表1　二次がんと初発がんとの関係（文献2を改変）

二次がん	初発がん	潜伏期（中央値）	危険因子
乳がん	ホジキン病	15～20年	放射線
	骨腫瘍		女性
	軟部肉腫		
	急性リンパ性白血病		
	脳腫瘍		
	ウイルムス腫瘍		
	非ホジキンリンパ腫		
脳腫瘍	急性リンパ性白血病	9～10年	放射線
	脳腫瘍		若年者
	ホジキン病		
骨髄異型性症候群と急性骨髄性白血病	急性リンパ性白血病	3～5年	トポイソメラーゼⅡ阻害剤
	ホジキン病		アルキル化剤
	骨腫瘍		
甲状腺がん	急性リンパ性白血病	13～15年	放射線
	ホジキン病		若年者
	神経芽腫		女性
	軟部肉腫		
	骨腫瘍		
	非ホジキンリンパ腫		
骨腫瘍	網膜芽腫（遺伝性）	9～10年	放射線
	他の骨腫瘍		アルキル化剤
	ユーイング肉腫		脾摘
	急性リンパ性白血病		
軟部肉腫	網膜芽腫（遺伝性）	10～11年	放射線
	軟部肉腫		若年者
	ホジキン病		アントラサイクリン系薬剤
	ウイルムス腫瘍		
	骨腫瘍		
	急性リンパ性白血病		

ん，甲状腺がんが女性に多いことによる．また，放射線治療による誘発がんとしては，幼児期に放射線治療を受けていること，放射線線量の増加に伴い，さらにフォローアップ期間が長いほど誘発がんが増加している．放射線誘発がんとしてホジキン病から乳がん，ホジキン病・急性リンパ性白血病から甲状腺がん，ホジキン病から肺がん，脳腫瘍・急性リンパ性白血病から脳腫瘍，網膜芽腫から骨肉腫が発生することは有名である（表1）[2]．

しかし，注意しなければならないのが遺伝子異常をもたらす疾患である．両側性網膜芽腫では染色体異常（13q-14 deletion）が認められ，がん抑制遺伝子RB gene遺伝子変異のため治療後の誘発がんが非常に多く認められ[3]，診断から50年累積発生率は，放射線照射を受けなかった患者では26%，放射線照射を受けた患者では58%である[4]．がん抑制遺伝子p53遺伝子の変異が原因で起こる誘発がんもある．Li-Fraumeni症候群（リ・フラウメニ症候群）が有名で，その家系内に脳腫瘍，乳がん，白血病，肉腫，小児がんなどの様々な悪性腫瘍が多発する稀な遺伝疾患である．また，神経線維腫症1型（NF-1）はNF1遺伝子（がん抑制遺伝子）異常を伴うために脳神経系に腫瘍が発生しやすい遺伝性疾患であり，これらの遺伝子異常は誘発がんの検討からは外すべきと考えられている．

1. 放射線単独治療による影響

放射線誘発がんの最初の報告は，レントゲンによるX線発見6年後に，4年間レントゲン管を扱っていた33歳の技術者の手にできた皮膚扁平上皮がんであった．その後，放射線誘発がんは放射線治療，広島・長崎の原爆などから様々な報告がある．そのなかで，小児放射線被曝で常に問題となっているものに甲状腺がんの発生がある．これに関しては非常に少ない線量140cGyから2600cGy（平均512cGy）の線量で発生しており[5]，最近ではチェルノブイリ原子炉事故においても問題になっている．

われわれが放射線治療で用いている超高圧X線治療装置に至る前には深部X線治療装置が使用されていた時代があった．Bostonグループでの深部X線治療による小児放射線治療患者の調査では悪性腫瘍発生リスク17%と高いものであった[6]．

本邦における放射線治療の歴史からみて，この深部X線治療装置を用いた時期は短く，コバルト60遠隔治療装置から超高圧X線治療装置（リニアック）に移行している．その時期，1979年，1984年の2回の全国アンケート調査「良性疾患の放射線治療後における発がん」では，良性疾患の放射線治療後における誘発がん症例236例が集計された．潜伏期間は2～63年，平均28.9年であり，発生した食道がん・下咽頭がんなどでは平均30年以上の長い潜伏期間を有していた．1955年以前に放射線治療を受けていた群では放射線誘発がん発生頻度は平均1.9%であるが，1956年以降の放射線治療群では0.1～0.4%と低い[7]．しかし，このリスク評価は母数を放射線治療総数としているため，統計学的には低い評価を行っていると考えられる．

また同じく，1979年，1984年の2回の全国アンケート調査による「悪性腫瘍の放射線治療後における二次発がん」調査では，248例が集計された．この248例中147例が放射線誘発がんの定義「被照射組織より新たに発生したがんであり，第二のがん病理組織像と発生臓器がともに第一がんとは明らかに異なっているもの」に合致するものである．発生した白血病（23例）の潜伏期は0.5年～14年，平均5.7年であり，固形がんでは5～35年，平均15.0年であった．発生頻度の母数を照射患者数とすると1955年以前の照射群では0.50%，1956年以降の照射群では0.06～0.22%と低くなっている．しかし，母数を5年生存者数とすると1955年以前では1.57%，1956年以降では0.15～0.64%となる[8]．この推定値においてもまだ生命表，期待値などを用いていないために統計学的には低い評価を行っていると考えられる．

2. 放射線療法，化学療法併用による影響

まず，化学療法による誘発がんについて考えてみたい．小児悪性リンパ腫は化学療法が主体であり，放射線治療は低線量で良いとされている．発がん性を小児ホジキン病のみでみると，6回のMOPP治療による二次白血病が5.5%に認められ，これは放射線治療による固形腫瘍発生の22倍の危険性である[9]．また，突然変異頻度（glycophorin A mutation assay）は，治療前と放射線治療群はコントロール群と変わらなかったが，化学療法群において著明に増加していた[10]．これら化学療法剤による誘発がんは，アルキル化剤，トポイソメラーゼII阻害剤などによるものが有名で，これらはDNA二重鎖切断などを起こすもので，その結果として染色体損傷が起こりがん抑制遺伝子の欠失，がん遺伝子の活性化などを引き起こすためと考えられている．

