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多発溶骨性病変の一例：2年後の経過報告

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【症　例】69歳、女。

【主　訴】右股関節痛。

【現病歴】2008年8月に原発不明癌として当院に紹介された。多発性の溶骨性病変を伴い、両大腿骨頸部は切迫骨折状態であった(図1)。病理診断未定ながら骨折予防の処置は必須のため、右大腿骨近位部は広範切除＋人工骨頭置換術、左大腿骨近位部は腫瘍掻爬＋整復固定術(CHS)＋セメント充填を行った。

本症例は第64回の本研究会において検討された結果、病理診断としてGorham-Stout disease、臨床的には“Skeletal Cystic Angiomatosis(SCA)”との結論となった。

【前回報告後の経過】病理所見で破骨細胞の極めて旺盛な増殖が見られたこと、文献的にもSCAに対する有効例が報告されていることから、monthlyにビスホスホネートの点滴を開始した。この結果、全身骨痛は軽減し、骨融解病巣の辺縁硬化も出現して、1年間はsatable diseaseであった。その後、骨融解が徐々に拡大、新規骨病変も出現したため、悪性腫瘍を再検討することが必要となった。以前よりあった子宮病変が増大してきたこともあり、平滑筋肉腫の可能性も考慮し、2009年9月子宮付属器切除を行った。病理診断は“atypical leiomyoma”であった。しかし、手術後より全身骨融解がさらに進行した。このため子宮手術の1年後(大腿骨腫瘍切除後2年)に左腸骨より切開生検を再度施行。この結果“Spindle cell sarcoma”の診断となった。これ以後、肺転移、肝転移が進行してきたため、子宮平滑筋肉腫に対する有効性が報告されているDOCE/GEM[1),2)]による全身化学療法を12月より開始した。これにより肺病変はいったん縮小、ALPを病勢の指標とする血液検査所見も改善した。

しかし効果は3ヵ月間で、その後、癌性腹膜炎を発症、5月に死亡した(図2)。

【画像所見】単純レントゲン所見：右大腿骨近位部は人工骨頭により置換されているが、大腿骨幹および臼蓋部の骨融解は認めない。左大腿骨近位は、掻爬術後2年で骨融解巣の再発進行を認めず、ラグスクリューの緩みも認めない(図3)。腸骨は、海綿骨の広範な融解が進行しているが、骨皮質の輪郭が比較的保たれている。胸椎・腰椎の骨融解も進行したが、2年間で圧迫骨折の進行は経度であった。

CT所見：当初より、脊椎、骨盤骨に反応性骨変化を伴わない溶骨性変化が多発していた。治療開始後1年時点では骨融解の進行は経度で、ビスホの効果により骨密度が上昇し、病変は辺縁骨硬化を伴って境界が明瞭になった。しかし、2年後からは海綿骨の骨融解が再び進行し、病変は椎体全体に及んだ。さらに2ヵ月後には骨皮質の破壊も進行した(図4)。

この時点で、肺転移、肝転移病巣が明らかとなった。

PETの経過：初回は、多発骨透亮像の割に集積はごくわずかであった。1年後には、集積を脊椎に複数認めたが、新規病巣への集積ではなく、当初から骨融解を示した病巣に徐々に増加した。2年後では、脊椎、骨盤に強い集積を示しているが、これらの多発骨病変は、もともと骨融解を示していた病変が主体である(図5)。

【病理所見】大腿骨病変の広範切除材料(図6)：骨髄内に太い網目状に増殖する線維性組織がみられ、この中に小型で異型軽度の紡錘計画を有する紡錘形細胞を認める。線維性組織におかされていない造血細胞を入れた骨髄領域との境界線には異常な数のosteoclastが1列に並び(矢印)、骨梁を溶かして線維組織に置き換えているように見える。osteoblastはほとんど認められず、骨形成反応を欠いた骨融解

機転異常な亢進状態で、Gorham-Stout diseaseあるいはSkeletal Cystic Angiomatosis(SCA)の診断となった。腸骨再切開生検標本(図7)：膠原線維からなる特定の構造を示さない間質性組織であるが、一部に異型小型紡錘核を有する細胞の増加を認め、fibroblastないしleio系のspindle cell sarcomaとすべき所見である。免疫染色ではSMA(+), CD34(+), desmin(−), HHF35(−), caldesmon(−)。当初はlow grade sarcomaとの診断であったが、臨床経過がaggressiveな病態であるため、前回の子宮腫瘍の再検討をおこなった。結果、atypical leiomyomaとされた子宮組織に骨生検標本と同様な所見をみとめた。このため、本研究会では多発骨融解を初発症状として、2年後に判明した子宮平滑筋肉腫の播種性転移として報告した。

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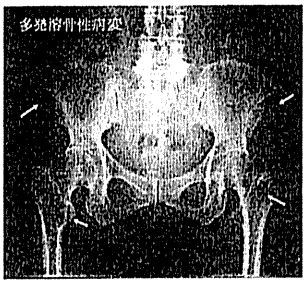

図1　多発性溶骨性病変

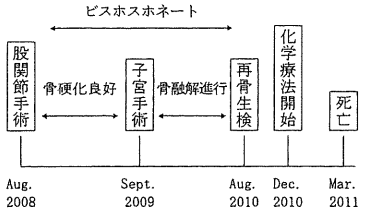

図2

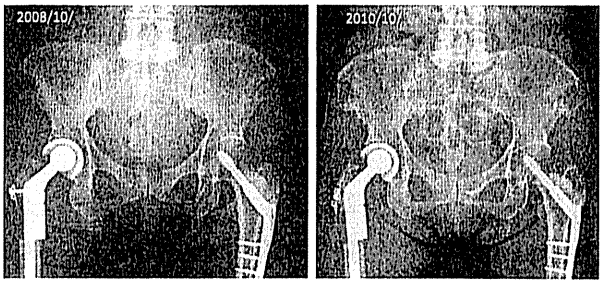

図3　単純レントゲン所見

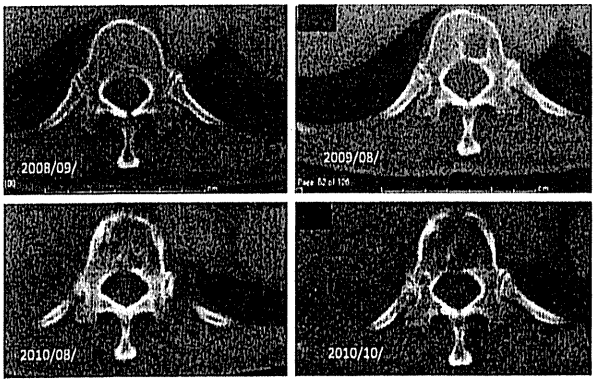

図4　CT所見

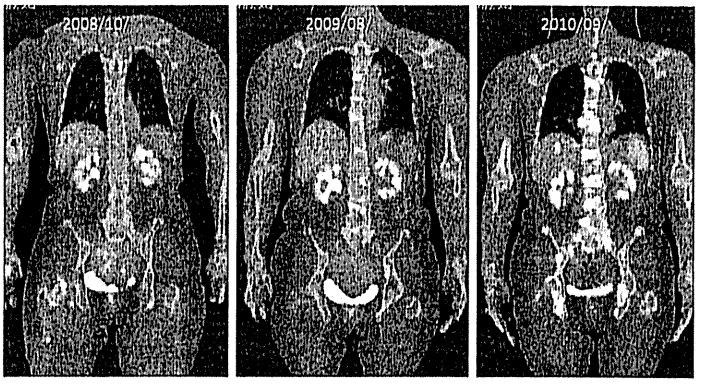

図5　PETの経過

図6　大腿骨病変の広範切除材料

図7　腸骨再切開生検標本

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慢性創傷から発生したinvasive squamous cell carcinoma（SCC）の根治性と機能温存の検討

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清原　祥夫[1]　中川　雅裕[2]　永松　将吾[2]　茅野　修史[2]
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要旨　慢性創傷に続発する有棘細胞癌（以下：SCC）は緩徐に進行するため，癌の発見が遅れ，そのため発見時にはすでに進行期にあることが多いとされる。我々はこれまで慢性創傷から発生し，深部組織に浸潤したSCCを8例経験した。今回それらについての治療成績をまとめ，根治性と機能温存の可能性を検討する。

対象は2002年9月から2009年12月まで当施設で手術を行った症例8例で，男性5例，女性3例で，年齢は平均65.5歳であった。

発生部位は大腿部3例，下腿3例，手指2例であった。前駆病変は小児期の骨髄炎，褥瘡，熱傷瘢痕によるものがみられ，8例中4例は患肢温存可能で，残りの4例は患肢切断術を選択した。SCCの潰瘍部には高率に感染・炎症の波及を認めるが，切除範囲の正確な評価と，年齢・PSを考慮した治療により根治的治療とQOLの維持を計ることができると考える。また過去の瘢痕に難治性の潰瘍をみたら病期が進行する前に悪性化を疑い生検を行うことが必要である。

Surgery for squamous cell carcinoma arising from a chronic wound

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Most squamous cell carcinoma (SCC) in the extremities involves only the dermis and epidermis. However, SCC can occur as a rare complication in chronic inflammatory wounds. These lesions are often found as an advanced condition and involve bone and deep soft tissues. The aim of this study is to clarify the clinical results of the patients with invasive SCC treated surgically in our institute.

