintain telomere length through alternative lengthening of telomeres (ALT). However, it remains unclear whether OBP-301 is cytopathic for nonepithelial malignant cells. Here, we evaluated the antitumor effect of OBP-301 on human bone and soft tissue sarcoma cells.

Experimental Design: The cytopathic activity of OBP-301, coxsackie and adenovirus receptor (CAR) expression, and telomerase activity were examined in 10 bone (OST, U2OS, HOS, HuO9, MNNG/HOS, SaOS-2, NOS-2, NOS-10, NDCS-1, and OUMS-27) and in 4 soft tissue (CCS, NMS-2, SYO-1, and NMFH-1) sarcoma cell lines. OBP-301 antitumor effects were assessed using orthotopic tumor xenograft models. The fiber-modified OBP-301 (termed OBP-405) was used to confirm an antitumor effect on OBP-301–resistant sarcomas.

Results: OBP-301 was cytopathic for 12 sarcoma cell lines but not for the non–CAR-expressing OUMS-27 and NMFH-1 cells. Sensitivity to OBP-301 was dependent on CAR expression and not on telomerase activity. ALT-type sarcomas were also sensitive to OBP-301 because of upregulation of human telomerase reverse transcriptase (*hTERT*) mRNA following virus infection. Intratumoral injection of OBP-301 significantly suppressed the growth of OST and SYO-1 tumors. Furthermore, fiber-modified OBP-405 showed antitumor effects on OBP-301–resistant OUMS-27 and NMFH-1 cells.

Conclusions: A telomerase-specific oncolytic adenovirus is a promising antitumor reagent for the treatment of bone and soft tissue sarcomas. *Clin Cancer Res; 17(7); 1828–38. ©2011 AACR.*

Introduction

Bone and soft tissue sarcomas are annually diagnosed in 13,230 patients in the United States (1). They are the third most common cancer in children and account for 15.4% of all childhood malignancies. Treatment of patients with bone and soft tissue sarcomas requires a multidisciplinary approach that involves orthopedic oncologists, musculoskeletal radiologists and pathologists, radiation oncologists, medical and pediatric oncologists, and microvascular surgeons (2, 3). Despite major advances in the treatment of bone and soft tissue sarcomas, such as neoadjuvant and adjuvant multiagent chemotherapy and aggressive surgery, about one fourth of the patients show a poor response to conventional therapy, resulting in subsequent recurrence and leading to a poor prognosis (1). Therefore, the development of a novel therapeutic strategy is required to cure patients with bone and soft tissue sarcomas.

Recent advances in molecular biology have fostered remarkable insights into the molecular basis of neoplasia. More than 85% of all human cancers, but only a few normal somatic cells, show high telomerase activity (4–6). Telomerase activity has also been detected in 17% to 81% of bone and soft tissue sarcomas (7–10). Telomerase activation is considered to be a critical step in cancer development, and its activity is closely correlated with the expression of human telomerase reverse transcriptase

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

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doi: 10.1158/1078-0432.CCR-10-2066

Published OnlineFirst February 16, 2011; DOI:10.1158/1078-0432.CCR-10-2066

Translational Relevance

Bone and soft tissue sarcomas frequently occur in young children and show aggressive progression, resistance to conventional chemotherapy, and poor prognosis, indicating a requirement for novel antitumor therapy to improve the clinical outcome. Telomerase-specific replication-selective oncolytic virotherapy is emerging as a promising antitumor therapy. We developed an oncolytic adenovirus, OBP-301, that efficiently kills human epithelial malignant cells in a telomerase-dependent manner. However, alternative lengthening of telomeres (ALT)-type nonepithelial malignant cells show low telomerase activity, suggesting lower effectiveness of OBP-301 in these cells. Here, we showed that OBP-301 has antitumor effects on both non–ALT-type and ALT-type sarcoma cells through upregulation of human telomerase reverse transcriptase mRNA. Furthermore, coxsackie and adenovirus receptor–negative sarcoma cells were efficiently killed by fiber-modified OBP-301 (termed OBP-405) through virus–integrin binding. Thus, a telomerase-specific oncolytic adenovirus would greatly improve the clinical outcome of young patients with advanced sarcomas.

(*hTERT*; ref. 11). Recently, telomerase-specific replication-selective oncolytic virotherapy has emerged as a promising antitumor therapy for induction of tumor-specific cell death. We previously developed an oncolytic adenovirus, OBP-301, in which the *hTERT* promoter drives the expression of the *E1A* and *E1B* genes linked to an internal ribosome entry site (IRES; ref. 12). We determined that OBP-301 efficiently induced the selective killing of a variety of human malignant epithelial cells, such as colorectal, prostate, and non–small cell lung cancers, but not of normal cells (12, 13). Furthermore, a phase I clinical trial of OBP-301, which was conducted in the United States on patients with advanced solid tumors, indicated that OBP-301 is well tolerated by patients (14).

There are 2 known telomere-maintenance mechanisms in human malignant tumors (15, 16): telomerase activation (4–6) and telomerase-independent alternative lengthening of telomeres (ALT; ref. 17–19). The ALT-type mechanism is more prevalent in tumors arising from nonepithelial tissues than in those of epithelial origin (20, 21). Therefore, ALT-type nonepithelial malignant cells frequently show low telomerase activity, suggesting that they have a low sensitivity to OBP-301, which kills cancer cells in a telomerase-dependent manner. However, it remains to be determined whether OBP-301 can exert an antitumor effect on human nonepithelial and on epithelial malignancies.

Adenovirus infection is mainly mediated by interaction of the virus with the coxsackie and adenovirus receptor (CAR) expressed on host cells (22). Therefore, while CAR-expressing tumor cells are the main targets for oncolytic adenoviruses, tumor cells that lack CAR can escape from being killed by oncolytic adenoviruses. It has been reported that CAR is frequently expressed in human cancers of various organs such as the brain (23), thyroid (24), esophagus (25), gastrointestinal tract (26), and ovary (27). Bone and soft tissue sarcomas also express CAR (28–30). However, some populations of tumor cells lack CAR expression, suggesting a requirement for the development of a novel antitumor therapy against CAR-negative tumor cells. We recently developed fiber-modified OBP-301 (termed OBP-405), which can bind to not only CAR but also integrin molecules ($\alpha v\beta 3$ and $\alpha v\beta 5$) and efficiently kill CAR-negative tumor cells (31).

In the present study, we first investigated the *in vitro* cytopathic efficacy of OBP-301 against 14 human bone and soft tissue sarcoma cells. Next, the relationship between the cytopathic activity of OBP-301, CAR expression, and telomerase activity in human sarcoma cells was assessed. The *in vivo* antitumor effect of OBP-301 was also confirmed using orthotopic animal models. Finally, the antitumor effect of OBP-405 against OBP-301–resistant sarcoma cells was evaluated *in vitro* and *in vivo*.

Materials and Methods

Cell lines

The human osteosarcoma (HuO9; ref. 32), chondrosarcoma (OUMS-27; ref. 33), and synovial sarcoma (SYO-1; ref. 34) cell lines were previously established in our laboratory. The human osteosarcoma cell lines OST, HOS, and SaOS-2 were kindly provided by Dr. Satoru Kyo (Kanazawa University, Ishikawa, Japan). The human clear cell sarcoma cell line CCS was maintained in our laboratory. These cells were propagated as monolayer cultures in Dulbecco's modified Eagle's medium (DMEM). The human osteosarcoma cell line U2OS was obtained from the American Type Culture Collection (ATCC) and was grown in McCoy's 5a medium. The human osteosarcoma cell line MNNG/HOS was purchased from DS Pharma Biomedical and was maintained in Eagle's minimum essential medium containing 1% nonessential amino acids. The human osteosarcoma cell lines NOS-2 and NOS-10 (35), the human dedifferentiated chondrosarcoma cell line NDCS-1 (36), the human malignant peripheral nerve sheath cell line NMS-2 (37), and the human malignant fibrous histiocytoma cell line NMFH-1 (38) were kindly provided by Dr. Hiroyuki Kawashima (Niigata University, Niigata, Japan) and were grown in RPMI-1640 medium. The transformed embryonic kidney cell line 293 was obtained from the ATCC and maintained in DMEM. All media were supplemented with 10% heat-inactivated FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin. The cells were maintained at 37°C in a humidified atmosphere with 5% CO_2.