小児がん長期フォローアップにおいて放射線治療，化学療法のデータが取れているミネソタ大学病院単一施設の報告がある．1953年～1975年，University of Minnesota Hospitalにおいて超高圧放射線を用いた小児放射線治療患者330人（189人が化学療法併用）の調査，この調査から両側網膜芽腫と神経線維腫症患児は誘発がんのリスクが高いため除かれている，では14例の二次腫瘍が5～21年後に認められた（4.2%：14/330）．30年累積発生率（standard life-table methods）は

9.6%であった．そのうち10例が非悪性腫瘍であった．9例が照射野内発生し，そのうち5例は化学療法併用である．2例が化学療法併用で照射野外発生している．3例が化学療法使用していない患児において照射野外発生している．照射野内発生腫瘍の30年累積発生率は8.2%であり，照射野内発生の悪性腫瘍のリスクは1.0%である[11]．これは化学療法も含まれているのでやや高めに評価されてはいるが，放射線治療の誘発がんのリスクとしては正確なものと考えてよい．

その後，同じミネソタ大学病院から新たに追加された症例を含めての解析では，1954～1980年，University of Minnesota Hospital，現在はFairview University Medical Centerとなっているが，において超高圧放射線を用いた17歳以下で放射線治療後最低5年を経過している小児放射線治療患者446人（302人が化学療法併用），両側網膜芽腫と神経線維腫症は除いて解析した．5年生存以上を採用基準としているため5年生存率は100%であり，Kaplan-Meier生存評価は10年で91%，20年で85%，30年で80%であった．37人（調査対象の8.3%）に二次（悪性）腫瘍が発生しており，このなかで20症例が化学療法併用である．37症例中，22例が照射野内発生で，9例が照射野周囲発生で，5例が照射野外発生で，潜伏期は中間値15.5年（3.8～31.8年）であった．二次（悪性）腫瘍としては脳腫瘍（8例），乳がん（8例：SI 23.9%），皮膚悪性腫瘍（8例）が多く発生している．なお37例以外に22例の良性腫瘍が発生したが，この検討からは除外してある．全二次（悪性）腫瘍の30年累積リスクは13%，標準化発生率standardized incidence ratio（SIR: observed/expected ratio）は5.2%である[12]．

小児がん生存者の長期フォローアップ調査the childhood cancer survivor study（CCSS）はアメリカの大規模研究組織ではあるが，放射線療法の詳細をデータとして持っていないために，放射線治療による誘発がんの検討には限界がある．1970～1986年，CCSS登録患者，21歳未満の悪性腫瘍患者で5年以上生存者，20,245名のうち，適格症例13,581名（放射線療法7,780名）の調査で298名に二次悪性腫瘍314例が認められた．標準化発生率（SIR）は6.38%で，20年累積推定発生率cumulative estimated incidenceは3.2%，中間潜伏期11.7年であった．5年ごと30年間の調査では，白血病発生リスクは5～9年にかけて最も高く，それ以降減少する．乳がん・甲状腺がんを含む固形がん発生リスクは全観察期間にわたり著明に増加する．二次悪性腫瘍の多いものは乳がん（60例，SIR＝16.18）で女性ホジキン病生存者に発生しており，その次は甲状腺がん（43例，SIR＝11.34）で白血病と中枢神経腫瘍生存者に認められており，中枢神経悪性腫瘍（36例，SIR＝9.85）は急性リンパ性白血病頭蓋照射患者に多く認められている．分析した時点で二次悪性腫瘍を発生していない群の91.9%が生存しているのに比べ，二次悪性腫瘍を発生した群は59.4%の生存であった[13]．

同じCCSSの新しい解析では，1970～1986年に診断された21歳未満の悪性腫瘍患者で5年以上生存者14,372名に二次悪性腫瘍677例が発生している．そのうち調査許可を得た13,136名ではがん（cancer）が71例に認められた．これらの調査の初発患者から網膜芽腫，非中枢神経胚細胞腫瘍，肝腫瘍は除かれている．潜伏期は6～28年，中間値15年で，がん発症年齢は10～44歳，中間値27歳であった．誘発がん（cancer）全体の標準化発生率（SIR）は4.0%（初発時の中枢神経腫瘍以外）であり，中枢神経腫瘍においては2.1%と非常に低いものであった．化学療法施行群SIR 5.4%，放射線療法施行群SIR 5.1%となっているが両者併用か単独治療かの区別はできていない．二次がんは多くは照射野内に発生しているが，71症例中22例（33%）は照射されていない部位から発生している．また，化学療法，放射線療法ともに受けていない患者から2名の二次がんが発生している[14]．

3. 中枢神経腫瘍治療後の誘発がんについて

1984～2002年，St. Jude Children's Research Hospitalにおいて治療された22歳未満の中枢神

経腫瘍1,283名，1型神経線維腫症（NF1）は二次腫瘍が発生しやすいので除く，のうち24名に二次腫瘍が発生した．初発時年齢は中間値4.6歳（0.4～20.1歳），二次腫瘍発生時は15.9歳（2.5～31.6歳），潜伏期は7.9年（0.3～21年）であった．24症例の内7症例に遺伝子異常があり除外し解析した10年推定累積発生率は二次腫瘍で1.9%，二次悪性腫瘍で1.4%である．また15年推定累積発生率は二次腫瘍で5.3%，二次悪性腫瘍で4.0%である．二次腫瘍の発生を治療別に見ると放射線療法単独では278症例中3例，化学療法単独では57症例中0例，放射線療法・化学療法併用では486症例中5例，化学療法・放射線療法とも施行しない群では218症例中1例の発生を認めているが，これら4群間には有意差は無い[15]．