Between 2002 and 2008, 189 patients with SCC were surgically treated in our institute. Among them, 7 cases (5 men and two women), of squamous cell carcinoma arose in chronic wounds and required orthopedic surgical procedures due to bone or deep tissue invasion. The mean age was 63.8 (53-81) years. The precedent lesion was post-traumatic osteomyelitis in four patients, decubitus in one, deep burn scar in one, and unknown in one. The mean time interval between the precedent lesion and the diagnosis of the SCC was 35years.

At the primary operation, four patients underwent limb salvage surgery and three underwent amputation. One patient after the limb salvage surgery had local recurrence, which eventually required

hindquarter amputation. To accomplish curative treatment, we required resection of infectious lesions and the free flap cover because of the large defect. The 5-year survival rate was 85.7%. Most cases with invasive SCC are treated with amputation in the literature. Because of the tendency for the disease to metastasize early, poor results may ensue after local excision. In this report, we determined the extent of resection based on accurate image assessment. As a result, we succeeded with local excision and limb salvage surgery, and the oncological outcome of the patients was good. [*Skin Cancer (Japan)* 2011 ; 26 : 62-68]

Key words : Squamous cell carcinoma, Limb-sparing surgery, Chronic wound, Marjolin's ulcer, Scar cancer

はじめに

慢性創傷に続発する有棘細胞癌（以下：SCC）は緩徐に進行するため，癌の発見が遅れそのため発見時にはすでに進行期にあることが多いとされる。具体的には治療法として90％で肢切断を選択し，そのうち20～50％において局所再発率を認めたとの報告がある[1]。我々はこれまで慢性創傷から発生し，深部組織に浸潤したSCCを8例経験した。今回の報告では患肢温存例と切断例を供覧し，治療成績をまとめ，根治性と機能温存の可能性を検討する。

目　的

慢性創傷より発生した進行性SCC症例（ここではTNM7thでT3以上の症例とする）に対する患肢切断術と温存術の根治性と機能温存について比較・検証する。

対象と方法

対　象：当院にて2002年8月～2009年8月に加療した慢性創傷由来の進行性SCC 8例

腫瘍学的評価と残存機能評価を以下の項目で行った。

評価項目：

①腫瘍学的評価：

1）5年無病生存期間（Kaplan-Meire法による）

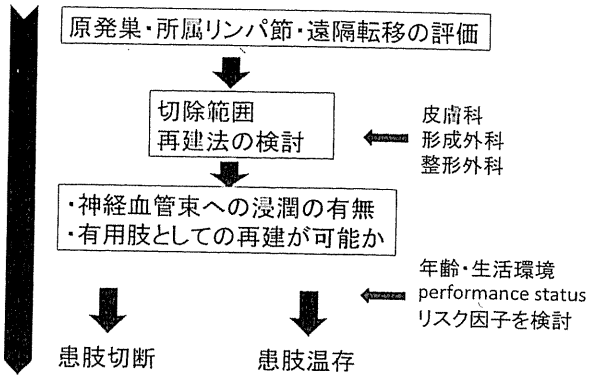


図1．当院における手術方法の検討

2）5年全生存期間（Kaplan-Meire法による）

3）局所再発・遠隔転移の有無

②残存機能評価：MSTS（muskloskeltal toumor society score）

TESS（tront extrimities salvage score）

当施設における治療方針の決定については図1に示すように，CT・MRIによる切除範囲の検討を行いその上で患肢温存か切断術かの選択については・神経血管束への浸潤の有無・再建を行った場合，患肢が有用肢として機能するかの2点について十分な検討を行い決定した。

症例供覧

患肢温存例（症例5）

小児期の骨髄炎を発生母地に，60年の経過を経て発生した有棘細胞癌症例。

初診時レントゲン・CTではすでに骨皮質へ

の浸潤が疑われたが，骨髄までの浸潤はないと判断し腫瘍浸潤が疑われる部位の脛骨部分切除，腸骨移植，遊離広背筋皮弁，分層植皮による患肢温存術を選択した。術後6ヵ月で全荷重負荷が可能となり，現在は術後20ヵ月無再発生存中である（図2a，b）。

患肢切断例（症例2）

小児期の術後対麻痺による車いす生活のためにできた坐骨部褥瘡から44年の経過を経て発生した有棘細胞癌症例。

初診時すでに股関節は破壊された状態で，最大4 cmのリンパ節転移を来していた。腫瘍の浸潤状態より患肢温存は困難であること，肢切断によるADL低下の程度は少ないことから股関節離断，鼠径リンパ節郭清を行い大腿の後方よりfilet flapで閉創した。術後も術前のperformance statusと変わりなく自立した生活を送っており，術後38ヵ月無再発生存でしている（図3a，b，図4a，b）。