Recombinant adenoviruses

The recombinant tumor-specific, replication-selective adenovirus OBP-301 (Telomelysin), in which the promoter

Published OnlineFirst February 16, 2011; DOI:10.1158/1078-0432.CCR-10-2066

element of the *hTERT* gene drives the expression of *E1A* and *E1B* genes linked with an IRES, was previously constructed and characterized (12, 13). OBP-405 is a telomerase-specific replication-competent adenovirus variant that was previously generated to express the RGD peptide in the fiber knob of OBP-301 (31). The *E1A*-deleted adenovirus vector dl312 and wild-type adenovirus serotype 5 (Ad5) were used as the control vectors. Recombinant viruses were purified by ultracentrifugation using cesium chloride step gradients, and their titers were determined by a plaque-forming assay by using 293 cells and they were stored at −80°C.

Cell viability assay

Cells were seeded on 96-well plates at a density of 1×10^3 cells/well 20 hours before viral infection. All cell lines were infected with OBP-301 or OBP-405 at multiplicity of infections (MOI) of 0, 0.1, 1, 10, 50, or 100 plaque forming units (PFU)/cell. Cell viability was determined on days 1, 2, 3, and 5 after virus infection, using a Cell Proliferation kit II (Roche Molecular Biochemicals) that was based on an XTT, sodium 3′-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis (4-methoxy-6-nitro)benzene sulfonic acid hydrate, assay, according to the manufacturer's protocol. The ID_{50} value of OBP-301 for each cell line was calculated using cell viability data obtained on day 5 after virus infection.

Flow cytometric analysis

The cells (5×10^5) were labeled with mouse monoclonal anti-CAR (RmcB; Upstate Biotechnology), anti-human integrin αvβ3 (LM609; Chemicon International), or anti-human integrin αvβ5 (P1F6; Chemicon International) antibody for 30 minutes at 4°C. The cells were then incubated with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse IgG second antibody (Zymed Laboratories) and were analyzed using flow cytometry (FACS Array; Becton Dickinson). The mean fluorescence intensity (MFI) of CAR and integrin αvβ3 or αvβ5 for each cell line was determined by calculating the difference between the MFI in antibody-treated and nontreated cells from 3 independent experiments.

Quantitative real-time PCR analysis

U2OS cells, seeded on 6-well plates at a density of 5×10^5 cells/well 20 hours before viral infection, were infected with Ad5, OBP-301, or dl312 at an MOI of 10 or 100 PFUs/cell. Mock-infected cells were used as controls. Furthermore, to confirm the modulation of *hTERT* mRNA expression by OBP-301 infection, CAR-positive and *hTERT* mRNA-expressing human sarcoma cell lines were seeded on 6-well plates at a density of 5×10^4 cells/well 20 hours before viral infection and were infected with OBP-301 at an MOI of 100 PFUs/cell. Total RNA was extracted from the cells 2 days after virus infection by using the RNA-Bee reagent (Tel-Test Inc.). After synthesis of cDNA from 100 ng of total RNA, the levels of *hTERT* and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) mRNA expression were determined using quantitative real-time PCR and a Step One Plus Real Time PCR System (Applied Biosystems) and TaqMan Gene Expression Assays (Applied Biosystems). The relative levels of *hTERT* mRNA expression were calculated by using the $2^{-\Delta\Delta Ct}$ method (39) after normalization with reference to the expression of *GAPDH* mRNA.

To compare the *E1A* copy number between OBP-301- and Ad5-infected U2OS cells, U2OS cells, seeded on 6-well plates at a density of 5×10^5 cells/well 20 hours before viral infection, were infected with OBP-301 or Ad5 at an MOI of 10 PFUs/cell. Genomic DNA was extracted from serially diluted viral stocks, and tumor cells were infected with OBP-301 or Ad5 by using the QIAmp DNA Mini Kit (Qiagen). *E1A* copy number was also determined using TaqMan real-time PCR systems (Applied Biosystems).

In vivo OST and OUMS-27 xenograft tumor models

Animal experimental protocols were approved by the Ethics Review Committee for Animal Experimentation of Okayama University School of Medicine. The OST and OUMS-27 cells (5×10^6 cells per site) were inoculated into the tibia or the flank of female athymic nude mice aged 6 to 7 weeks (Charles River Laboratories). Palpable tumors developed within 14 to 21 days and were permitted to grow to approximately 5 to 6 mm in diameter. At that stage, a 50 μL volume of solution containing OBP-301, OBP-405, dl312, or PBS was injected into the tumors. Tumor size was monitored by measuring tumor length and width by using calipers. The volumes of OUMS-27 tumors were calculated using the following formula: $(L \times W^2) \times 0.5$, where L is the length and W is the width of each tumor. The volumes of OST tumors were calculated using the formula: $(L + W) \times L \times W \times 0.2618$, as previously reported (40).

X-ray examination

The formation of osteolytic lesions was monitored using radiography (FUJIFILM IXFR film; FUJIFILM Co.) and an X-ray system (SOFTEX TYPE CMB; SOFTEX Co.).

Histopathologic analysis

Tumors were fixed in 10% neutralized formalin and embedded in paraffin blocks. Sections were stained with hematoxylin/eosin (H&E) and analyzed by light microscopy.

Statistical analysis

Data are expressed as means ± SD. Student's t test was used to compare differences between groups. Pearson's product–moment correlation coefficients were calculated using PASW statistics version 18 software (SPSS Inc.). Statistical significance was defined when the P value was less than 0.05.

Results

In vitro cytopathic efficacy of OBP-301 against human bone and soft tissue sarcoma cell lines

To evaluate the *in vitro* cytopathic effect of OBP-301 against nonepithelial malignant cells, 14 tumor cell lines

Published OnlineFirst February 16, 2011; DOI:10.1158/1078-0432.CCR-10-2066

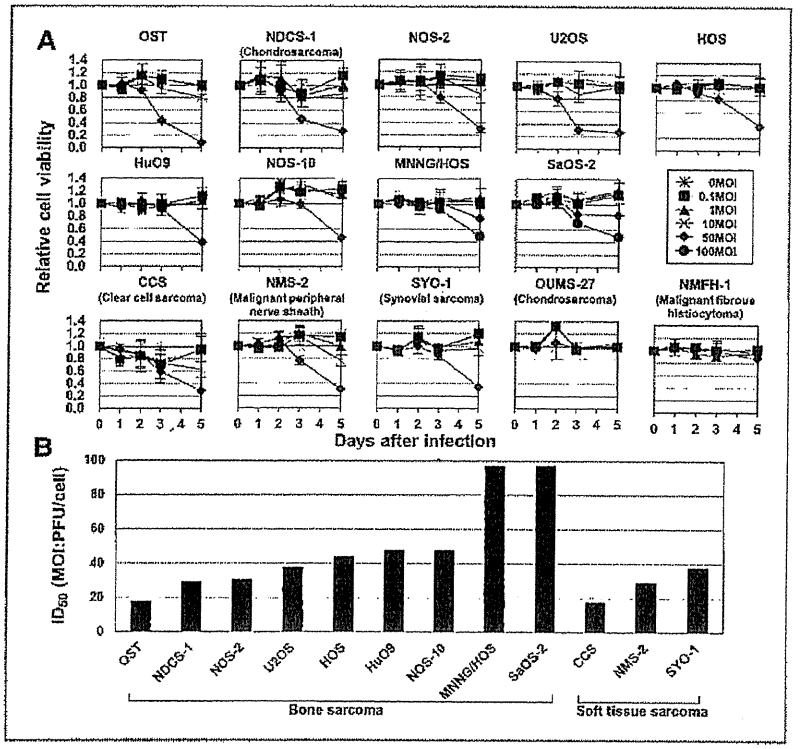

Figure 1. Cytopathic effect of OBP-301 on human bone and soft tissue sarcoma cell lines. A, cells were infected with OBP-301 at the indicated MOI, and cell survival was quantified over 5 days using the XTT assay. The cell viability of mock-treated group on each day was considered 1.0, and the relative cell viability was calculated. Data are means ± SD. The types of tumor except for osteosarcoma were shown in parentheses. B, the 50% inhibiting doses of OBP-301 on cell viability 5 days after infection were calculated and are expressed as ID_{50} values.

derived from human bone and soft tissue sarcomas were infected with various doses of OBP-301. The cell viability of each cell line was assessed over 5 days after infection by the XTT assay. OBP-301 infection induced cell death in a time-dependent manner in all sarcoma cell lines except for the OUMS-27 and NMFH-1 cell lines (Fig. 1A). Calculation of the ID_{50} values revealed that, of the 12 OBP-301–sensitive sarcoma cell lines, MNNG/HOS and SaOS-2 cells were relatively less sensitive than the other 10 sarcoma cell lines (Fig. 1B). Furthermore, to rule out the possibility that cytopathic effect of OBP-301 is due to nonspecific toxicity based on the high uptake of virus particles into tumor cells, we examined the cytopathic activity of replication-deficient dl312 in U2OS and HOS cells. dl312 did not show any cytopathic effect in U2OS and HOS cells, even when these cells were infected with dl312 at high dose (50 and 100 MOIs; Supplementary Fig. S1). These results indicate that OBP-301 is cytopathic for most human bone and soft tissue sarcoma cells line but that some sarcoma cell lines are resistant to OBP-301.