中枢神経予防照射についてみると，1962～1992年，St. Jude Children's Research Hospitalにて治療を受けた急性リンパ性白血病で10年以上無病生存している白血病再発のない856症例の調査において，56例に重大な合併症が起き，内訳は8例の寛解中死亡，4例の再発，44例の二次腫瘍であり（そのうち41例は放射線治療に関連していた），二次腫瘍のほとんどは良性あるいは軽度悪性であった．予防的頭蓋照射（597名）による二次腫瘍推定累積リスクは導入化学療法寛解20年後では20.9%とされ，頭蓋照射を行わない群（259名）の0.95%よりも高いリスクであった[16]．従来，急性リンパ性白血病による二次腫瘍累積リスクは，診断後15～20年後において4%以下とされていた．今回の調査では遅い時期の二次がんは良性腫瘍とされており，予後不良とされる発がんは初期の10年に認められているとの報告と一致している．誘発がん（cancer）発生の標準化発生率（SIR）は，予防的頭蓋照射群（22/597）で6.65%，非照射群（1/259）で2.08%，脳腫瘍発生SIRは27.74%となっているが，甲状腺がん（4），口腔・咽頭がん（3），肝がん（2），骨髄腫瘍（2），膵臓がん（1），膀胱がん（1），卵巣がん（1），乳がん（1），ホジキン病（1：非照射群）も報告されており[16]，脊髄照射群が少ないことも加味して本当に照射野内から発生したものを捉えられているのか疑問視せざるを得ない．このように化学療法併用時の誘発がんを考えるとき，化学療法施行しているにもかかわらず放射線治療群として選別することの意義に放射線腫瘍医として問題を提起したい．

まとめ

集学的治療が行われている小児がんにおいて，小児がん生存者の長期フォローアップ調査では放射線療法単独での誘発がんを調査できるものはほとんどない現状である．このような調査において，放射線療法・化学療法併用患者と化学療法単独患者の誘発がんの比較が行われているが，これは化学療法がベースになっているところで放射線治療を加えたことのリスクというべきで，放射線治療の方が誘発がんのリスクが高いということを証明するものではない．両者併用では当然のことながら，誘発がんのリスクは相乗的に増加するものと考えられている．

筆者個人が小児がん放射線治療に関わって25年となり，国立小児病院，国立成育医療センターで扱った患児は567名で，放射線治療単独というのは網膜芽腫と一部の脳腫瘍のみである．他はほとんど化学療法併用の集学的治療を行って，その治療成績を向上させてきており，半数以上が長期生存している．その中で1名の両側網膜芽腫患児の照射野内から横紋筋肉腫が発生したのが唯一の放射線誘発がんである．経過観察が不十分な症例もあると思うが，この間化学療法誘発がんがわれわれの施設から報告されていることを考えると，放射線誘発がんは比較的少ないのが事実ではないだろうか．本邦の成人主体の放射線治療報告では，放射線誘発がん発生頻度は1955年以前では平均1.9%，1956年以降では0.1～0.4%[7]，アメリカでの小児がん治療における放射線治療における誘発がん標準化発生率は5.2%～6.38%[12,13]である．脳腫瘍治療による誘発がんリスクは低いとされているが，標準化発生率（SIR）2.1%[14]，15年推定累積発生率は4.0%[15]であり，中枢神経以外の小児がん放射線治療による照射野内発生の悪性腫瘍30年累積発生率は1.0%～13%[11,12]と

長期フォローアップにより発生頻度が高まるとされている．これらの報告は化学療法がベースに使用されていることを考慮することが必要であるが，この長期フォローアップにより発生頻度が高まるということが重要で，今後本邦においても小児がん長期フォローアップ体制を全国的に行うことが行政的な課題と考える．

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総会記録

小児がん登録キャンペーンシンポジウム「小児がん登録の現状と分析，そしてこれから」

藤本純一郎[1)]，池田　均[2)]

まず，福澤らは「小児がん全数把握登録事業：開始の経緯と現状」と題して小児がんの正確な患者数および罹患率を把握することが，小児がんに対する治療戦略を立てる上で必須の命題であることを強調し，それを実現するために日本がん学会が取り組んでいる小児がん全数把握登録事業について，その経緯ならびに現状を報告した．本登録は，疫学研究指針，個人情報保護法を配慮し，可能な限り簡略化した匿名化登録であるとの説明があった．また，この登録事業が，関連7学会と連携して実施されているとの紹介があった．

坂本は「小児がん登録の疫学」と題し，疫学的な立場から，疾病をコントロールするには，信頼度の高い統計情報，特に，罹患数・罹患率および死亡数・死亡率が必須であり，疾病の実態把握が可能となるとの説明があった．また，この情報に治療内容や転帰情報を合わせることにより，医療の向上や患者のQOLの向上に資することが可能になると報告した．小児がんのごとく発生数が少ない希少疾患の場合は，悉皆性を確保しながら個々の情報の信頼性も担保することが必要であり，そのためにはもれなく情報を収集するとともに，複数の異なる情報源との照合によるデータクリーニングが必須であると報告した．

掛江は「小児がん登録における倫理的諸問題」と題し重複登録や誤登録を排除するためには，診断病名の登録以外にある程度の個人情報を収集することが必要になると説明した．その際，疾患登録の持つ「公共性」と「患者のプライバシー権（自己の情報をコントロールする権利）」のバランスを考慮しながら小児がん実数把握登録を実施する必要があると強調した．実はこの課題は，地域がん登録事業でもしばしば議論されたことであり，解決が困難な部分もある．また，疫学研究指針の範囲内となる可能性も考慮しておく必要もあることから，本事業を実施するに当たっては，登録する医師らは安心して登録できる体制整備を行うことが重要であると報告した．