結　果

男性5例，女性3例で，年齢は平均65.5歳（53～81歳）であった。

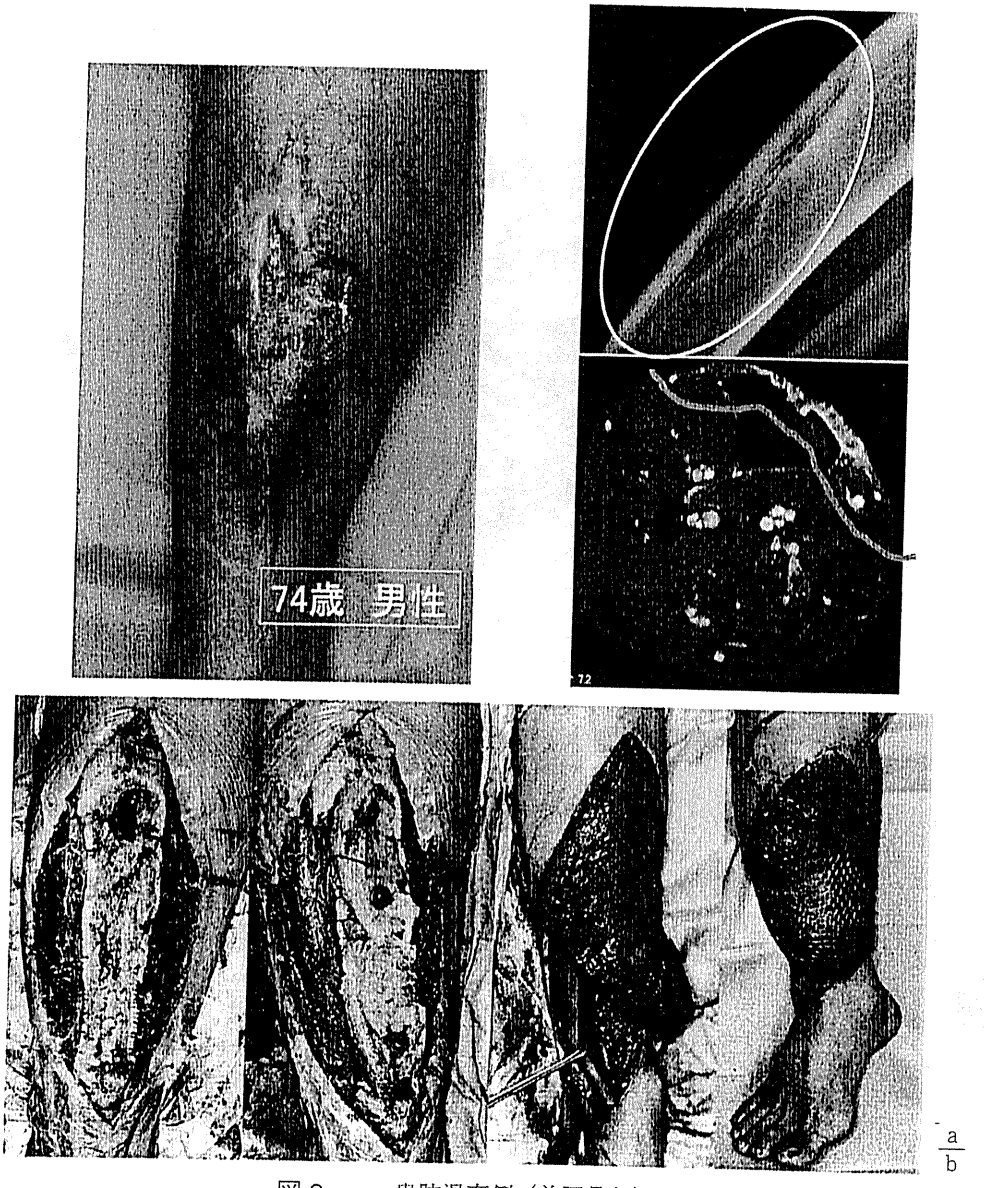
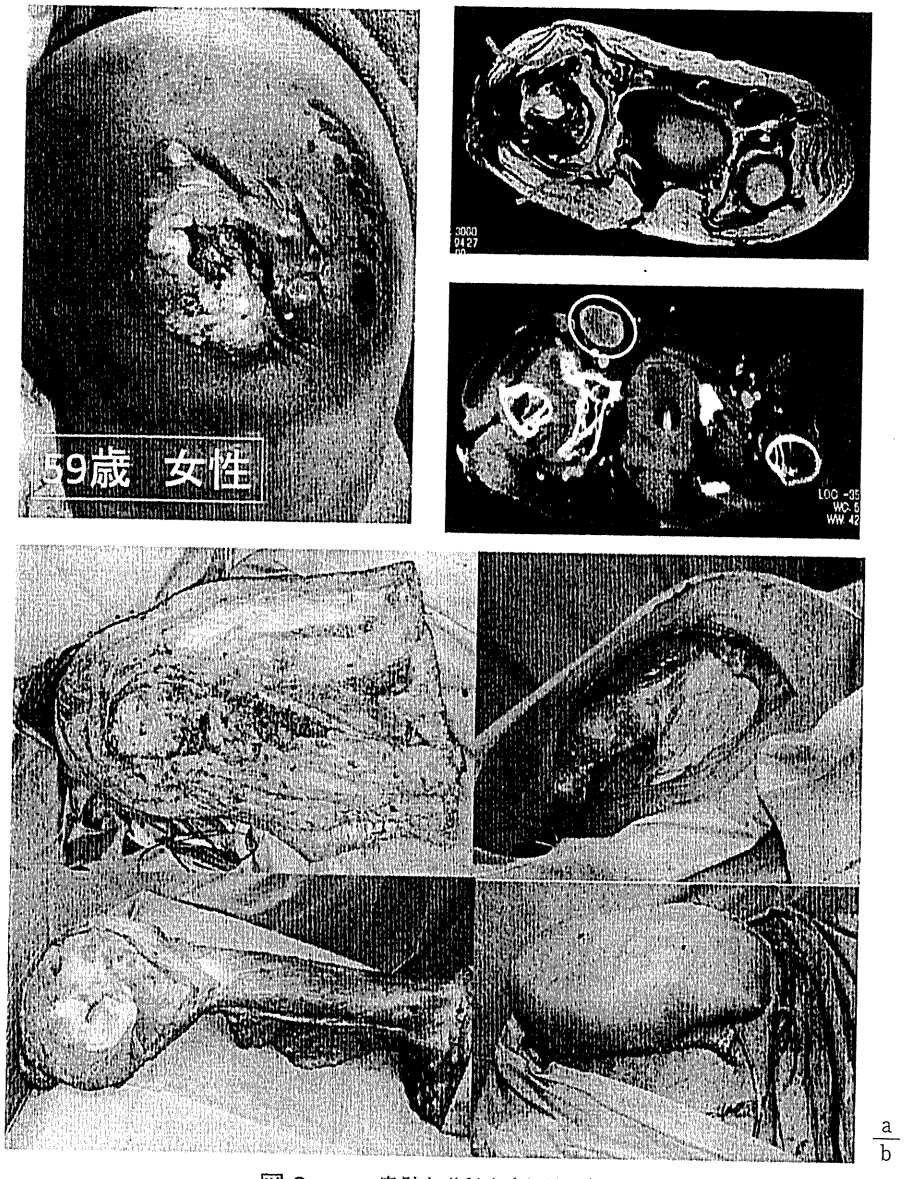
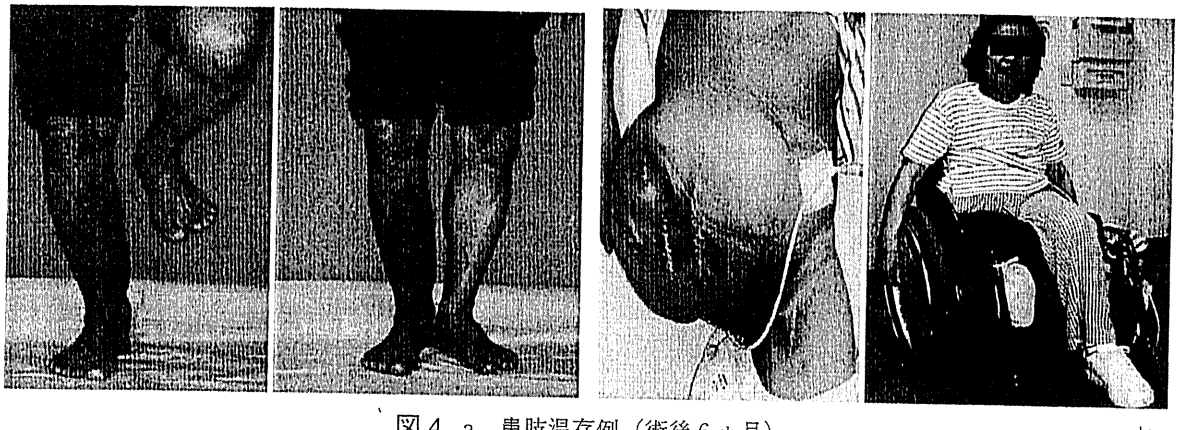


図2．a. 患肢温存例（前脛骨部）
b. 術中写真（2008/06）



a
b

図3．a. 患肢切断例（坐骨部）
b. 術中写真（2006/12）



a | b

図4．a. 患肢温存例（術後6ヵ月）
b. 患肢切断例（術後6ヵ月）

表１．８症例の臨床学的特徴

	症例	年齢/性別	部位	発生母地	手術	起炎菌	切除縁	観察期間
切断	1	53/M	手	不明	肩甲骨離断 腋窩郭清	-	陰性	15 ★
	2	65/M	大腿部	骨髄炎	骨盤半砕	MRSA	陰性	38
	3	59/F	坐骨部	褥瘡	股関節離断 鼠径郭清	γ-streptococcus	陰性	38
温存	4	53/F	膝窩部	熱傷瘢痕	拡大切除 鼠径郭清	P. Aeruginosa	神経断端 陽性	22 ☆
	5	74/M	前脛部	骨髄炎	脛骨部分切除 LD	MRSA α-streptcoccus	陰性	20
	6	81/F	手	外傷後	CM関節離断 ALT	S.maltophilia	陰性	16
	7	62/M	前脛部	熱傷瘢痕	拡大切除 全層植皮	-	陰性	62
	8	77/F	前脛部	骨髄炎	脛骨部分切除 髄内釘・LD	P. Mirabilis E. faecalis	陰性	6

★は原病死，☆は術後2ヵ月で局所再発後の患肢切断，他は無再発生存

表２．腫瘍学的評価

	局所再発	遠隔転移
切断(n=3)	0/3	1/3
温存(n=5)	1/5	0/5

■5年　無再発生存率:72.9%

■5年　全生存率:85.7%

発生部位は大腿部３例，下腿３例，手指２例であった。前駆病変は小児期の骨髄炎３例，熱傷瘢痕２例，外傷後２例，褥瘡１例で，前駆病変が発生してから受診に至るまでに平均54年（44～70年）の経過を要しており，病変が急速に拡大してから受診に至るまでの期間は平均６ヵ月（４～12ヵ月）であった（表１）。

８例中５例に患肢温存術を選択し，３例に患肢切断術を選択した。８例中７例は病理組織学的に切除断端陰性であることを確認したが，神経断端に腫瘍細胞が陽性であった患肢温存例１例に局所再発を認め，追加治療としてabove knee amputationを行った。

平均観察期間は27ヵ月（６～62ヵ月）で，切断例１例に術後６ヵ月での遠隔転移，温存例に術後２ヵ月での局所再発を認めた。

８症例全体の５年無病生存率は71.4%，５年全生存率は85.7%であった（表２）。

現在，骨軟部腫瘍術後の機能評価として最も広く用いられているmuskloskeltal tumor society score＝ISOLSによる患肢機能評価法（MSTS）とtront extrimities salvage score（TESS）によりscoringを行い，（表３）に示す。（症例１は原病死のため評価不能で，症例４は切断例と

表３．機能評価

結果3:切断例の機能評価

切断	MSTS(%)	TESS(%)
症例2	36.6	43.3
症例3	36.6	53.7
症例4	56.6	59.8

平均値

MSTS:　43%

TESS:　52%

結果4:温存例の機能評価

温存	MSTS(%)	TESS(%)
症例5	97.6	85.7
症例6	86.6	97
症例7	93.3	100
症例8	63.3	48

平均値

MSTS:　85%

TESS:　83%

MSTS:
muskloskeltal toumor society score
= ISOLSによる患肢機能評価法
TESS:
tront extrimities salvage score

して分類する）

患肢切断例ではMSTS：43%，TESS：52%，患肢温存例ではMSTS：85%，TESS：83%という結果であった（表3）。

考　察

慢性創傷を発生母地とし，長期経過を経て悪性化する腫瘍は1828年にJean-Nicholas Marjolinが外傷後瘢痕より発生した癌を報告して以来，欧米ではMaljolin's ulcerと呼ばれ本邦では瘢痕癌と称されることが多い。

多くの場合30～40年以上の長い経過を経て悪性化し[1)2)]，発見時にはすでに進行期にあるため予後が不良であり，悪性化してからの腫瘍の進行が急速であることから転移を来しやすく，局所の再発率も高いとされている。実際に患肢切断術後の局所再発率が20～50%であるという報告や[3)]，一般的なSCCの転移率が0.5～3.0%であるのに対して，瘢痕より生じたSCCは30%であったという報告がある[4)]。