Expressions of the adenovirus receptor and *hTERT* mRNA on human bone and soft tissue sarcoma cell lines

Because adenovirus infection efficiency depends mainly on cellular CAR expression (22), we determined the expression level of CAR on the 14 sarcoma cell lines by flow cytometry. The 12 OBP-301–sensitive sarcoma cell lines showed CAR expression, determined as MFIs, at various levels, whereas the OBP-301–resistant OUMS-27 and NMFH-1 cells did not express CAR (Fig. 2A and Supplementary Fig. S2).

OBP-301 contains the *hTERT* gene promoter, which allows it to tumor specifically regulate the gene expression of *E1A* and *E1B* for viral replication. Thus, OBP-301 can efficiently replicate in human cancer cells with high telomerase activity but not in normal cells without telomerase activity (12). Recently, some populations of human sarcoma cells have been shown to possess low telomerase activity and to maintain telomere lengths through an ALT mechanism (17–19). Thus, it is probable that OBP-301 cannot efficiently replicate in, and kill, ALT-type human sarcoma cells because of their low telomerase activity. To assess whether the telomerase activity of human sarcoma cells affects the cytopathic activity of OBP-301, we analyzed *hTERT* mRNA expression levels in the 14 sarcoma cell lines by quantitative real-time reverse transcriptase PCR (RT-PCR) analysis. Thirteen of the sarcoma cell lines had detectable *hTERT* mRNA expression at variable levels, and only SaOS-2 cells did not express *hTERT* mRNA (Fig. 2B).

Published OnlineFirst February 16, 2011; DOI:10.1158/1078-0432.CCR-10-2066

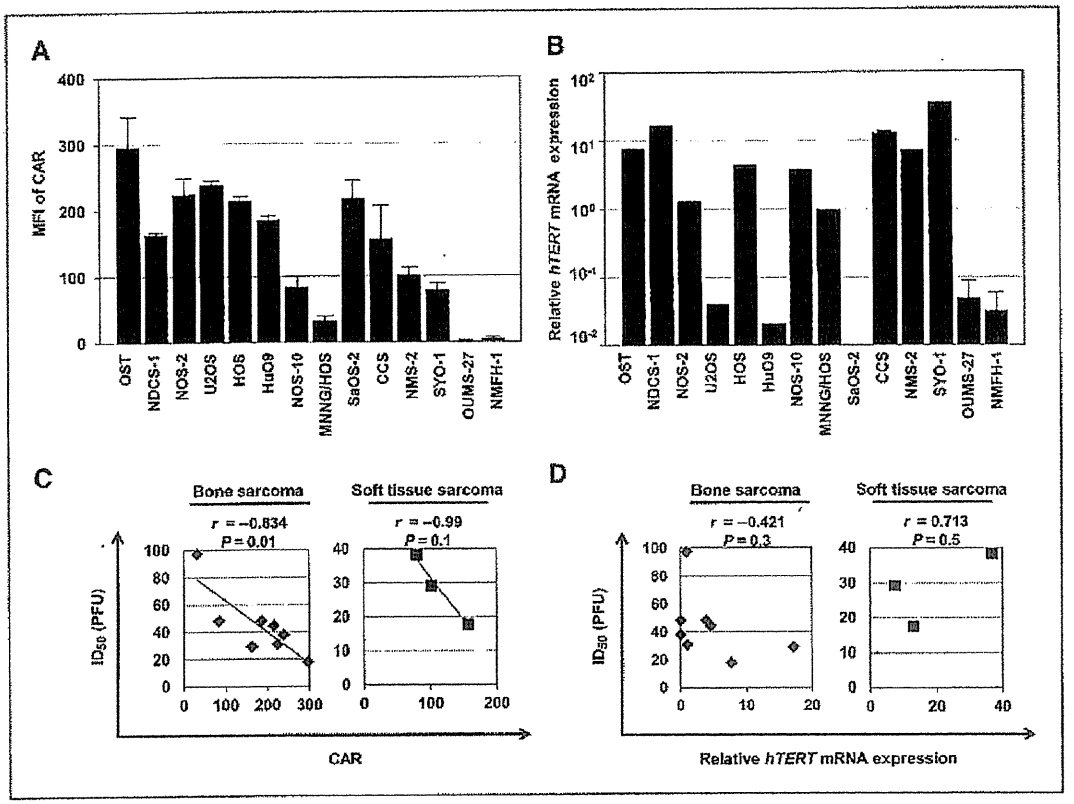

Figure 2. Relationship between the expression levels of CAR and *hTERT* mRNA and the cytopathic activity of OBP-301 against human bone and soft tissue sarcoma cell lines. A, the MFI of CAR expression on human bone and soft tissue sarcoma cells. The cells were incubated with a monoclonal anti-CAR (RmcB) antibody, followed by flow cytometric detection using an FITC-labeled secondary antibody. B, expression of *hTERT* mRNA in human bone and soft tissue sarcoma cells by quantitative real-time PCR. The relative levels of *hTERT* mRNA were calculated after normalization with reference to the expression of *GAPDH* mRNA. C, correlation between the MFI of CAR and the ID_{50} of OBP-301 on human bone and soft tissue sarcoma cells. D, correlation between *hTERT* mRNA expression and the ID_{50} of OBP-301 on human bone and soft tissue sarcoma cells. Statistical significance was determined as $P < 0.05$, after analysis of Pearson's correlation coefficient.

We next investigated the relationship between CAR and *hTERT* mRNA expressions and the cytopathic activity of OBP-301 among the 11 CAR-positive sarcoma cell lines with *hTERT* gene expression. CAR expression levels significantly ($r = -0.834$; $P = 0.01$) correlated with the cytopathic activity of OBP-301 against 8 of the bone sarcoma cell lines (Fig. 2C). CAR expression in 3 of the soft tissue sarcoma cell lines also correlated ($r = -0.99$) with the cytopathic effect of OBP-301, but the differences did not reach significance ($P = 0.1$) because of the low number of cell lines assayed. In contrast, there was no significant correlation between *hTERT* mRNA expression and the cytopathic activity of OBP-301 (Fig. 2D). These results indicate that the cytopathic activity of OBP-301, at least in part, depends on CAR expression.

Furthermore, SaOS-2 and U2OS cells have already been shown to be ALT-type sarcoma cell lines with low telomerase activity (9, 17). Among these ALT-type sarcoma cells, U2OS cells showed a sensitivity to OBP-301 that was similar to that of non–ALT-type sarcoma cells such as HOS and NOS-10 (Fig. 1B). These results indicate that ALT-type human sarcoma cells are sensitive to OBP-301 and that a low telomerase activity does not detract from the cytopathic activity of OBP-301.

Enhanced virus replication and cytopathic activity of OBP-301 through *hTERT* mRNA upregulation in ALT-type sarcoma cell lines

The high sensitivity of ALT-type sarcoma cells to OBP-301 prompted us to hypothesize that OBP-301 may activate the *hTERT* gene promoter, thereby enhancing the viral replication rate and subsequently inducing cytopathic activity in ALT-type sarcoma cells. Furthermore, it has been previously shown that the adenoviral E1A