以上，前半では日本小児がん学会が実施中の小児がん全数把握登録事業とそこでの疫学的ならびに倫理的配慮に関する発表を行った．後半は，近畿地区で行われている府県連携の小児がん登録の取り組みについての発表が行われた．

井上らは「大阪府における小児がん全数登録の取り組み：大阪府小児がん登録推進委員会からの報告」と題して，大阪府における取り組みを紹介した．大阪府は「大阪府がん登録」として地域がん登録に実績があり，その枠組みの中で小児がん登録も実施してきたが，登録の精度向上，生存率の把握のために，小児がんを扱う機会の多い医療施設が連携して「小児がん登録委員会」を組織しているとの報告があった．この委員会が集計した結果では，2004年罹患数は127件（期待罹患数の80.3%）で，白血病，リンパ腫，肝芽腫のみで期待罹患数が80%を超えたが，小児慢性特定疾患治療研究事業との比較では，神経芽腫の把握が低いなどの問題点が判明したことが報告された．

太田らは「滋賀県における小児がん登録の試み」として，2004年および2005年における滋賀県小児がん登録委員会の調査結果を報告した．2004年，同登録では22例の登録例があったのに対し，

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小児慢性特定疾患登録における把握例は28例で，最終的な新規発生例は32例であった．また2005年はそれぞれ35例，40例で，新規発生は43例であった．太田は以上のデータから，小児慢性特定疾患登録による新規発生例の把握率は88%～93%であり，乳幼児医療費助成制度が適応される年齢では小児慢性特定疾患登録への登録率が低いこと，また想定外の施設から登録例があることなどから，小児がんの全数把握の難しさを指摘した．

足立らは京都府小児がん登録委員会を代表して「近畿地区における小児がん全例登録の取り組み」と題し，2005年の登録例の集計結果を報告した．すなわち全登録症例数は43例で，内訳は白血病15例（35%），脳腫瘍10例（23%），悪性リンパ腫8例（19%），骨軟部組織腫瘍5例（12%），胚細胞性腫瘍2例（5%）などであり，それぞれの疾患別発生率は小児人口10万人あたり2.9人，1.9人，1.5人，1.0人，0.4人であった．足立らは京都府における疾患別発生率は全国と同等であると結論している．

小阪は「近畿地区における小児がん全例登録の取り組み―兵庫県―」と題して，兵庫県における小児がん登録の試みを紹介した．主要施設から集計された2003年の新規発症例は92例で，内訳は白血病30例（33%），脳腫瘍17例（18%），骨軟部腫瘍13例（14%），神経芽腫7例（8%）などであった．また発症率は小児人口8900人あたり1人で，疾患別頻度および発症率ともに既報の集計結果と同様であったとしている．この結果から小阪らは兵庫県における登録システムは新規発症例の多数を把握していると推測しているが，思春期以降の造血器腫瘍が様々な施設で治療を受けており，全数把握は困難であると結論した．

岸本らは「奈良県における小児がん登録状況」と題して，奈良県小児がん登録推進委員会の調査結果を報告した．すなわち2004年度は施設登録例が15例で，小児がんの発生頻度は小児人口10万人あたり7.39人であったと報告した．岸本らは比較的人口の少ない奈良県においては小児がん診療を行う施設が限られているため，症例の全数把握が可能であろうと述べている．

神波らは「和歌山県における小児がん全数把握の現状」と題して，和歌山県における取り組みを紹介した．同県では小児がん登録委員会を設置し，県内の主要施設から登録を行った．2004年度の小児がん発症数は23例で，内訳はALL 5例，AML 3例，脳腫瘍5例，骨腫瘍3例，網膜芽腫2例などであった．小児慢性特定疾患の登録データと照合するとしているが，同登録による症例把握がリアルタイムでないこと，また他府県へ流れた患者の把握が困難なことなどから全数把握が困難となる問題点を指摘した．

以上のように，小児がん登録キャンペーンシンポジウムでは日本小児がん学会が取り組む小児がん全数把握登録事業とその基礎となる疫学的方法論が解説され，さらにがん登録に関連する倫理的諸問題が提示された．また近畿地区を中心とする小児がん登録の試みが紹介され，今後，小児がん全数把握登録事業が解決すべき問題点と将来的展望を含め，その意義をキャンペーンし得たと考えている．

Clear Cell Sarcoma of Tendons and Aponeuroses

A Study of 75 Patients

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BACKGROUND. Clear cell sarcoma (CCS) of tendons and aponeuroses (malignant melanoma of soft parts) is a rare melanocytic soft tissue sarcoma. The objective of this study was to determine the clinical features, prognostic factors, and optimal treatment policy for patients with this rare disease.

METHODS. Seventy-five consecutive patients with histologically confirmed CCS who received treatment between 1980 and 2004 were analyzed retrospectively.

RESULTS. There were 41 men and 34 women, and the median age was 36 years. Sixty-five tumors were located in the extremities, and 10 tumors were located in the trunk. The median tumor size was 4 cm. Seventy-one patients underwent surgical excision, and 56 patients received chemotherapy. Sixteen patients developed local recurrences, and 52 patients developed metastasis. The overall patient survival rates was 47% at 5 years and 36% at 10 years. Univariate analysis showed that sex ($P = .018$), tumor size ($P = .001$), tumor depth ($P = .002$), TNM classification ($P = .001$), and surgical margin ($P = .042$) were significant prognostic factors. Among the 52 patients who presented with localized disease, sex ($P = .023$), tumor size ($P = .002$), tumor depth ($P = .011$), TNM classification ($P = .004$), and chemotherapy ($P = .032$) were identified as significant prognostic factors. Multivariate analysis showed that tumor size remained an independent prognostic factor in both groups.