そして，治療法に関しても病変が骨に至った症例では治療法として90%が肢切断を選択しており，患肢温存した場合には，20～50%において局所再発率を認めたとの報告がある[2)]。

はじめに根治性の点において検証する。当施設での治療成績は全体で5年無病生存率71.4%全生存率が85.7%であり，評価法が同じでないために一概に比較することは難しいが，これまでの報告に比して同等かそれ以上の結果であると考える。

我々はレントゲン・MRIによる原発巣の浸潤評価，CTによる遠隔転移の検索を行い全症例の50%（4/8）に患肢温存による治療を選択したが，患肢温存例の治療成績は局所再発率20%，5年全生存100%で観察期間中に遠隔転移は認めていない。

次に機能温存の点について検討する。

両者のスコア平均を比較するとMSTSという客観的評価，TESSという主観的評価のいずれも患肢温存例のスコアが高く患肢温存術を選択した結果，患肢が有用肢として機能しているということがいえる。

これらのことより従来の進行性のSCCは患肢切断という考えに固執することなく，適切な病変の評価を行い，再建法を工夫することで患肢温存した上での根治的な治療が可能な症例も今後増やすことができると考えられる。

また今回は治療法としての手術療法に主眼を置いて検討したが，予後改善のためには早期の診断と進行期例に対する集学的治療が必要不可欠であることはいうまでもない。前者においては悪性化を疑った際の組織生検は必須であり，その際には悪性化していない病変の端を生検すれば偽陰性が容易に起こりうると考えられるため，より正確な結果を得るために十分な組織量を採取し，疑わしい部位からは複数箇所検体を採取するという姿勢が必要である[1)2)]。また後者においては病変が局所にとどまっている場合でも，切除縁に関しての十分な検討を行った上で，必要に応じて後照射を追加することを考慮する必要があると考える。

ま と め

骨軟部組織への浸潤を伴ったSCCに対して腫瘍の浸潤，そして感染・炎症の範囲を十分に評価し手術した結果，8例中6例で一期的な根治術が可能であった。そのうち4例は患肢温存が可能で良好な機能予後が得られた。

T3以上の進行性のSCCであっても切除範囲の正確な評価と，年齢・PSを考慮した治療により根治的治療とQOLの維持を図ることができると考えられる。また過去の瘢痕に難治性の潰瘍をみたら病期が進行する前に悪性化を疑い生検を行うことが必要である。

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PET/CT Allows Stratification of Responders to Neoadjuvant Chemotherapy for High-Grade Sarcoma

A Prospective Study

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Purpose: The aim of the present study was to determine whether metabolic reduction is capable of reflecting the histopathologic response and outcome after neoadjuvant chemotherapy in patients with high-grade sarcoma.

Patients and Methods: Forty-two patients with histologically proven high-grade sarcoma underwent neoadjuvant chemotherapy followed by surgical resection. Quantitative F-18 fluorodeoxyglucose (F-18-FDG) positron emission tomography (PET)/computed tomography scans were acquired before and after the first cycle and after completion of neoadjuvant chemotherapy. Standardized uptake values (SUVs) and metabolic reduction rates were compared with histopathologic response, progression-free survival, and overall survival.

Results: Baseline SUVmax was 10.9 ± 3.6 (range, 3.8–19.6). Therapeutic effect resulted in 10 patients (24%) with a satisfactory response and in 32 patients (76%) with an unsatisfactory response after completion of neoadjuvant chemotherapy. The SUV decreased to 7.8 ± 3.4 after the first cycle (t1) of chemotherapy and to 5.2 ± 3.4 after completion (t2) of chemotherapy. Histopathologic response and percentage SUV (t2) reduction rate were independent predictors of progression-free survival and overall survival in the multivariate analyses.

Conclusion: Metabolic reduction after neoadjuvant chemotherapy evaluated by F-18 FDG PET or computed tomography can be used for stratification of the histopathologic response in patients with high-grade sarcoma.

Key Words: PET/CT, sarcoma, neoadjuvant chemotherapy

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The majority of patients with high-grade sarcomas are found to have locally advanced tumors at the time of the initial diagnosis. However, surgical resection of the tumors often results in inadequate margin, and there is a high propensity to develop local recurrence and distant metastasis.[1–3]

For patients with high-grade sarcoma, the main treatment option with the potential cure is definitive neoadjuvant chemotherapy followed by surgery. Induction treatment protocols using preoperative chemotherapy or combined chemoradiotherapy followed by resection yield a complete remission rate of 59% at 5 years in high-grade sarcoma.[4–8] Neoadjuvant chemotherapy is under investigation for the treatment of high-grade sarcomas. Prior studies have demonstrated that neoadjuvant chemotherapy followed by standard surgery is associated with comparative response.[4–8]

Imaging metabolic activity offers an alternative method of visualizing the effects of treatment. Malignant transformation of cells is frequently associated with increased metabolic activity. Positron emission tomography (PET) using F-18 fluorodeoxyglucose (F-18 FDG) has been used to evaluate the prognosis in patients with sarcomas.[9–12] The glucose analog F-18 FDG reflects exogenous glucose consumption, because it is phosphorylated by intracellular hexokinases through glucose transporter proteins. F-18 FDG uptake expressed semi-quantitatively as the standardized uptake value (SUV) has been found to be strongly associated with outcome.[13–15] The degree of F-18 FDG uptake in sarcomas is associated with histologic tumor aggressiveness and glucose transporter protein 1 expression.[16,17] Identification of PET findings that affect the prognosis of patients with high-grade sarcoma may be useful to determine preoperative value.

Several studies have revealed that reduction in tumor metabolism often occurs early in the course of therapy and precedes with the reduction of tumor size. Conventional methods for monitoring of treatment response are based on size reduction as revealed by computed tomography (CT). However, CT is not an accurate basis for predicting outcome, because morphologic changes in tumors occur much later than the changes in metabolic response.[6–8] Quantification of tumor glucose metabolism is a highly accurate method of monitoring the effects of chemotherapy.[18–21] Recent studies have revealed that sequential F-18 FDG PET scans are sensitive to detect early response to chemotherapy in malignant tumors.[22–31] The reduction rate of F-18 FDG uptake technique reflects the histopathologic response to chemotherapy.

However, the role of sequential F-18 FDG PET scans in measuring the reduction of metabolic response after neoadjuvant chemotherapy in high-grade sarcomas has not been fully elucidated. The PET/CT can improve the diagnostic accuracy in patients with sarcomas because they record anatomic and molecular information simultaneously. Our hypothesis is that a sequential F-18 FDG PET/CT scans reflect the changes of metabolic process that may be related to the therapeutic response. The purpose of the present study was to clarify whether F-18 FDG PET/CT scans are capable of reflecting the histopathologic response and outcome after neoadjuvant chemotherapy of high-grade sarcomas.

PATIENTS AND METHODS

Patients

All patients underwent initial staging based on a review of their medical history, physical examinations, and imaging studies,

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including PET/CT. The criteria for eligibility were histologically proven or highly suggestive high-grade soft-tissue sarcoma without any treatment before the study, age greater than 20 years, and adequate organ function. The inclusion criteria for performance status (PS) were PS 0, that is, fully active, able to perform all predisease activities without restriction or PS 1, that is, physically strenuous activity restricted but ambulatory and able to carry out work of a light or sedentary nature.[32] The exclusion criteria were diabetes and pregnancy. Patients who presented with metastatic disease or concomitant malignancy were ineligible according to the protocol. This study was conducted in accordance with the amended Helsinki declaration and approved by the local ethics committees after all the patients had given informed consent.

Treatment

The neoadjuvant therapy consisted of 2 cycles of ifosfamide (10 g/m^2) followed by 2 cycles of doxorubicin (60 mg/m^2) and cisplatin (80 mg/m^2). After completion of the neoadjuvant chemotherapy, all patients underwent surgical resection. All specimens were analyzed by one pathologist, who was blinded to the PET/CT data. According to the criteria based on the histopathologic response, histopathologic responders received adjuvant chemotherapy after surgery with the following regimen: 3 cycles of ifosfamide (10 g/m^2) followed by doxorubicin (60 mg/m^2) and cisplatin (80 mg/m^2). Histopathologic nonresponders received 4 cycles of the same regimen. During the postoperative follow-up, 9 (21%) patients who had developed localized bone metastasis received external beam radiation therapy.

PET/CT

Patients received an intravenous injection of 380 to 401 MBq of F-18 FDG after at least 6 hours of fasting, followed by an uptake phase of approximately 60 minutes. Studies were performed with a lutetium oxyorthosilicate-based whole-body PET/CT scanner (Aquiduo, Toshiba Medical Systems). Data acquisition was performed for each patient from the top of the skull to the leg at a scan length of 120 seconds per one bed position. A total of 10 to 12 bed positions, which depend on the patient's height, were needed for whole-body scans, and the total examination time was approximately 30 minutes. All PET images were reconstructed using an iterative algorithm: attenuation-weighted ordered-subsets expectation maximization, 4 iterations; 14 subsets; 7-mm Gaussian filter. All reconstructed images were reviewed and analyzed on a Vox-base SP1000 workstation (J-MAC systems).