Published OnlineFirst February 16, 2011; DOI:10.1158/1078-0432.CCR-10-2066

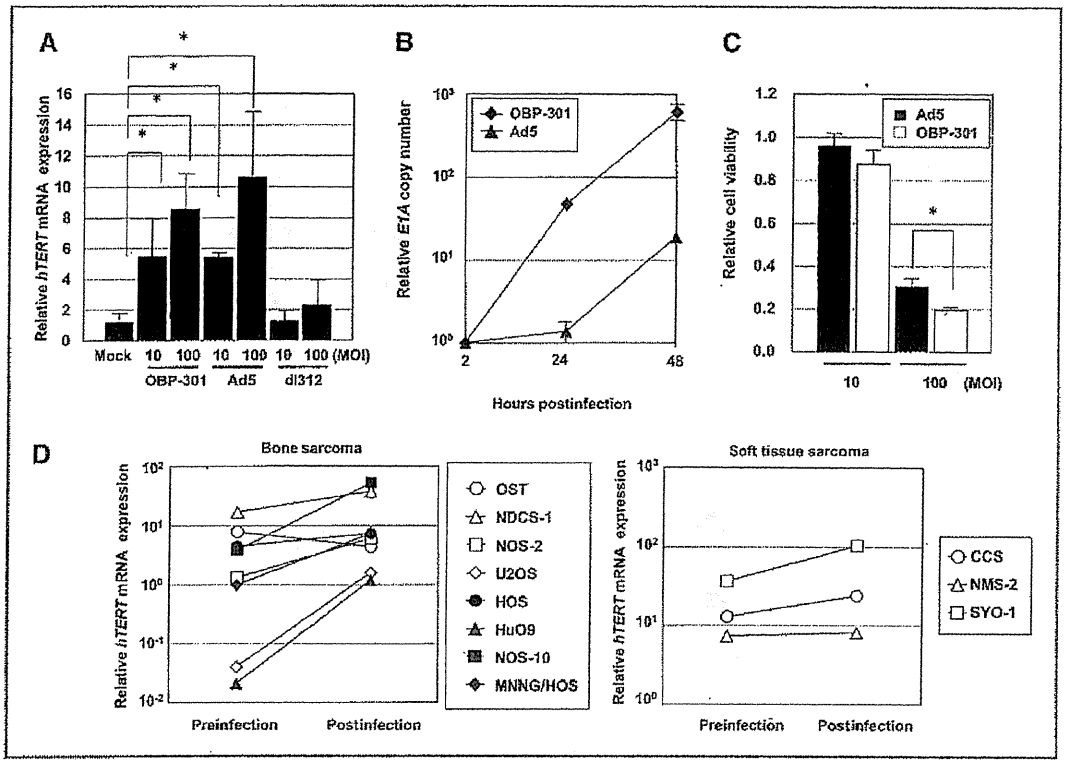

Figure 3. Upregulation of *hTERT* gene expression in ALT-type human sarcoma cell lines enhances the replication and the cytopathic effect of OBP-301. A, expression of *hTERT* mRNA in U2OS cells that were mock-infected or were infected with OBP-301, Ad5, or dl312. The cells were infected with OBP-301, Ad5, or dl312 at the indicated MOIs for 48 hour, and *hTERT* mRNA expression was analyzed using quantitative real-time RT-PCR. The value of *hTERT* mRNA expression in the mock-infected cells was set at 1, and relative mRNA levels were plotted. B, quantitative measurement of viral DNA replication in U2OS cells infected with OBP-301 or Ad5. The cells were infected with OBP-301 or Ad5 at an MOI of 10 PFUs/cell, and *E1A* copy number was analyzed over the following 2 days by quantitative real-time PCR. The value of the *E1A* copy number at 2 hours after infection was set at 1, and relative copy numbers were plotted. C, comparison of the cytopathic effect of OBP-301 and Ad5 in U2OS cells. The cells were infected with OBP-301 or Ad5 at the indicated MOIs, and cell survival was quantified 5 days after infection by using an XTT assay. D, expression of *hTERT* mRNA after infection of human bone (left) and soft tissue (right) sarcoma cell lines with OBP-301 at an MOI of 100 PFUs/cell. Statistical significance (*) was determined as $P < 0.05$ (Student's *t* test).

protein can activate the promoter activity of the *hTERT* gene (41, 42). Therefore, to determine whether OBP-301 infection activates *hTERT* mRNA expression, we examined the expression level of *hTERT* mRNA in ALT-type U2OS cells after infection with OBP-301 at MOIs of 10 and 100 PFUs/cell (Fig. 3A). Compared with mock-infected U2OS cells, OBP-301–infected U2OS cells showed a 6- to 8-fold increase in *hTERT* mRNA expression in a dose-dependent manner. Ad5 infection also increased *hTERT* mRNA expression in U2OS cells, whereas there was no increase in U2OS cells infected with *E1A*-deleted dl312. These results suggest that OBP-301 is cytopathic for ALT-type sarcoma cells through E1A-mediated activation of the *hTERT* gene promoter.

We next compared viral replication rates after infection of ALT-type U2OS cells with OBP-301 or Ad5. As expected, the viral replication rate of OBP-301 was significantly higher than that of Ad5 (Fig. 3B). Furthermore, the cytopathic activity of OBP-301 was significantly higher than that of Ad5 against the ALT-type U2OS cells (Fig. 3C). Finally, to determine whether OBP-301 activates *hTERT* mRNA expression in both ALT-type and non–ALT-type human sarcoma cell lines, we infected 11 CAR-positive human sarcoma cells with OBP-301 at 100 MOI. Ten of the 11 CAR-positive human sarcoma cell lines showed an increase in the expression level of *hTERT* mRNA after OBP-301 infection that ranged from a 1.1- to 50.0-fold increase (Fig. 3D and Supplementary Table S1). In addition, the expression level of *hTERT* mRNA was also upregulated when OST cells were infected with 5 or 50 MOI of OBP-301 (Supplementary Fig. S3). These results suggest that OBP-301 is cytopathic for both ALT-type and non–ALT-type human sarcoma cells through activation of the *hTERT* gene promoter.

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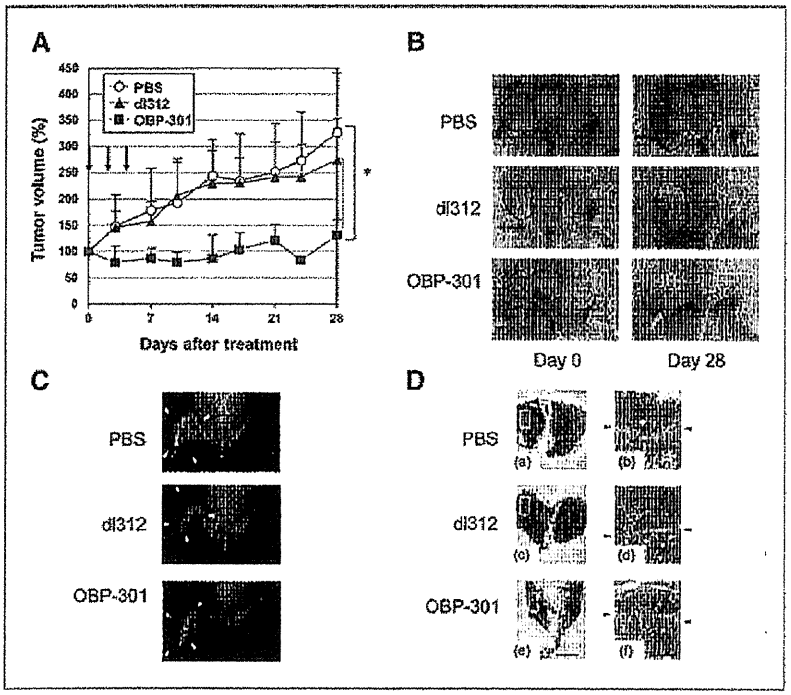

Figure 4. Antitumor effect of OBP-301 in an orthotopic OST bone sarcoma xenograft model. A, athymic nude mice were inoculated intratibially with OST cells (5×10^6 cells/site). Fourteen days after inoculation (designated as day 0), OBP-301 (■) or OBP-405 (▲) was injected into the tumor, with 1×10^8 PFUs on days 0, 2, and 4. PBS (○) was used as a control. Four mice were used for each group. Tumor growth was expressed as mean tumor volume ± SD. Statistical significance (*) was determined as $P < 0.05$ (Student's *t* test). B, macroscopic appearance of OST tumors in nude mice on days 0 and 28 after treatment with PBS, dl312, or OBP-301. Tumor masses are outlined by a dotted line. C, X-ray photographs of mice bearing OST tumors. The white arrowheads indicate the space occupied by the tumor mass. D, histologic analysis of the OST tumors. Tumor sections were obtained 28 days after inoculation of tumor cells. Paraffin-embedded sections of OST tumors were stained with H&E. The black arrowheads indicate growth plate cartilages. a, c and e, are low-magnification images and b, d and f are high-magnification images of the area outlined by a white square. Left scale bar, 5 mm. Right scale bar, 500 μm.