CONCLUSIONS. The current results supported the contention that early diagnosis and initial wide excision are essential for a favorable outcome of CCS. The role of chemotherapy for CCS should be investigated further. *Cancer* **2007;109:109–16.**

KEYWORDS: clear cell sarcoma, malignant melanoma of soft parts, prognostic factors, tumor size, chemotherapy.

Clear cell sarcoma (CCS) of tendons and aponeuroses is a rare malignant tumor that occurs most commonly in the extremities of young adults. Since its first description as a distinct clinicopathologic entity by Enzinger in 1965,[1] a number of studies of its histogenesis have been conducted. Because of its close histologic kinship with malignant melanoma (the presence of melanin, ultrastructural evi-

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TABLE 1
Clinicopathologic Studies of Clear Cell Sarcoma

Reference	Year	No. of patients	Five-year survival	Prognostic factors
Sara et al., 1990[8]	1990	17	40	Size
El-Naggar et al., 1991[9]	1991	11	NA	DNA content
Lucas et al., 1992[10]	1992	35	67	Size, necrosis
Montgomery et al., 1993[11]	1993	58	63	Size,* necrosis*
Deenik et al., 1999[12]	1999	30	30	Size, radiotherapy
Finley et al., 2001[13]	2001	8	55	Size
Ferrari et al., 2002[14]	2002	28	66	Size, site, IRS group
Takahira et al., 2004[15]	2004	14	33	Mitosis
Current study		75	47	Size,* depth, sex, TNM stage, surgical margin

NA indicates not available; IRS group, Intergroup Rhabdomyosarcoma Study group.
* Multivariate analysis.

dence of melanosomes, and immunohistochemical staining for S-100 protein and melanoma-associated antigen HMB-45), Chung and Enzinger proposed the name malignant melanoma of soft parts.[2] More recent molecular genetic characterization of the 2 diseases (the presence of translocation t[12;22][q13;q12], resulting in a chimeric EWS/ATF1 gene only in CCS and not in cutaneous malignant melanoma; the presence of activating mutations in the kinase domain of the BRAF gene only in cutaneous malignant melanoma and not in CCS) have led to the supposition that CCS and malignant melanoma constitute 2 separate entities despite their histologic resemblance.[3–6] Compared with the extensive literature regarding the histopathologic nature of the disease, very little is known about the clinical features of CCS.

The main obstacle to gaining a thorough understanding of the clinical behavior of CCS is the rarity of the disease. Even among studies from major referral centers, the majority have dealt with less than a few dozen patients who were treated over a long period (Table 1).[7–15] These limitations have impeded the establishment of well-founded prognostic factors and treatment policies for CCS. Here, we report a series of 75 patients with CCS who received treatment during the era of modern multidisciplinary therapy that provides useful new information on the clinical behavior of the disease and promising treatment strategies.

MATERIALS AND METHODS

Between January 1980 and March 2004, 75 patients with histologically confirmed CCS were admitted to our Japanese Musculoskeletal Oncology Group-affiliated institutions. Complete data on patients, tumor characteristics, treatment, and follow-up were available for all patients. The histologic criteria for the diagnosis of CCS were those described by Enzinger.[1] Confirmation of the diagnosis was made by immunohistochemistry (immunoreactivity for S-100 protein, HMB-45, and neuron-specific enolase) and, in selected patients (diagnostically difficult cases for which snap-frozen tumor tissues had been preserved), by molecular genetic techniques (reverse transcriptase-polymerase chain reaction for detection of the chimeric EWS/ATF1 gene) and/or molecular cytogenetic techniques (fluorescence in situ hybridization for the detection of t[12;22][q13;q12]).[3,5,16]

The following demographic and treatment factors were examined for prognostic importance: patient age (≤30 years, >30 years), sex, tumor size (≤5 cm, >5 cm), location, depth, symptoms, clinical staging, microscopic surgical margin, chemotherapy, and radiotherapy. With respect to tumor location, proximal sites included the trunk and proximal extremities (upper arm and thigh), and distal sites included distal extremities (below or at the elbow and below or at the knee joint). Tumor depth was classified as superficial or deep in relation to the investing muscle fascia and was determined by surgery and radiographic findings. The microscopic surgical margin was determined histologically on the resected specimens and was classified as negative (no tumor cells at the inked margin) or positive (tumor cells at the inked margin). Clinical staging was assessed according to the American Joint Committee on Cancer/International Union Against Cancer TNM staging system.[17]

Patients received multimodality treatment, including surgery, radiotherapy, and chemotherapy. Wide excision of the primary tumor with a negative surgical margin was attempted whenever possible. If necessary, amputation/disarticulation was planned to achieve a negative surgical margin. For potential margin-positive limb-sparing resection, we preferred amputation/disarticulation in the hope of obtaining a negative surgical margin.

Radiotherapy was received mainly by patients who were at risk of local recurrence, because a negative surgical margin could not be obtained despite this surgical principle. Different chemotherapeutic regimens were used, partly because many different hospitals were involved. The chemotherapeutic agents used included doxorubicin, ifosfamide, cisplatin, dacarbazine, cyclophosphamide, vincristine, bleomycin, interferon-α, and caffeine.

Tumor response was judged from plain radiography, computed tomography (CT), and magnetic resonance imaging (MRI) findings according to World Health Organization criteria.[18] A complete response was defined as the total disappearance of all clinically and radiologically detectable disease for ≥4 weeks. A partial response was defined as a reduction ≥50% in the size (sum of the products of the 2 greatest perpendicular dimensions) of measurable lesions with no new lesions appearing. Stable disease was defined as a reduction <50% or an increase <25% in the size of measurable lesions with no new lesions appearing. Progressive disease was defined as an increase ≥25% in the size of measurable lesions or the appearance of new lesions.