Image Interpretation

PET and CT images in all standard planes were reviewed on the workstation (e-soft, Siemens). Images were analyzed visually and quantitatively by 2 reviewers who recorded their findings after reaching a consensus. Reviewers were blinded to the results of other modalities. In the visual analysis, abnormal F-18 FDG uptake was defined as substantially greater activity than in aortic blood on attenuation-corrected images. A region of interest (ROI) was outlined within areas of increased F-18 FDG uptake and measured on each slice. When the lesion was extensively heterogeneous, the ROI was set so as to cover all of the components of the lesion. The maximum SUV measured on every scan was used for prognostic stratification.

Morphologic and Metabolic Response

MRI was used as a treatment monitoring method. MRI of the primary site was performed using a 1.5 Tesla system (Signa Horizon; GE Medical Systems, Milwaukee, WI, or Visart; Toshiba Medical Systems). Tumor size on MRI after the first cycle of neoadjuvant chemotherapy (t1) and after completion of neoadjuvant chemotherapy (t2) was compared with tumor size on the baseline study (t0). The percentage of size (t1 or t2) reduction rate was calculated by the formula: [Size (t1 or t2) − Size (t0)]/Size (t0) × 100%. Evaluation of metabolic response was accomplished by comparing the relative change in SUV and percent SUV (%SUV) reduction rate. The SUVs from PET/CT after the first cycle of neoadjuvant chemotherapy (t1) and after completion of neoadjuvant chemotherapy (t2) was compared with the SUVs on the baseline study (t0). The percentage SUV (t1 or t2) reduction rate was calculated by the following formula: [SUV (t1 or t2) − SUV (t0)]/SUV (t0) × 100%.

Histopathologic Response and Reference Standard

Histologic examinations were performed by an expert pathologist. Whenever necessary, immunohistochemical staining was carried out to confirm the diagnosis or tumor type according to the World Health Organization classification, TNM classification of the UICC for sarcoma of bone, and AJCC staging protocol for sarcoma of soft tissue.[33,34] In this study, the histologic grade of the tumors was determined using the grading system established by Hasegawa et al.[35–37] Histopathologic response to neoadjuvant chemotherapy was evaluated based on the grading system.[37–39] A favorable response to chemotherapy was defined as ≤10% viable tumor cells and an unfavorable response as >10% viable tumor cells. Two board-certified radiologists retrospectively reviewed the medical records and follow-up imaging studies. We evaluated follow-up imaging findings based on visual analysis as standard of reference. The designation of relapse was defined as local recurrence, nodal metastasis, or distant metastasis.

Statistical Analysis

The standard deviations (SD) of SUV (t1) and SUV (t2) are compared between metabolic responders and nonresponders as measures of heterogeneity by Student *t* test. The proportion of metabolic responders is compared based on histologic types by Kruskal-Wallis test. Overall survival (OS), which was from the time of baseline PET/CT study, for which the event was the first documentation of death, was chosen as the end point for assessment of prognostic value. Progression-free survival (PFS) was defined as the period between the time of the baseline PET/CT study and the occurrence of relapse or death, whichever came first. The Cox proportional-hazard model was applied to test the independence of established prognostic factors as outcome predictors. All P values calculated were 2-sided and adjusted for multiple testing. A $P < 0.05$ was considered indicative of statistical significance. Statistical analysis was performed with the PASW Statistics 18 software program (SPSS Inc, Chicago, IL).

RESULTS

The analyses were based on data obtained from 42 patients (Table 1). The most common anatomic site of the primary tumor was trunk (50%), followed by extremities (43%), and head and neck (7%). Of the tumors, 24% were less than 5-cm, 48% were in the 5- to 10-cm, and 28% were larger than 10-cm range. Pleomorphic sarcoma (n = 17, 40%) was the most common histologic subtype, followed by myxoid liposarcoma (n = 11, 26%), myxofibrosarcoma (n = 5, 12%), leiomyosarcoma (n = 3, 7%), dedifferentiated liposarcoma (n = 2, 5%), fibrosarcoma (n = 2, 5%), epithelioid sarcoma (n = 2, 5%), and alveolar soft part sarcoma (n = 1, 2%). The mean follow-up period of all 42 patients was 31 months (range, 5–52 months).

Distributions of SUV, tumor size, and reduction rate are summarized in Table 2. Baseline MRI showed tumors with a mean size (t0) ± SD of 77.5 ± 39.9 mm. After the first cycle (t1) and after completion (t2) of neoadjuvant chemotherapy, size was 65.6 ± 36.3 mm at (t1) and 54.1 ± 36.0 mm at (t2). The percent reduction rates

TABLE 1. Patient Characteristics

Characteristic	No. Patients
Age, yr	
Mean	54
SD	11
Range	32–72
Follow-up, mo	
Mean	31
SD	16
Range	5–52
Gender	
Male	26 (62)
Female	16 (38)
Size, cm	
0–5	10 (24)
5–10	20 (48)
>10	12 (28)
Distribution	
Trunk	24 (57)
Extremities	18 (43)

SD indicates standard deviation.

TABLE 2. Distributions of SUV, Tumor Size, and Reduction Rate

Baseline	
SUV (t0)	Size (t0), mm
10.9 ± 3.6 [3.8–19.6]	77.5 ± 39.9 [24.8–204.9]
Postneoadjuvant chemotherapy	
SUV (t1)	Size (t1), mm
7.8 ± 3.6 [1.9–13.8]	65.6 ± 36.3 [18.0–160.0]
SUV (t2)	Size (t2), mm
5.2 ± 3.4 [1.1–12.6]	54.1 ± 36.0 [5.0–155.0]
Reduction rate	
%SUV (t1) reduction rate	%Size (t1) reduction rate
28.8 ± 17.3 [2.0–78.0]	14.4 ± 20.0 [−18.2–72.7]
%SUV (t2) reduction rate	%Size (t2) reduction rate
51.7 ± 24.9 [2.0–94.0]	29.9 ± 26.4 [−18.2–92.9]

Data are presented as mean ± standard deviation (SD) with range.

SUV indicates standardized uptake value; t0, baseline; t1, after the first cycle; t2, after completion of neoadjuvant chemotherapy.

TABLE 3. Metabolic Responder and Nonresponders by Histologic Type

	%SUV (t1) Reduction Rate		%SUV (t2) Reduction Rate	
	Metabolic Nonresponder	Metabolic Responder	Metabolic Nonresponder	Metabolic Responder
MFH	12 (29)	5 (12)	8 (19)	9 (21)
MLS	7 (17)	4 (10)	4 (10)	7 (17)
LMS	3 (7)	0	2 (5)	1 (2)
MFS	2 (5)	3 (7)	1 (2)	4 (10)
FS	1 (2)	1 (2)	1 (2)	1 (2)
ASPS	1 (2)	0	0	1 (2)
DDL	1 (2)	1 (2)	0	2 (5)
ES	0	1 (2)	0	1 (2)

The numbers of parentheses are percentages.

The proportion of metabolic responders do not depend on histologic types with %SUV (t1) reduction rate ($P = 0.545$) and %SUV (t2) reduction rate ($P = 0.671$) by Kruskal-Wall test.

MFH indicates malignant fibrous histiocytoma; MLS, myxoid liposarcoma; LMS, leiomyosarcoma; FS, fibrosarcoma; ASPS, alveolar soft part sarcoma; DDL, dedifferentiated liposarcoma; ES, epithelioid sarcoma.

of size (t1) and size (t2) were 14.4 ± 20.0% at (t1) and 29.9 ± 26.4% at (t2). Baseline PET/CT showed high FDG uptake with a mean SUV (t0) ± SD of 10.9 ± 3.6. After the first cycle of neoadjuvant chemotherapy, the SUV had decreased to 7.8 ± 3.4, and then it had decreased further to 5.2 ± 3.4 after completion of neoadjuvant chemotherapy (Table 2). The SDs of SUV (t1) and SUV (t2) compared between metabolic responders and nonresponders as measures of heterogeneity showed that no significant difference was found in SUV (t1) (metabolic responder vs. nonresponder, 3.0 vs. 2.9, $P = 0.534$) and SUV (t2) (metabolic responder vs. nonresponder, 2.9 vs. 2.5, $P = 0.711$). The proportion of metabolic responders compared on the basis of histologic types revealed that there was no significant difference in %SUV (t1) reduction rate ($P = 0.545$) and %SUV (t2) reduction rate ($P = 0.671$) (Table 3). By using a previously defined threshold of 35% decrease in SUV, 15 of the 42 patients (36%) were classified as metabolic responders with a mean ± SD %SUV (t1) reduction rate of 43.8 ± 18.0% and the other 27 patients (64%) were classified as metabolic nonresponders who had a mean ± SD %SUV (t1) reduction rate of 20.5% ± 9.6% after the first cycle of neoadjuvant chemotherapy. After completion of neoadjuvant chemotherapy, 26 of the 42 patients (62%) were classified as metabolic responders with a mean ± SD %SUV (t2) reduction rate of 63.9% ± 18.6%. Sixteen patients (38%) were classified as metabolic nonresponders who had a mean ± SD %SUV (t2) reduction rate of 28.6% ± 14.3%. The correlation between %SUV (t1) reduction rate and %size (t1) reduction rate, and between %SUV (t2) reduction rate and %size (t2) reduction rate were not significant (t1: $r = -0.092$, $P = 0.561$; t2: $r = 0.304$, $P = 0.050$).