Antitumor effect of OBP-301 against 2 orthotopic tumor xenograft models

To evaluate the *in vivo* antitumor effect of OBP-301 against human bone and soft tissue sarcomas, we used 2 types of orthotopic tumor xenograft models: the OST bone sarcoma xenograft and the SYO-1 subcutaneous soft tissue sarcoma xenograft. We first identified a dose of OBP-301 that was suitable for induction of an antitumor effect in the subcutaneous OST bone sarcoma xenograft model (determined as $>10^7$ PFUs; Supplementary Fig. S4). We next assessed the antitumor effect of OBP-301 on the orthotopic OST bone sarcoma xenograft model. OBP-301 was injected into the tumor once a day for 3 days, with 10^8 PFUs per day (10). Replication-deficient adenovirus dl312 or PBS was also injected into control groups. Tumor growth was significantly suppressed by OBP-301 injection compared with injection of dl312 or PBS (Fig. 4A). Macroscopic analysis of the tumors indicated that OBP-301–treated tumors were consistently smaller than dl312- or PBS-treated tumors on day 28 after treatment (Fig 4B). We further determined whether OBP-301–treated tumors were less destructive to surrounding normal tissues than control tumors, using X-ray and histologic analyses (Fig. 4C and D). X-ray examination revealed that OBP-301–treated tumors resulted in less bone destruction than dl312- or PBS-treated tumors. Histologic findings were consistent with the X-ray results, showing that some tumor tissue had penetrated over the growth plate cartilage in dl312- and PBS-treated tumors but not in OBP-301–treated tumors.

With future clinical application in mind, we sought to establish a suitable protocol for repeated intratumoral injection of OBP-301 by using an orthotopic SYO-1 soft tissue sarcoma xenograft model. Doses of OBP-301 that were suitable for induction of an antitumor effect on SYO-1 tumors ($>10^8$ PFUs) were determined in a manner similar to that of OST bone sarcoma cells (data not shown). OBP-301 was injected 3 times into the tumor, with 10^9 PFUs and intervals of 1 day, 2 days, or 1 week between injections (Supplementary Fig. S5). A total of 3 OBP-301 injections, with intervals of 2 days or 1 week between injections, induced a significant

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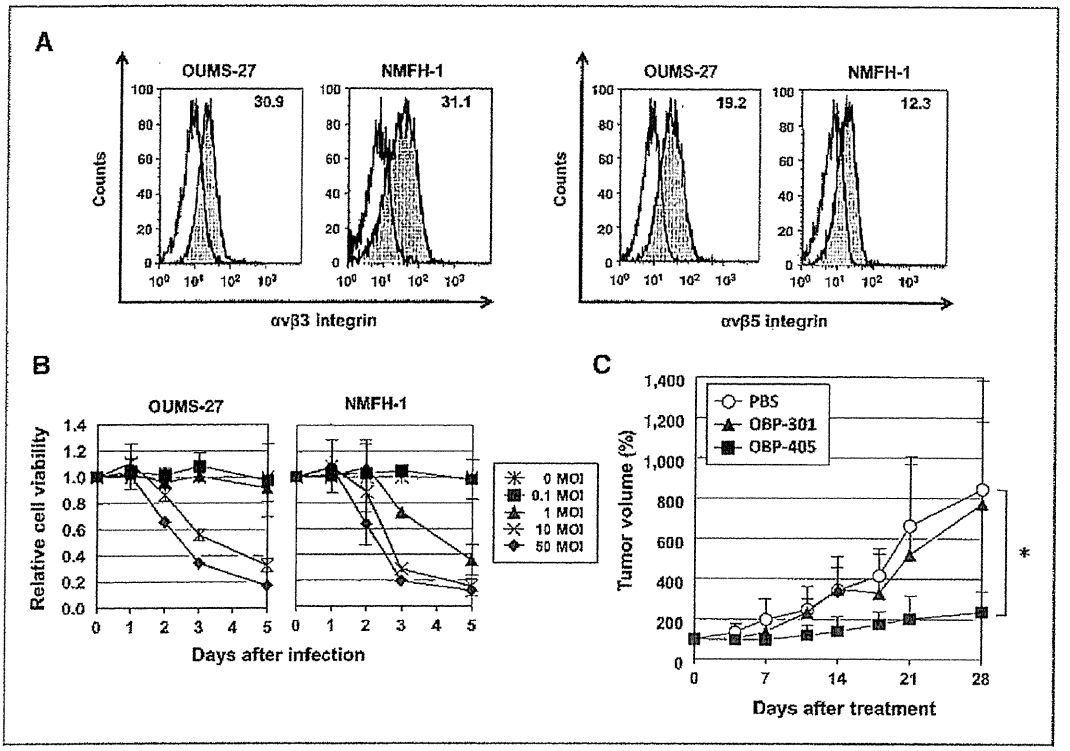

Figure 5. *In vitro* and *in vivo* antitumor effects of OBP-405 on OBP-301–resistant human sarcoma cell lines. A, expression of the integrins αvβ3 (left) and αvβ5 (right) on OUMS-27 and NMFH-1 cells. The cells were incubated with a monoclonal anti-αvβ3 integrin (LM609) or an anti-αvβ5 integrin (P1F6), followed by flow cytometric detection using an FITC-labeled secondary antibody. The gray histogram represents integrin antibody staining. The number at the top right-hand corner of each graph is the MFI. B, cytopathic effect of OBP-405 on OUMS-27 and NMFH-1 cells. The cells were infected with OBP-405 at the indicated MOI values, and cell survival over 5 days was quantified using an XTT assay. C, antitumor effect of OBP-405 in a subcutaneous OUMS-27 xenograft tumor model. Athymic nude mice were inoculated subcutaneously with OUMS-27 cells (5×10^6 cells/site). Fourteen days after inoculation (designated as day 0), OBP-301 (▲) or OBP-405 (■) was injected into the tumor, with 1×10^8 PFUs on days 0, 2, and 4. PBS (○) was used as a control. Ten mice were used for each group. Tumor growth was expressed as the mean tumor volume ± SD. Statistical significance (*) was determined as $P < 0.05$ (Student's *t* test).

suppression of tumor growth, whereas intervals of 1 day between injections were not effective. These results suggest that an interval of more than 2 days between injections is necessary to efficiently suppress tumor growth by repeated injections of OBP-301.

Antitumor effect of OBP-405 on OBP-301–resistant sarcoma cell lines

OUMS-27 and NMFH-1 cells are resistant to OBP-301 because they lack CAR expression (Fig. 1A and Supplementary Fig. S2). We previously developed a fiber-modified OBP-301, termed OBP-405, which can enter not only CAR-positive cancer cells but also CAR-negative cancer cells through binding to the cell surface integrins αvβ3 and αvβ5 (31). We therefore sought to evaluate the antitumor effect of OBP-405 on the OBP-301–resistant OUMS-27 and NMFH-1 cells. We first examined the expression levels of the integrins αvβ3 and αvβ5 on the surface of these cells by flow cytometry (Fig. 5A). OUMS-27 and NMFH-1 cells expressed both integrin molecules. We next examined the effect of OBP-405 on OUMS-27 and NMFH-1 cell viability by using the XTT assay (Fig. 5B). OBP-405 efficiently suppressed cell viability of both of these cell lines in a dose- and time-dependent manner. We further assessed whether OBP-405 has an *in vivo* antitumor effect by assaying the effect of 3 intratumoral injections of OBP-301 or OBP-405, with 10^8 PFUs or of control PBS, into subcutaneous OUMS-27 tumor xenografts. As shown in Figure 5C, administration of OBP-405 resulted in significant suppression of tumor growth compared with OBP-301- or PBS-treated tumors 28 days after treatment. These results suggest that fiber-modified OBP-405 is a potential antitumor reagent that is effective against CAR-negative human sarcoma cells.

Published OnlineFirst February 16, 2011; DOI:10.1158/1078-0432.CCR-10-2066

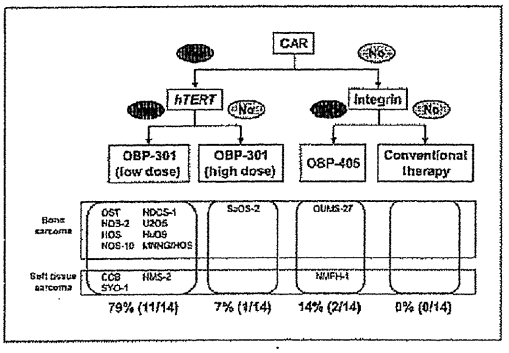

Figure 6. Outline of a therapeutic strategy for the use of telomerase-specific replication-selective oncolytic adenoviruses for human bone and soft tissue sarcoma cells. Assessment of CAR expression in tumor cells could serve as an indicator for OBP-301 or OBP-405 treatment. Of the 14 human sarcoma cell lines shown, the 12 CAR-expressing sarcoma cells (86%) should be treated with OBP-301 and the 2 sarcoma cells (14%) that lack CAR expression should be treated with OBP-405. The tumor expression level of *hTERT* mRNA would be useful in deciding the dose of OBP-301 to be used for treatment. The expression level of integrins on the tumor surface should be confirmed prior to OBP-405 treatment.