All time-to-event endpoints were computed by using the Kaplan-Meier method.[19] Patients who died of causes unrelated to CCS were censored at the time of death. Potential prognostic factors were identified by univariate analysis using the log-rank test. Independent prognostic factors were evaluated with Cox proportional-hazards regression using a stepwise selection procedure.[20] To arrive at a parsimonious multivariate model, covariates were selected into the model only if they contributed significantly to the fit of the model. The criterion used to select covariates into the model was the score chi-square statistic. Differences at $P < .05$ were considered significant. Statistical analyses were performed using the Statview 5 statistical package (SAS Institute Inc., Cary, NC).

RESULTS

Tumor Characteristics

The study group comprised 41 men (55%) and 34 women (45%), and patients ranged in age from 10 years to 71 years (median, 36 years). Sixty percent of patients were aged <40 years, and 91% were aged <60 years.

Sixty-five tumors (87%) were located in the extremities, and 10 tumors (13%) were located in the trunk. The most common tumor site was the foot (22 tumors), followed by the hand (13 tumors), and the thigh (9 tumors). Twenty-four tumors (32%) were considered proximal, and 51 tumors (68%) were considered distal. There were 2 CCS tumors arising from bone (1 in the ulna and 1 in the tibia).[21] The tumor depth was superficial in 24 patients (32%) and deep in 51 patients (68%). The tumors ranged in size from 1 cm to 11 cm (median, 4 cm). The tumors measured <5 cm in 40 patients (53%), between 5 cm and 10 cm in 25 patients (33%), and >10 cm in 5 patients (7%). Tumor size was unknown in 5 patients. All patients had a mass that had been enlarging slowly for periods ranging from a few months to 23 years (median, 12 months). Pain and/or tenderness were observed in 16 patients (21%). No patients had a history of melanoma or unusual pigmented skin lesions. A chimeric EWS/ATF1 gene was detected in 7 of 9 patients examined.

Fifty-two patients (69%) presented with localized disease, and 23 patients (31%) presented with metastatic disease, including 12 patients with disease metastatic to lymph nodes, 6 patients with disease metastatic to the lung, and 5 patients with disease metastatic to both lymph nodes and lung. According to the TNM staging system,[17] 31 patients were classified with stage IIA CCS (tumors ≤5 cm, no metastases), 2 patients were classified with stage IIB CCS (tumors >5 cm and superficial, no metastases), 14 patients were classified with stage III CCS (tumors >5 cm and deep, no metastases), and 23 patients were classified with stage IV CCS (tumors with metastases).

Treatment

In most (71) patients, the principal treatment consisted of surgical excision of the primary tumor. Four patients who did not undergo surgery had advanced disease with multiple distant metastases. Fifty-six patients underwent limb-sparing surgery, and 15 patients underwent amputation/disarticulation. A negative microscopic surgical margin was achieved in 60 patients, and a positive margin was observed in 11 patients. Surgical excision of the metastatic tumors was performed 35 times in 20 patients (19 lymph node resections, 8 pulmonary resections, and 8 other resections).

Fifty-six patients (75%) received chemotherapy. Thirty patients received chemotherapy with measureable disease, including 27 patients with measurable metastatic disease and 3 patients with advanced locoregional disease. Twenty-nine patients received chemotherapy as adjuvant treatment before or after excision of the primary tumor. Various chemotherapeutic regimens for musculoskeletal sarcomas were employed, including doxorubicin as a single agent (60–90 mg/m^2); combinations of doxorubicin and ifosfamide (doxorubicin 60 mg/m^2 and ifosfamide 5–9 g/m^2) or doxorubicin and cisplatin (doxorubicin 60 mg/m^2, cisplatin 120 mg/m^2); combined sodium mercaptoethanesulfonate (mesna) (10 g/m^2), doxorubicin (60 mg/m^2), ifosfamide (7.5 g/m^2), and dacarbazine (900 mg/m^2); and

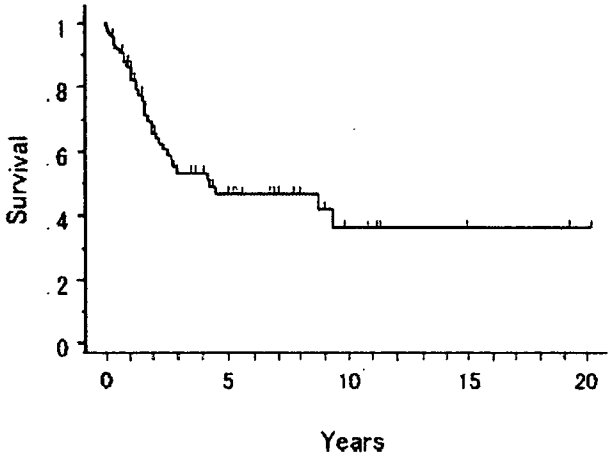

FIGURE 1. Overall survival of 75 patients with clear cell sarcoma.

combined cyclophosphamide (500 mg/m^2), vincristine (1.5 mg/m^2), doxorubicin (50 mg/m^2), and dacarbazine (1200 mg/m^2). Regimens for malignant melanomas also were employed, including combined dacarbazine (500 mg/m^2), nimustine (60 mg/m^2), and vincristine (1.5 mg/m^2); and interferon-α (5 × 10^6 U/m^2 per day subcutaneously for 5 days).[22–24] Caffeine-assisted chemotherapy using cisplatin and doxorubicin was received by 7 patients.[25,26] Three to five courses of cisplatin (120 mg/m^2), doxorubicin (30 mg/m^2 per day for 2 days), and caffeine (1.5 g/m^2 per day for 3 days) were administered to 4 patients with measurable disease and to 3 patients without macroscopic disease. The most common chemotherapeutic agents used in the regimens were doxorubicin (n = 48), ifosfamide (n = 27), and cisplatin (n = 25).

Radiotherapy (external-beam irradiation with megavoltage beams) was received by 17 patients (preoperatively in 6 patients, postoperatively in 11 patients). The median radiotherapy dose was 45 grays (Gy) (33 Gy for preoperative irradiation, 60 Gy for postoperative irradiation).