Therapeutic response evaluated by histopathology resulted in 10 patients (24%) with satisfactory response and 32 patients (76%) with unsatisfactory response after completion of neoadjuvant chemotherapy (Fig. 1). The SUV (t1) and SUV (t2) of the histopathologic nonresponders were significantly higher as compared with those of the histopathologic responders ($P = 0.007$ and $P = 0.033$, respectively). However, no significant associations were found between the histopathologic response and size (t0), size (t1), size (t2), and SUV (t0). Metabolic responders defined as having at least 35% decrease in %SUV reduction rate showed more frequent histopathologic response compared with metabolic nonresponders ($P = 0.009$ for %SUV (t1) reduction rate and $P = 0.004$ for %SUV (t2) reduction rate, respectively), whereas there were no associations between histopathologic response and %size reduction rate. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of %SUV reduction rate for favorable histologic response were 46.7%, 88.9%, 70.0%, 75.0%, and 73.8% for %SUV (t1) reduction rate, respectively, and 38.5%, 100%, 100%, 50.0%, and 61.9% for %SUV (t2) reduction rate, respectively. No significant association was found between histologic response and %size reduction rate. Fourteen of the 42 patients (33%) have developed recurrence, with a mean time to recurrence of 9.0 months. The 2-year and 4-year actuarial PFS rates were 68.6% and 27.1%, respectively. Univariate analyses of potential prognostic factors demonstrated that PFS was associated with histopathologic response, SUV (t2), %SUV (t1) reduction rate, and %SUV (t2) reduction rate (Fig. 2, Table 4). Age, glucose transporter protein 1,

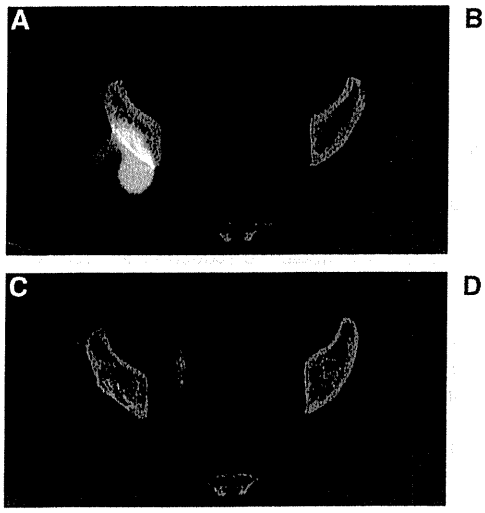

FIGURE 1. Responder of neoadjuvant chemotherapy. (**A, B**) Transaxial F-18 FDG PET/CT images demonstrate significant uptake in the tumor of the right buttock which is pathologically confirmed as pleomorphic sarcoma. (**C, D**) Transaxial F-18 FDG PET/CT images demonstrate metabolic reduction in the tumor of the right buttock after neoadjuvant chemotherapy.

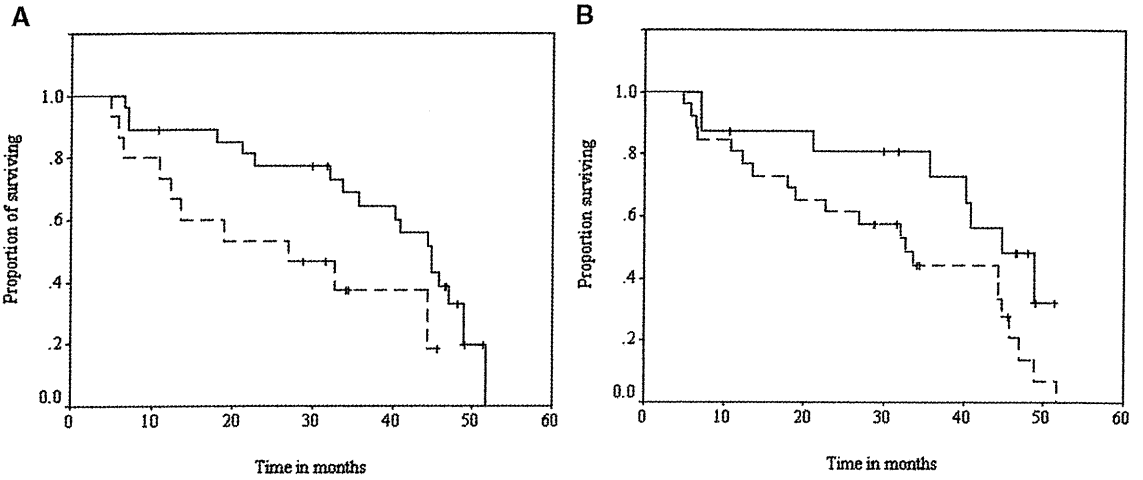

FIGURE 2. A, The PFS of %SUV (t1) reduction rate after the first cycle of neoadjuvant chemotherapy. Solid line: %SUV (t1) reduction rate is ≥30%. Dash line: %SUV (t1) reduction rate is less than 30%. **B,** The PFS of %SUV (t2) reduction rate after completion of neoadjuvant chemotherapy. Solid line: %SUV (t2) reduction rate is ≥60%. Dash line: %SUV (t2) reduction rate is less than 60%.

size (t0, t1, t2), SUV (t0, t1, t2), and %size (t1, t2) reduction rate failed to show a predictive value. A multivariate analysis of factors related to disease progression was performed. In order of relative risk, histopathologic response and %SUV (t2) reduction rate were independent predictors of PFS (Table 4). The SUV (t2) and %SUV (t1) reduction rate were not found to be identical order of relative risk.

The OS for the 42 patients was 83.3% at 2 years and 32.9% at 4 years. Univariate analyses showed that OS had a significant association with histopathologic response, %SUV (t1) reduction rate, and %SUV (t2) reduction rate (Fig. 3, Table 4). However, size (t0, t1, t2), SUV (t0, t1, t2), and %size (t1, t2) reduction rate failed to show a predictive value. In the multivariate analysis, histopathologic response and %SUV (t2) reduction rate showed strong independent value for the prediction of OS (Table 5). The %SUV (t1) reduction rate failed to show independent prognostic properties.

DISCUSSION

In this study, we examined the therapeutic effect and prognostic value of F-18 FDG PET/CT after neoadjuvant chemotherapy in patients with high-grade sarcoma. We found that the histopathologic response and metabolic reduction rate had significant association with PFS and OS. Tumors of histopathologic responders showed high metabolic reduction, which was similar to the results of recent studies.[40]

The prognostic importance of metabolic reduction after neoadjuvant chemotherapy has been suggested in the literature.[22–30] In clinical setting for patients with soft-tissue sarcomas of the extremities, metabolic reduction in F-18 FDG uptake after neoadjuvant chemotherapy was at high risk of systemic disease recurrence.[41] In a study of 36 patients with Ewing sarcoma family tumors, neoadjuvant chemotherapy-induced metabolic reduction after 3 to 7 cycles was noted and SUV after neoadjuvant chemotherapy <2.5 was predictive of PFS.[40] Furthermore, a change in the size of the tumor after neoadjuvant chemotherapy is frequently used in clinical practice to evaluate therapeutic response in patients with high-grade sarcoma. The size reduction evaluated by standard CT has not been correlated consistently with histologic response or with outcome.[6–8] This may relate to the limited availability of this diagnostic modality. In contrast, PET or PET/CT allows semi-quantitative assessment of uptake that is convenient to evaluate tumor viability during

TABLE 4. Univariate Analyses of PFS and OS

Characteristic	No. Patients	2-yr PFS (%)	4-yr PFS (%)	*P*	2-yr OS (%)	4-yr OS (%)	*P*
Age, yr							
≥56	22	59	12	0.181	74	20	0.234
<56	20	74	43		94	51	
Histologic response							
Nonresponder	32	58	12	0.0004	81	16	0.0007
Responder	10	90	75		90	77	
Glut-1 expression							
Positive	25	68	28	0.539	86	27	0.596
Negative	17	64	33		80	41	
Size (t0), mm							
≥72	21	66	20	0.58	78	28	0.52
<72	21	66	32		89	40	
Size (t1), mm							
≥56	22	63	19	0.45	84	28	0.59
<56	20	69	33		83	40	
Size (t2), mm							
≥45	23	65	30	0.89	85	40	0.95
<45	19	68	20		82	24	
%Size (t1) reduction rate							
≥6.2	21	71	34	0.164	84	45	0.122
<6.2	21	61	18		83	23	
%Size (t2) reduction rate							
≥29.5	21	76	30	0.19	94	38	0.128
<29.5	21	56	22		72	29	
SUV (t0)							
≥11.7	22	71	24	0.7	90	28	0.88
<11.7	20	60	29		73	39	
SUV (t1)							
≥6.6	20	68	25	0.96	84	31	0.91
<6.6	22	63	29		83	38	
SUV (t2)							
≥3.9	21	51	8	0.007	81	13	0.14
<3.9	21	81	40		86	43	
%SUV (t1) reduction rate							
≥30	15	92	69	0.0012	92	71	0.005
<30	27	56	9		75	13	
%SUV (t2) reduction rate							
≥60	26	96	40	<0.0001	96	43	0.0001
<60	16	26	0		51	0	

PFS indicates progression-free survival; OS, overall survival; Glut-1, glucose transporter protein-1; t0, baseline; t1, after the first cycle; t2, after completion of neoadjuvant chemotherapy.

therapy. Moreover, good correlations have been demonstrated between histologic response and changes in F-18 FDG accumulation after neoadjuvant chemotherapy in patients with various histologic kinds of tumors.[22–30] Tumor response determined by reduction of F-18 FDG uptake after neoadjuvant chemotherapy was found to be an early indicator of improved PFS or OS.[26–30] Our data demonstrate that significant metabolic reduction after neoadjuvant chemotherapy evaluated by PET/CT has a possibility to portend a favorable outcome and provides compelling support for the routine use of this technique in patients with high-grade sarcoma.