Discussion

Telomerase-specific replication-selective oncolytic adenoviruses are emerging as promising antitumor reagents for induction of tumor-specific cell death. We previously reported that OBP-301 has a strong antitumor effect on a variety of human epithelial malignant cells that have high telomerase activity (12, 13). However, nonepithelial malignant cells often show low telomerase activity and instead maintain telomere length through an ALT mechanism (20, 21). The effect of OBP-301 on human bone and soft tissue sarcoma cells has not been extensively examined. In this study, we showed that OBP-301 induced cell death in 12 of 14 human bone and soft tissue sarcoma cell lines (Fig. 1) and that the cytopathic activity of OBP-301 significantly correlated with tumor CAR expression (Fig. 2A). Furthermore, 2 ALT-type sarcoma cells showed low *hTERT* mRNA expression (Fig. 2B) but a similar sensitivity to OBP-301 compared with non–ALT-type cells because of *hTERT* mRNA upregulation by OBP-301 infection (Fig. 3). In contrast, 2 OBP-301–resistant sarcoma cells that lack CAR expression were highly sensitive to OBP-405, which can infect cells by binding to surface integrin molecules (Fig. 5). On the basis of these results, and with future clinical application in mind, we established a therapeutic strategy for the use of telomerase-specific oncolytic adenoviruses to treat patients with bone and soft tissue sarcomas (Fig. 6). This strategy involves assessment of the expression levels of CAR, *hTERT*, and integrins on human sarcoma cells, which would then allow easy selection of the most effective protocol for the treatment of patients by using oncolytic adenoviruses. Furthermore, as OBP-301 and OBP-405 show the profound antitumor effect in the combination of various chemotherapeutic agents (43, 44), further evaluation for the strategy using OBP-301 and OBP-405 in combination with chemotherapy should be warranted.

The cytopathic activity of OBP-301 significantly correlated with CAR expression, but not with telomerase activity, of human sarcoma cells (Fig. 2). These results suggest that the cytopathic activity of OBP-301 depends primarily on infection efficiency rather than virus replication. Primary epithelial and nonepithelial malignant tumors frequently express CAR (23–30). However, CAR expression can often be downregulated by tumor progression (45, 46) or under hypoxic conditions (47), possibly leading to a low infection efficiency and resistance to OBP-301. Thus, for future clinical application of OBP-301, it may be necessary to overcome the resistance to OBP-301 that arises during tumor progression. A histone deacetylase (HDAC) inhibitor has been previously shown to enhance CAR expression on human cancer cells (48–50). Therefore, for the treatment of OBP-301–resistant sarcomas, it may be necessary to either upregulate CAR expression on tumor cells in combination with an HDAC inhibitor or use OBP-405 to kill tumor cells in an integrin-dependent manner (31).

ALT-type sarcoma cells that express a low level of *hTERT* mRNA showed sensitivity to OBP-301 that was similar to that of non–ALT-type sarcoma cells (Figs. 1 and 2). We further showed that OBP-301 infection upregulates *hTERT* gene expression and subsequently activates virus replication and cytopathic activity in ALT-type sarcoma cells (Fig. 3). These results suggest that the *hTERT* gene promoter is a useful tool for enhancement of the oncolytic adenoviruses not only because it induces tumor-specific virus replication but also because it enhances virus replication after infection. Indeed, the ALT-type sarcoma SaOS-2 cells that lack *hTERT* gene expression were relatively less sensitive to OBP-301 than the other ALT-type sarcoma U2OS cells that express low levels of *hTERT* mRNA (Figs. 1 and 2). We further observed that *hTERT* mRNA expression was not upregulated after OBP-301 infection of SaOS-2 cells (data not shown). These results suggest that if *hTERT* gene expression cannot be detected in tumor cells, then ALT-type sarcoma cells should be treated with high doses of OBP-301, or with OBP-405, to enhance OBP-301 infection efficiency (Fig. 6).

It is also worth noting in terms of future clinical application that an interval of more than 2 days between injections is necessary in order for repeated injections of OBP-301 to induce a strong antitumor effect in an SYO-1 animal xenograft model (Supplementary Fig. S5). We first expected that continuous injection of OBP-301 at intervals of 1 day, when tumors are of a minimum size, might be more effective in inducing an antitumor effect than injection at intervals of 2 days or 1 week. Surprisingly, continuous injection of OBP-301 at intervals of 1 day, for 3 days, could not induce an antitumor effect. There are 2 possible explanations for these results. The

Published OnlineFirst February 16, 2011; DOI:10.1158/1078-0432.CCR-10-2066

first possibility is that 3 days of continuous injections may not provide enough time for OBP-301 to replicate and reach the minimal dose required for induction of an antitumor effect within tumor tissues. The second possibility is that OBP-301 may be less effective against more slowly proliferating tumor cells than it is against rapidly proliferating tumor cells because its replication rate would be lower in the more slowly proliferating cells. Although it remains unclear why continuous injection of OBP-301 was less effective, it is clear that repeated infection with OBP-301 at intervals of more than 2 days would be sufficient to exert an antitumor effect against human sarcoma tissues.

In conclusion, we have clearly shown that OBP-301 has strong *in vitro* and *in vivo* antitumor effects against human bone and soft tissue sarcoma cells. Telomerase-specific replication-selective oncolytic virotherapy would provide a new platform for the treatment of patients with bone and soft tissue sarcomas.

Disclosure of Potential Conflict of Interest

Y. Urata is an employee of Oncolys BioPharma, Inc., the manufacturer of OBP-301(Telomelysin). The other authors disclosed no potential conflicts of interest.

Acknowledgments

The authors thank Dr. Satoru Kyo (Kanazawa University) for providing the OST, HOS, and SaOS-2 cells, Dr. Hiroyuki Kawashima (Niigata University) for providing the NOS-2, NOS-10, NDCS-1, NMS-2, and NMFH-1 cells, and Tomoko Sueishi for her excellent technical support.

Grant Support

This study was supported by grants-in-aid from the Ministry of Education, Science, and Culture, Japan (T. Fujiwara) and grants from the Ministry of Health and Welfare, Japan (T. Fujiwara).

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Received August 1, 2010; revised November 4, 2010; accepted November 15, 2010; published OnlineFirst February 16, 2011.

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Thallium-201 Scintigraphy Is an Effective Diagnostic Modality to Distinguish Malignant From Benign Soft-Tissue Tumors

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Background: The aim of this study is to evaluate whether thallium-201 (201-Tl) scintigraphy can differentiate malignant from benign soft-tissue tumors.
Methods: Between April 1995 and December 2005, 192 patients with soft-tissue tumors (85 malignant and 107 benign) underwent 201-Tl scintigraphy before treatment. Isotope uptake was used as a proxy for tumor-to-background ratio (TBR). The accuracy of TBR on early and delayed imaging was evaluated using the Mann-Whitney U and χ^2 tests.
Results: There was a statistically significant difference in mean TBR on early and delayed imaging of malignant and benign soft-tissue tumors (124% ± 109% vs. 22% ± 42%, and 82% ± 83% vs. 12% ± 25%, $P < 0.0001$). A TBR cutoff of 20% indicated the probability of malignancy on early and delayed imaging (82% sensitivity and 77% specificity; 82% sensitivity and 84% specificity, $P < 0.0001$). Well-differentiated liposarcomas showed low isotope accumulation, while pigmented villonodular synovitis and giant cell tumors of the tendon sheath showed high isotope accumulation.
Conclusions: Thallium-201 scintigraphy can distinguish malignant from benign tumors with relatively high accuracy. With the exception of low grade liposarcomas and locally aggressive benign tumors, 201-Tl scintigraphy may be an effective diagnostic modality to differentiate malignant from benign soft-tissue tumors.

Key Words: thallium-201, scintigraphy, soft-tissue, tumor, sarcoma

(*Clin Nucl Med* 2011;36: 982–986)

Distinguishing malignant from benign soft-tissue tumors presents an important diagnostic dilemma. Several radiographic modalities are useful for initial investigation, including magnetic resonance imaging (MRI), which produces detailed images of soft-tissue abnormalities relative to adjacent anatomy, without the use of ionizing radiation.[1,2] However, the ability of MRI to differentiate benign from malignant soft-tissue tumors is controversial.[3–6] MRI could demonstrate some biologic characteristics of soft-tissue tumors, such as myxoid or fibrous tumors, better than the tumor activity or viability. Computed tomography (CT) and ultrasound are also available to demonstrate anatomic location of soft-tissue tumors. However, the quality and resolution of these imaging are not adequate enough to distinguis malignant from benign soft-tissue tumors.