Treatment Outcome

Follow-up ranged from 2 months to 243 months (average, 44 months). Metastatic disease developed in 52 patients; and local recurrence developed in 16 patients, including 3 patients who developed metastasis earlier than local recurrence, 3 patients who simultaneously developed local recurrence and metastasis, and 7 patients who developed metastasis after local recurrence. The site of the first metastasis was the lymph nodes in 24 patients, lung in 15 patients, bone in 3 patients, and multiple regions in 10 patients. Development of lymph node metastasis, which was the most frequent pattern of the first recurrence of the disease, generally preceded wide dissemination of the disease. At the time of the current analysis, 34 patients had died as a result of disseminated metastatic disease. The median time to metastasis, local recurrence, and cancer-specific death was 13 months, 19 months, and 20 months, respectively.

TABLE 2
Analyses of Overall Survival (All Patients)

Variable	5-year survival	Univariate *P*	Cox *P*	RR	95% CI
Sex					
Men	31				
Women	65	.018			
Age					
≤30 y	51				
>30 y	45	.589			
Location					
Proximal	39				
Distal	51	.281			
Depth					
Superficial	80				
Deep	29	.002			
Size					
≤5 cm	64				
>5 cm	19	.001	.021	5.65	1.29–24.71
Symptom					
Pain (negative)	43				
Pain (positive)	56	.574			
TNM stage					
II	72				
III	24				
IV	25	.001			
Microscopic margin					
Negative	47				
Positive	20	.042			
Chemotherapy					
Yes	53				
No	20	.180			
Radiotherapy					
Yes	35				
No	50	.060			

RR indicates relative risk; 95% CI, 95% confidence interval.

The overall survival rate was 47% at 5 years and 36% at 10 years (Fig. 1). Univariate analysis showed that sex (P = .018), tumor size (P = .001), tumor depth (P = .002), TNM classification (P = .001), and surgical margin (P = .042) were significant prognostic factors for survival (Table 2). Other tumor- or therapy-related variables (including identification of the EWS/ATF1 gene or t[12;22] translocation) were identified as not significant. On multivariate analysis, only tumor size (P = .021) emerged as a significant prognostic factor.

Of the 52 patients who presented with localized disease, the overall survival rate was 55% at 5 years and 41% at 10 years. Univariate analysis showed that

TABLE 3
Analyses of Overall Survival (Localized Disease)

Variable	5-year survival	Univariate *P*	Cox *P*	RR	95% CI
Sex					
Men	36				
Women	73	.023			
Age					
≤30 y	59				
>30 y	54	.913			
Location					
Proximal	41				
Distal	63	.183			
Depth					
Superficial	90				
Deep	39	.011			
Size					
≤5 cm	71				
>5 cm	28	.002	.026	3.76	1.16–12.17
Symptom					
Pain (negative)	51				
Pain (positive)	64	.606			
TNM stage					
II	72				
III	24	.004			
Microscopic margin					
Negative	59				
Positive	33	.539			
Chemotherapy					
Yes	66				
No	22	.032			
Radiotherapy					
Yes	51				
No	56	.248			

RR indicates relative risk; 95% CI, 95% confidence interval.

sex ($P = .023$), tumor size ($P = .002$), tumor depth ($P = .011$), TNM classification ($P = .004$), and chemotherapy ($P = .032$) were significant prognostic factors in this group of patients (Table 3). Women had a significantly better prognosis than men. Patients who had tumors in deep sites and patients with tumors >5 cm had a significantly worse prognosis compared with other patients (Fig. 2). Patients who received adjuvant chemotherapy appeared to have a better outcome than patients who did not receive chemotherapy. On multivariate analysis, only tumor size ($P = .026$) remained a significant prognostic factor.

The cumulative probabilities of recurrence at 5 years and at 10 years were 22.3% for local recurrence (recurrence) compared with 61.0% and 68.5% for distant recurrence (metastasis), respectively (Fig. 3). The cumulative risk for metastasis was significantly greater than that for local recurrence ($P = .004$).

Response to chemotherapy was evaluable in 30 patients who had measurable disease. Objective tumor regression (partial response) was observed in 7 patients (23.3%: 95% confidence interval, 8.2–38.5%). Seventeen patients had stable disease, and 6 patients had progressive disease. Regimens that achieved an objective response included caffeine-assisted chemotherapy using doxorubicin and cisplatin in 3 patients, doxorubicin combined with cisplatin in 2 patients, and a combination of doxorubicin, cisplatin, and ifosfamide in 2 patients.

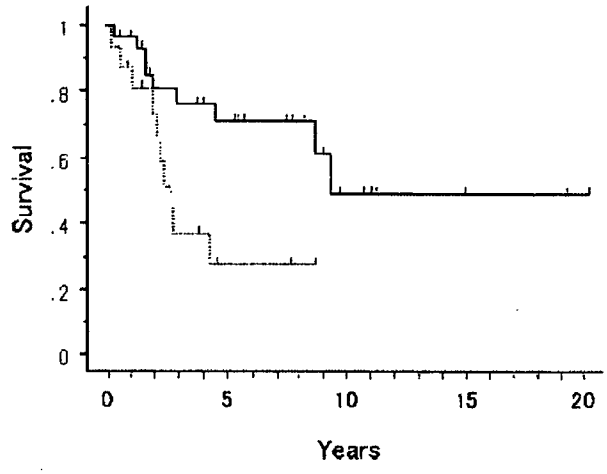

FIGURE 2. Overall survival of 52 patients who presented without metastatic disease according to tumor size ($P = .002$). Solid line indicates tumors ≤5 cm; dotted line, tumors >5 cm.