We demonstrate possible correlations of measures of fall in SUV after the first cycle and completion of neoadjuvant chemotherapy and survival. A decrease in SUV after neoadjuvant chemotherapy with median cutoff value of 30% or 60% has been used. The use of change in %SUV reduction rate after the first cycle and after completion of induction chemotherapy as a continuous variable confirmed the predictive ability of metabolic reduction to predict less favorable outcome. A prior study revealed that the ratio of pre- and postchemotherapeutic SUV was correlated with histologic response in patients with high-grade sarcoma.[20] Our data provide a support for this, as patients experiencing such a level of metabolic reduction exhibited favorable PFS and OS, although with a short follow-up duration and a wide confidence interval.

Treatment-induced pathologic necrosis has been demonstrated to be an independent predictor of outcome in patients who have received neoadjuvant chemotherapy for high-grade soft-tissue sarcoma.[1–5] Evaluation of pathologic specimens is warranted to perform precise assessment of response to neoadjuvant chemotherapy. In the prior studies using PET or PET/CT, metabolic reduction after neoadjuvant chemotherapy is capable of reflecting histopathologic response.[42–47] A decrease in SUV after neoadjuvant chemotherapy correlates with the percent of pathologic necrosis. However, such a correlation has been suggested, as yet there has been no studies assessing the direct association between metabolic reduction, histopathologic response, and outcome. In an attempt to resolve this issue in the present study, we investigated whether metabolic response could reflect histopathologic response and could have association with PFS or OS. Our data demonstrated that metabolic reduction after neoadjuvant chemotherapy showed significant correlation with histopathologic response and had a possibility to reflect favorable outcome.

The prognostic value of SUV likely reflects the cumulative effect of a maximum value of different ROIs within the heterogeneous tumor. The results of the previous study revealed a fair association between measures of preoperative SUV and poor outcome.[13] The other study documented that mean SUV on the preoperative PET images of patients with resectable soft-tissue sarcoma predicted outcome.[15] Given the prognostic value of postchemotherapeutic SUV in patients with Ewing sarcoma in a retrospective study,[47] a measurement of SUV after neoadjuvant chemotherapy in high-grade sarcoma may reflect in vivo chemotherapeutic sensitivity. The probable ability of SUV measurements to assess complicated metabolic interactions and predicting outcome makes %SUV reduction rate an important tool for management of patients with high-grade sarcoma. Further prospective studies should confirm whether %SUV reduction rate may be an independent prognostic factor.

Another novel finding in the present study is the documentation that a decrease in SUV after neoadjuvant chemotherapy is a common phenomenon in patients with high-grade sarcoma. Although most of the studies evaluating metabolic reduction have relied on 2-point PET studies at baseline and after neoadjuvant chemotherapy, several studies have assessed metabolic reduction based on sequential PET scans during neoadjuvant chemotherapy in patients with solid tumors.[48,49] Metabolic reduction after the first and third cycle of neoadjuvant chemotherapy was found to be correlated significantly with OS in patients with ovarian cancer.[48] Similarly, in a study of 11 patients with breast cancer, metabolic reduction after the first and second cycles of neoadjuvant chemotherapy was 28% and 46% in the responding lesions, respectively.[49] Because chemotherapeutic response depends on the therapeutic regimens and histologic type of tumor, the degree of

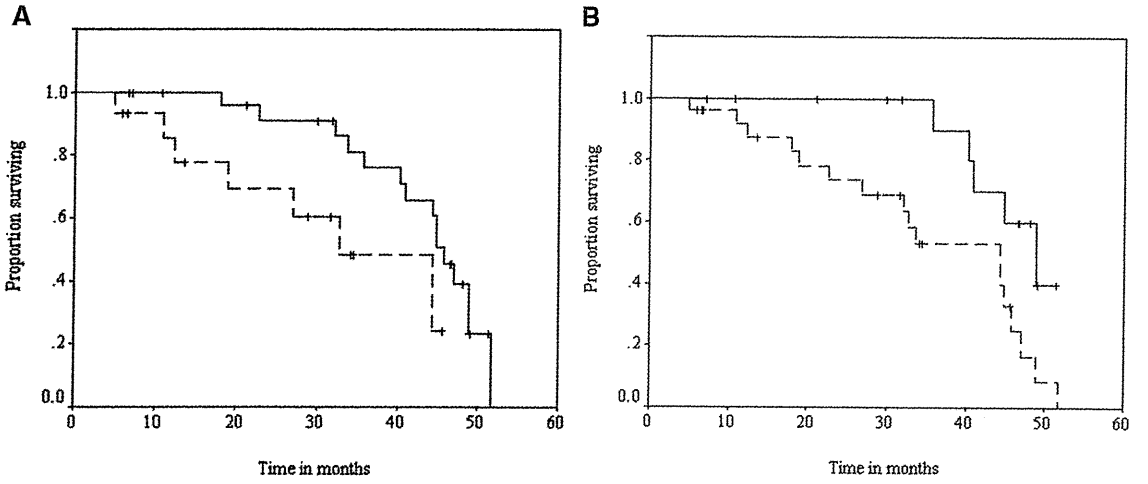

FIGURE 3. A, The OS of %SUV (t1) reduction rate after the first cycle of neoadjuvant chemotherapy. Solid line: %SUV (t1) reduction rate is ≥30%. Dash line: %SUV (t1) reduction rate is less than 30%. B, The OS of %SUV (t2) reduction rate after completion of neoadjuvant chemotherapy. Solid line: %SUV (t2) reduction rate is ≥60%. Dash line: %SUV (t2) reduction rate is less than 60%.

TABLE 5. Multivariate Analyses of PFS and OS

Characteristic	B	Wald	HR	LCI	HCI	*P*
PFS						
Histopathologic response	2.00	6.67	7.35	1.62	33.40	0.01
%SUV (t2) reduction rate	2.00	7.99	7.32	1.84	29.10	0.005
OS						
Histopathologic response	1.92	6.14	6.80	1.49	30.94	0.013
%SUV (t2) reduction rate	2.33	13.61	10.31	2.99	35.64	<0.0001

PFS indicates progression-free survival; OS, overall survival; B, regression coefficient; LCI, lower confidence interval; HCI, higher confidence interval; HR, hazard ratio.

metabolic reduction may be different after every cycle of chemotherapy. Importantly, our study examines patients with specific histologic diagnoses and confirms that metabolic reduction after induction chemotherapy is a common finding in patients with high-grade sarcoma.

The limitations of the present study included the small number of patients and relatively short duration of follow-up period. The diversity of histologic diagnoses had a potential of bias, but the proportion of metabolic responder and nonresponder based on each histologic subtype was not statistically significant. This study focused on patients with high-grade sarcoma who underwent neoadjuvant chemotherapy and the data cannot be extrapolated to patients with advanced stage. A validation study using sequential PET/CT scans is needed in a larger population with various histologic types and adequate follow-up period. The cost-effectiveness of sequential PET/CT for predicting the therapeutic efficacy after neoadjuvant chemotherapy needs to be considered. The role of sequential PET/CT in reducing the number of ineffective chemotherapies in nonresponders must be clarified.

In conclusion, our data also suggest that metabolic reduction after neoadjuvant chemotherapy evaluated using PET/CT can be used for stratification of patients with high-grade sarcoma in clinical trials, as 2 groups of responders and nonresponders exhibit a very different survival profile. Further research is required to determine the role of serial measures of PET/CT in the long-term follow-up period and whether metabolic reductions evaluated by PET/CT can be used as a surrogate for outcome.