Thallium-201 (201-Tl) scintigraphy is a functional scan th has been traditionally used as a measurement of myocardial perfi sion and has also been investigated in tumors.[7] Although 201-T scintigraphy is reported to be useful for imaging and differentiatir between malignant lesions and benign lesions, these studies wer composed of small patient numbers and therefore failed to achiev statistical significance.[8–12]

In this study, we investigated 201-Tl scintigraphy as a indicator of malignancy in 193 soft-tissue tumors. We confirmed ou findings by histologic examination of tumor biopsies, and report th sensitivity and specificity of 201-Tl scintigraphy to differentia between malignant and benign soft-tissue tumors.

PATIENTS AND METHODS

Patient Demographics and Histopathologic Diagnosis

Between April 1995 and December 2005, 192 patients (9 male, 94 female) with soft-tissue tumors who presented to our clini underwent 201-Tl scintigraphy before treatment. Before investiga tion of 201-Tl scintigraphy, all patients underwent MRI, and som of those also underwent CT for imaging assessment. Patient ag ranged from 2 to 82 (mean, 49.6) years. Lesions were locate primarily on the lower extremity (n = 117), followed by the upp extremity (n = 54) and trunk (n = 21).

The diagnosis of all malignant tumors and 67 benign soft-tissu tumors was confirmed by histologic examination. The diagnosis of th remaining 40 benign soft-tissue tumors was made with the help c ancillary imaging investigations with clinical follow-up of a minimu of 6 months; 10 hemangiomas, 5 schwannomas, 5 inflammatory l sions, 4 lipomas, 4 hematomas, 4 fibromas, and 8 others.

201-Tl Scintigraphy

Thallium-201 scintigraphy was performed per institution protocol: intravenous bolus of 74 MBq (2mCi) of 201-Tl wa administered, and scintigraphic images were obtained using gamma camera at 15 minutes (early imaging) and 2 hours (delaye imaging) after the injection. Interval change in 201-Tl uptake in th lesion was contrasted with the contralateral normal anatomic area c the area adjacent to the tumor. Early and delayed imaging obtaine prior to 2003 were printed, and imported to a computer. From 200 digital images were obtained that were exported directly to th computer. Images were analyzed using ImageJ software (Nationa Institutes of Health, Bethesda, MD). Two equally sized regions c interest (ROI) were compared on each image (Figure 1). The firs ROI was focused on the lesion itself and the second ROI was place on the contralateral side or the area adjacent to the tumor in a norma area to serve as a control.[13] When 201-Tl uptake was not detectabl on the image, the ROI was placed on the area corresponding t images obtained by CT or MRI (Figs. 2, 3). For each ROI, th average counts per pixel were calculated using ImageJ softwar

Received for publication September 2, 2010; revision accepted January 17, 2011.
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Conflicts of interest and sources of funding: supported in part by Grant-in-aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science and Technology (No. 20591781) and by Grants-in-Aid for Clinical Cancer Research and Grants-in-Aid for Cancer Research (14S-4 and -5) from the Ministry of Health, Labor and Welfare.
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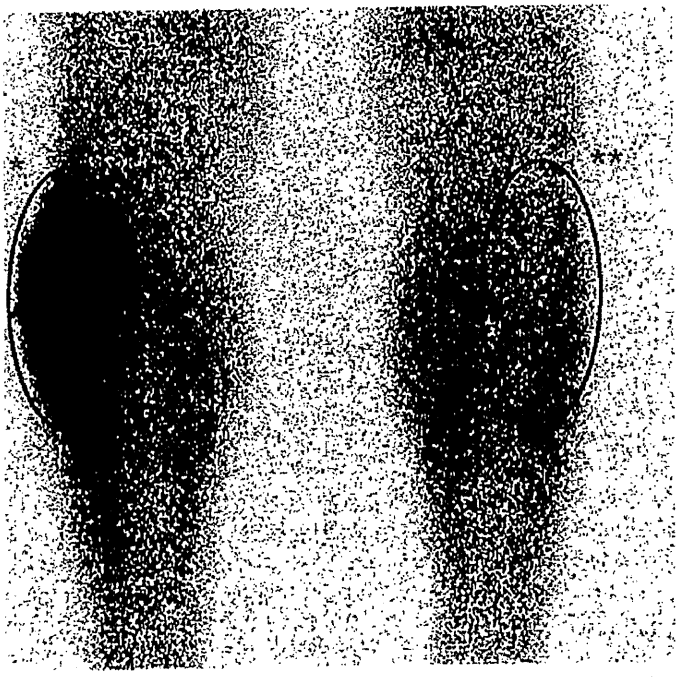
ISSN: 0363-9762/11/3611-0982



ıURE 1. Example of ROI placement on 201-Tl scintigraphy ıge. The first ROI (*) was placed on the outer border of lesion and the second ROI (**) was placed on the ıtralateral side, which served as the control.

ıluding background activity, the tumor-to-background ratio 3R) was calculated by dividing the average counts per pixel of I on the tumor area (T) by that of the background (BG);

$$\text{TBR} = [(\text{T} - \text{BG})/\text{BG}] \times 100.$$

The rate of change of TBR on early (TE) and delayed (TD) ıging was also calculated as:

$$\text{Rate of change of TBR} = [(\text{TE} - \text{TD})/\text{TE}] \times 100.$$

Statistical Analysis

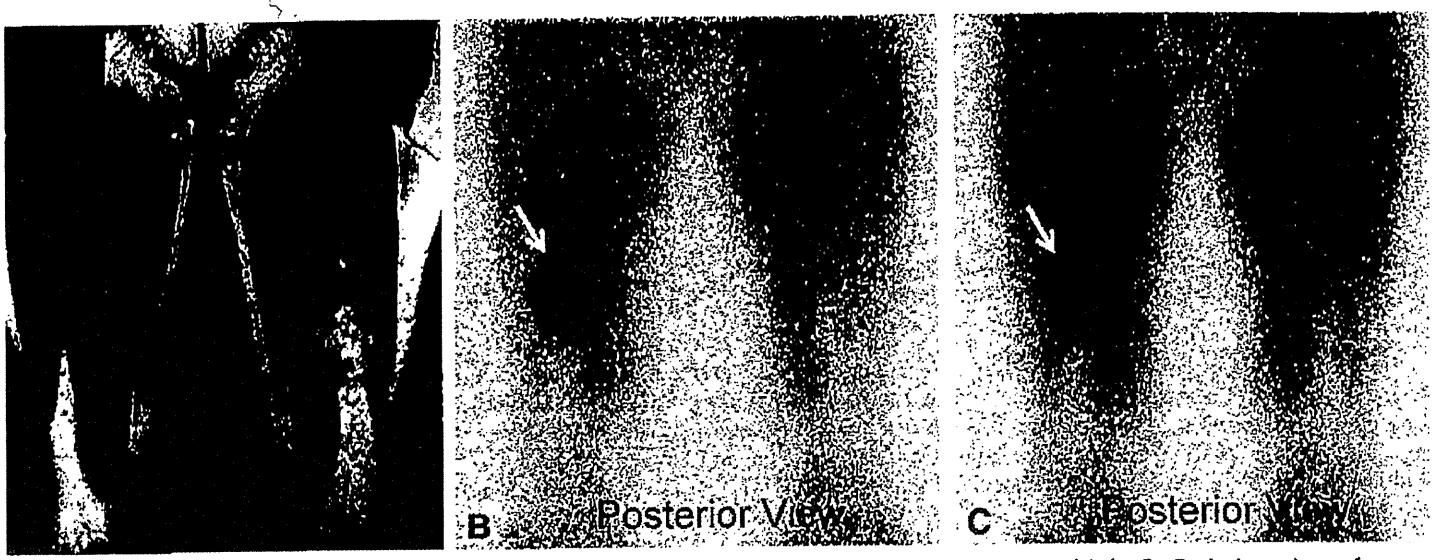
Statistical significance was ascertained by the Mann-Whitney's U test and the χ^2 test, using Stat View 5.0J statistical software (SAS Institute Inc, Cary, NC). $P = 0.05$ was considered statistically significant.