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Secernin-1 as a novel prognostic biomarker candidate of synovial sarcoma revealed by proteomics

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ABSTRACT

We aimed to develop prognostic biomarkers for synovial sarcoma employing a proteomic approach. We examined the proteomic profile of synovial sarcoma using two-dimensional difference gel electrophoresis (2D-DIGE). We identified 20 protein spots whose intensity was statistically different ($p<0.01$) between a group of eight patients who were alive and continuously disease-free for over five years and a group of five patients who died of the disease within two years post diagnosis. Mass spectrometric protein identification demonstrated that these 20 spots corresponded to 17 distinct gene products. Three of the 20 spots corresponded to secernin-1 and had higher intensity in the good prognosis group. The prognostic performance of secernin-1 was further examined immunohistochemically in 45 synovial sarcoma cases. The 5-year survival rate was 77.6% and 21.8% for patients with secernin-1 positive and negative primary tumors respectively ($p=0.0015$). The metastasis-free survival was significantly higher in the patient group with high secernin-1 expression compared to that with low expression ($p=0.0012$). Uni- and multivariate analyses revealed that secernin-1 expression was a powerful prognostic factor compared to other clinico-pathological parameters examined. These results indicate that secernin-1 may be used as a biomarker to predict the overall and metastasis-free survival in synovial sarcoma patients.

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1. Introduction

Synovial sarcomas are the third most common primary mesenchymal tumors, with a prevalence of 5–10% of all soft-tissue sarcomas, and more frequently affect young adults [1–4]. The clinical course of synovial sarcoma spans a wide spectrum from a curable disorder to a highly malignant disease that leads to metastasis and death, and the combination of surgery and chemotherapy has resulted in 60% 5-year survival rate [1–4]. Synovial sarcomas are classified into the biphasic subtype and the monophasic one [4]. Synovial sarcomas are characterized by the presence of a chromosomal translocation, t(X; 18)(p11.2;11.2), representing the fusion of the SYT gene with either SSX1 or SSX2 [5–8]. In addition to its diagnostic significance, the fusion type has recently been proposed as a prognostic factor [9–11]; patients with SYT-SSX1 fusion tumors have a significantly unfavorable prognosis, compared to patients with SYT-SSX2. However, the clinical utilities of histological classification and molecular characterization were not proven yet.

Recent comprehensive studies offered a global view of the molecular aberrations associated with the malignant spectrum of synovial sarcoma [12–16]. Global mRNA expression studies identified the genes that are involved in the signaling pathways specific to the cellular origin of synovial sarcoma, the genes associated with histological features denoting malignancy, and the genes differentially expressed corresponding to the different histological subtypes and fusion gene status [12–16]. These comprehensive studies improved our understanding of the biology of synovial sarcoma and may lead to the development of practical biomarkers to support individualized therapy. Proteomic approaches have identified many candidate proteins associated with early diagnosis [17], differential diagnosis [18], prognosis [19–22], and response to chemotherapy [23] in various diseases. Therefore, employing a proteomic approach should be useful in identifying novel prognostic factors in synovial sarcoma; the proteomic profile of synovial sarcoma has not been established to date.

In this report, we performed a proteomics study on synovial sarcoma samples using two-dimensional difference gel electrophoresis (2D-DIGE) and mass spectrometry. We found that the expression levels of 20 protein variants had significant difference corresponding to clinical outcomes. These proteins included three variants of secernin-1, a protein the high expression of which has been reported in gastric and colon cancers, but not in synovial sarcoma [24–26]. We examined the expression of secernin-1 in 45 synovial sarcoma cases by immunohistochemistry, and found that the expression of secernin-1 was significantly higher in patients with good prognosis. These results suggested secernin-1 to be a novel prognosis biomarker for synovial sarcoma.

2. Materials and methods

2.1. Patients and clinical information

We examined 13 frozen and 45 paraffin-embedded primary synovial sarcoma tissues from patients who underwent surgery or chemotherapy at the National Cancer Center Hospital from October 1979 to July 2005. This project was approved by the ethical review board of the National Cancer Center, after signed informed consent was obtained from all patients in this study. The histological features of the tissues were reviewed by four board-certified pathologists (N. T., K. S., A. Y. and T. H.). Diagnosis and classification were based on the WHO classification system for soft-tissue tumors, and included the examination of SYT-SSX1 and SYT-SSX2 expressions [4] (Table 1 and Supplemental Table 1).

Frozen samples were collected from biopsy specimens and resected primary tumors and stored in *liquid nitrogen*. We selected samples that did not have any potential interfering factors such as treatment with chemotherapy or radiotherapy for the 2D-DIGE analyses. Frozen tumor tissues were available for 13 synovial sarcoma cases which were examined for SYT-SSX1 and SYT-SSX2 expressions. Proteins were extracted on the same day and then were used for the proteomic analysis (Table 1). The origin of synovial sarcomas remains to be clearly elucidated, and synovial sarcoma is now regarded as a neoplasm of "uncertain differentiation" [13,27,28]. We employed a special strategy that compared the two different outcome groups, in order to identify the proteins corresponding to the tumor-specific characteristics and outcomes. We grouped the synovial sarcoma samples into two groups; samples from five patients who died of the disease (DOD) were defined as the poor-prognosis synovial sarcoma group (P-SS), and samples from eight patients who did not have metastases within five years post surgery were defined as the good-prognosis synovial sarcoma group (G-SS) (Table 1).

For the immunohistochemical study of secernin-1 expression, we examined 45 paraffin-embedded tissues from 45 patients, sampled prior to the initiation of chemotherapy. The clinical information concerning the cases examined immunohistochemically is summarized in Supplemental Table 1.

2.2. Protein expression profiling

The frozen samples were crushed to powder with a CryoPress (Microtech Nichion, Chiba, Japan) under cooling with liquid nitrogen according to our previous report [29]. The frozen powder was then treated with urea lysis buffer (6 M urea, 2 M thiourea, 3% CHAPS, and 1% Triton X-100). After centrifugation at 15,000 rpm for 30 min, the supernatant was used as the source of cellular proteins for protein expression studies.

2D-DIGE was performed as described previously [18–20,22,29,30]. In brief, the internal control sample was prepared by mixing a small portion of all individual samples. Five micrograms each of the internal control sample and the same amount of each individual sample were labeled with Cy3 and Cy5 respectively (CyDye DIGE Fluor saturation dye, GE Healthcare Biosciences, Uppsala, Sweden). The differently labeled protein samples were mixed and then separated by isoelectric focusing using IPG DryStrip gels for the first dimension separation (24 cm length, pI range between 4 and 7, GE Healthcare Biosciences) and then by SDS-PAGE for the second dimension separation (EttanDalt II, GE Healthcare Biosciences). The gels were scanned using laser scanners (Tyhoon Trio, GE Healthcare Biosciences) at the appropriate wavelengths (Fig. 1A and Supplemental Fig. 1). For all spots, the intensity of the Cy5 image was normalized by that of the

Table 1 – Clinicopathologic features of synovial sarcoma samples (13 cases).

No	Age	Gender	Primary location	Fusion gene	Subtype	MIB-1 score	Grade[a]	Tumor depth	Tumor size (cm)	Stage[b] TNM classification	Surgery	CTx.	RTx.	Local recurrence time after diagnosis (MO)	Metastasis		Follow-up	
															Site	Time after diagnosis (MO)	Time after diagnosis (MO)	Status
1	26	M	Calf	SYT/SSX2	Monophasic	3	3	Deep	5	III	Wide	Adjuvant	Paliative	–	Lung	3	21	DOD
2	49	F	Foot	SYT/SSX1	Biphasic	3	3	Deep	8	IV	Wide	Adjuvant	–	–	Lung	At diagnosis	14	DOD
3	54	M	Inguina	SYT/SSX1	Poor	3	3	Deep	7	IV	Wide	Paliative	Paliative	–	Bone	At diagnosis	16	DOD
4	19	F	Thigh	SYT/SSX2	Poor	3	3	Deep	8	IV	Wide	Adjuvant	–	–	Lung	At diagnosis	21	DOD
5	24	M	Elbow	not examined	Biphasic	3	3	Deep	15	IV	–	Paliative	–	–	Lung	At diagnosis	8	DOD
6	51	M	Hand	SYT/SSX1	Biphasic	3	3	Deep	5.4	III	Wide	–	–	–	Lung	56	97	NED
7	61	F	Thigh	SYT/SSX1	Biphasic	3	3	Deep	4.5	IIA	Wide	–	–	–	–	–	100	CDF
8	41	F	Foot	SYT/SSX1	Biphasic	3	3	Deep	5.4	III	Marginal	–	–	–	–	–	107	CDF
9	57	F	Knee	SYT/SSX1	Monophasic	3	3	Deep	13	III	Wide	–	–	–	–	–	122	CDF
10	10	F	Elbow	SYT/SSX2	Biphasic	2	2	Deep	2.5	IIA	Wide	–	–	–	–	–	125	CDF
11	14	M	Foot	SYT/SSX1	Monophasic	1	2	Deep	2.2	IIA	Additional wide	–	Adjuvant	2	–	–	138	NED
12	21	M	Knee	SYT/SSX1	Monophasic	2	2	Superficial	7.5	IIB	Wide	Adjuvant	–	–	–	–	113	CDF
13	33	M	Foot	SYT/SSX2	Monophasic	2	2	Deep	3	IIA	Wide	Adjuvant	–	–	–	–	123	CDF

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Fletcher CDM Unni KK, Mertens F. Pathology and genetics of Tumours of soft tissue and bone. Lyon: IARC Press; 2002. 4) poor: poorly differentiated.
[a] Modified FNCLCC (French Federation of Cancer Centers) system.
[b] Tumor-Nodes-Metastases Classification.