RESULTS

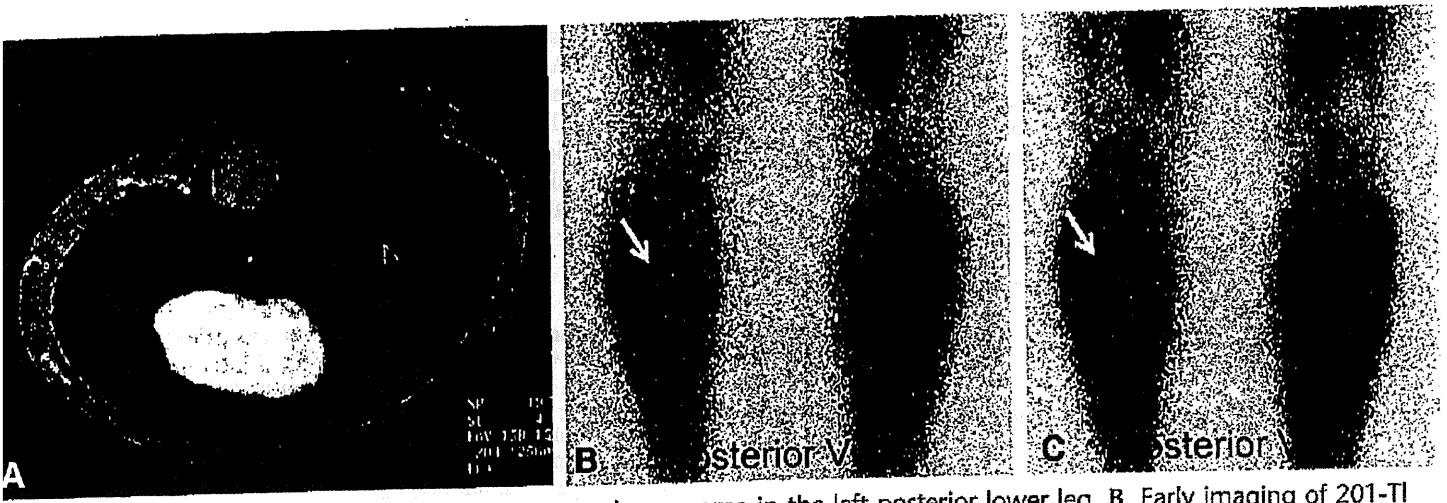
There were 85 malignant soft-tissue tumors and 107 benign soft-tissue tumors (Tables 1, 2). Of the malignant soft-tissue tumors, 63 were located in the lower extremity, 13 in the upper extremity, and 9 in the trunk. Of the 107 benign soft-tissue tumors, 54 were located in the lower extremity, 41 in the upper extremity, and 12 in the trunk. TBR for all malignant and benign soft-tissue tumors on early and delayed imaging was obtained.

Mean TBR on early imaging of all benign tumors was 22% ± 42% (Table 1), and of all malignant tumors was 124% ± 109% (Table 2). Mean TBR on early imaging of malignant tumors was significantly higher than that of benign tumors ($P < 0.0001$). In addition, there was a statistically significant difference in mean TBR between malignant and benign tumors on delayed imaging ($P < 0.0001$). Mean rate of change of TBR on early and delayed imaging was 69% ± 108% for all benign soft-tissue tumors, and 27% ± 40% for all malignant tumors. Mean rate of reduction in isotope accumulation on delayed imaging was significantly higher in benign versus malignant soft-tissue tumors ($P < 0.005$).

A TBR cutoff of 20% on early imaging was found to be 82% sensitivity and 77% specificity; on delayed imaging, specificity increased to 84% (Table 3). A TBR of 20% on both early and delayed imaging was a statistically significant indicator of malignancy ($P < 0.0001$). False-negative results (TBR of less than 20% on both early and delayed imaging) were found in 15 malignant tumors (11 well-differentiated liposarcomas, 3 myxoid liposarcomas, and 1 malignant peripheral nerve sheath tumor). False-positive results (TBR of >20% on early and delayed imaging) were found in 17 benign soft-tissue tumors (7 Giant cell tumor of tendon sheath, 4 pigmented villonodular synovitis, 3 inflammatory lesions, 2 desmoids, and 1 synovial osteochondromatosis), although not all benign tumors of these histologic subtypes were falsely positive. High accumulation of 201-Tl tracer in early imaging followed by marked reduc-



ıURE 2. A, MRI (T2-weighted image) of malignant fibrous histiocytoma in the left posterior thigh. B, Early imaging of 1-Tl scintigraphy (posterior view). C, Delayed imaging of 201-Tl scintigraphy (posterior view). Both images demonstrate vated accumulation of 201-Tl at the lesion (arrows).



IGURE 3. A, MRI (T2-weighted image) of intramuscular myxoma in the left posterior lower leg. B, Early imaging of 201-Tl :intigraphy (posterior view). C, Delayed imaging of 201-Tl scintigraphy (posterior view). Both images demonstrate decreased ccumulation of 201-Tl at the lesion (arrows).

ABLE 1. Mean TBR of 107 Patients With Benign oft-Tissue Tumors

	n	Early Image	Delayed Image
enign soft-tissue tumors	107	22% ± 42%	12% ± 25%
GCTTS	7	120% ± 59%	77% ± 21%
VS	4	77% ± 37%	54% ± 20%
nflammatory lesion	10	57% ± 45%	25% ± 22%
ynovial osteochondromatosis	2	38% ± 51%	14% ± 22%
)esmoid	5	22% ± 22%	26% ± 37%
Iemangioma	15	16% ± 32%	1% ± 2%
chwannoma	22	8% ± 20%	2% ± 5%
ibroma	4	4% ± 2%	0% ± 1%
Iematoma	6	2% ± 3%	1% ± 2%
eiomyoma	1	2%	3%
ipoma	12	1% ± 3%	0% ± 2%
pidermoid cyst	2	1% ± 3%	1% ± 3%
Blastofibroma	4	1% ± 1%	0% ± 2%
Glomus tumor	2	1% ± 0%	1% ± 1%
ntramuscular myxoma	1	1%	1%
Myofibroma	2	0% ± 2%	1% ± 0%
anglion	2	0% ± 0%	1% ± 0%
Others	6	1% ± 1%	1% ± 1%

n indicates number of tumors; GCTTS, giant cell tumor of tendon sheath; PVS, pigmented villonodular synovitis.

TABLE 2. Mean TBR of 85 Patients With Malignant Soft-Tissue Tumors

	n	Early Image	Delayed Image
Malignant soft-tissue tumors	85	124% ± 109%	82% ± 83%
Dedifferentiated liposarcoma	1	462%	346%
Clear cell sarcoma	4	236% ± 25%	192% ± 86%
Angiosarcoma	1	207%	120%
Solitary fibrous tumor	1	206%	101%
Unclassified sarcoma	2	196% ± 17%	158% ± 46%
Leiomyosarcoma	7	158% ± 103%	98% ± 45%
MPNST	4	155% ± 187%	111% ± 156%
MFH	29	138% ± 95%	91% ± 74%
Rhabdomyosarcoma	2	125% ± 68%	83% ± 6%
Synovial sarcoma	16	125% ± 89%	71% ± 71%
Myxoid liposarcoma	7	50% ± 79%	30% ± 37%
Well-differentiated liposarcoma	11	2% ± 1%	1% ± 1%

MPNST indicates malignant peripheral nerve sheath tumor; MFH, malignan fibrous histiocytoma.

TABLE 3. Comparison of TBR on Thallium Scintigraphy anc Diagnosis

Early Image	Malignant	Benign
TBR ≥20%	70	25
TBR <20%	15	82
	Sensitivity: 82%	Specificity: 77%
Delayed Image	**Malignant**	**Benign**
TBR ≥20%	70	17
TBR <20%	15	90
	Sensitivity: 82%	Specificity: 84%

TBR indicates tumor-to-background ratio.

tion (akin to rapid reduction) on delayed imaging occurred in 4 inflammatory lesions, 3 hemangiomata, and 1 schwannoma (Table 4).

DISCUSSION

Thallium-201 scintigraphy has been used in clinical oncology for estimating the presence and biologic activity of tumors, including those in bone and soft tissue. Since 201-Tl uptake is not only flow-dependent, but engages active transport mechanism, the 201-Tl accumulation may indicate tumor cell viability and metabolic activity.[14–16] Early articles reported a high affinity for 201-Tl in musculoskeletal sarcomas.[17–19] High accumulation of 201-Tl on scintigraphy could be a positive indicator of malignancy in the evaluation of soft-tissue tumors.

In our study, mean TBR on both early and delayed 201-T scintigraphy was found to be significantly higher in malignant versu benign soft-tissue tumors, with an acceptable level of accuracy.

Our results also showed a predictable trend in false negativity In our series, well-differentiated liposarcoma, a less aggressiv