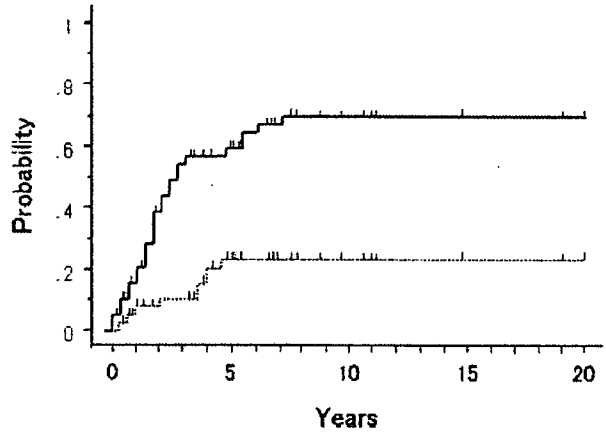

FIGURE 3. Cumulative probabilities of recurrence are illustrated according to types of failure ($P = .004$). Solid line indicates metastasis; dotted line, local recurrence.

DISCUSSION

CCS is a rare melanocytic tumor that accounts for 1% of all soft tissue sarcomas.[1,2] The rarity of the disease makes it difficult to draw conclusive statements

regarding its clinical behavior, prognostic factors, and ideal treatment. To our knowledge, the current study represents the largest reported series of patients with CCS treated during the era of modern multidisciplinary treatment. The results confirmed the known clinical features of CCS originally reported by Enzinger: arising primarily in young adults, predominance in distal extremities, and presenting as a slowly enlarging mass that has been evident for several months or even years.[1] Our findings also revealed several important factors that predict the clinical behavior of CCS and disclosed problems awaiting solution.

In our series, the 5-year survival rate was 47%, and the 10-year survival was only 36%. Survival data from other studies also suggest an unsatisfactory outcome, generally with a 5-year survival rate <50%. Lucas et al. reported a 5-year survival rate of 67%, but the rate decreased to 33% at 10 years.[10]

Development of late metastases after a long disease-free interval was not rare in patients with CCS. The pattern of metastasis, with a preference for lymph node metastasis compared with pulmonary metastasis, also was a unique feature. The finding that a substantial percentage of patients develop initial extrapulmonary metastasis has implications for the staging workup and subsequent monitoring for this specific subtype of sarcoma. In addition to the regular check-ups for pulmonary metastasis, close workups and monitoring of lymph node, abdomen/pelvis, and skeletal metastasis using CT, MRI, bone scan, and possibly positron emission tomography are recommended for patients with CCS.

The role of regional lymph node dissection in CCS has not been established to date. Some authors recommend prophylactic elective lymph node dissection, whereas others suggest lymphadenectomy only in patients who show clinical lymphadenopathy.[11,14] Consistent lymph node dissection was not performed in the current study, because it was a retrospective case series. Sentinel lymph node biopsy, which has been used successfully for the staging of patients with breast cancer and melanoma, may allow early detection of lymph node metastasis in patients with CCS.[27,28] It is quite possible that, with diligent staging using sentinel node biopsy, some patients who are believed to have localized disease in the current series will turn out not to have localized disease, and this would have a significant impact on the analyses. Further investigations of the usefulness of sentinel lymph node biopsy in CCS are warranted.

There is little information available in the literature with regard to prognostic factors in CCS. The only factors that have been associated significantly with survival are tumor size, site, the presence of necrosis, mitotic rate, DNA content, complete surgical resection, and perhaps adjuvant radiotherapy.[8–15] In our study, we observed that tumor size, tumor depth, sex, TNM classification, and surgical margin had prognostic value for patients with CCS in a univariate analysis. In a multivariate analysis, tumor size emerged as the only significant variable associated with survival.

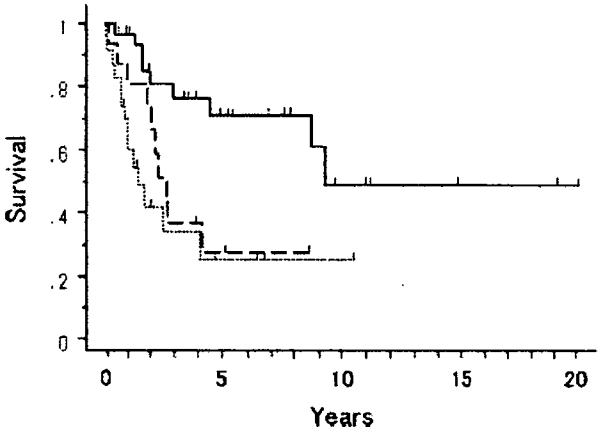

FIGURE 4. Overall survival of patients who had stage IIA tumors (solid line) compared with overall survival of patients who had stage IIB or III tumors (dashed line) and patients who had stage IV tumors (dotted line; $P = .002$).

Patients with CCS tumors >5 cm had a significantly worse prognosis than patients with smaller tumors. Considering the types of failure, tumors >5 cm had a significantly higher rate of metastases than smaller tumors (79% vs 48%, respectively; $P = .012$), whereas the rate of local recurrence did not differ significantly (13% vs 29%, respectively; $P = .287$). Figure 4 shows the survival curve of patients with stage IIA tumors (no metastasis, ≤5 cm) compared with the survival curve of patients with stage IIB or III tumors (no metastasis, >5 cm) and with stage IV tumors (with metastasis). The survival curve for patients with stage IIA tumors stands apart considerably from the curves for patients with tumors at other stages. Patients who had nonmetastatic tumors >5 cm had slightly better survival up to 2 years compared with patients who had metastatic tumors, but the 2 survival curves became equivalent after 3 years ($P = .318$). These results indicate that it probably should be assumed that patients with CCS tumors >5 cm have micrometastasis at the time of presentation, even if no findings of metastatic disease are evident at the time. These patients have a great propensity to develop macroscopic metastases within a few years, and they have a grim prognosis. Thus, stratification based on tumor size (≤5 cm or >5 cm) may help to identify patients with CCS at greater risk of metastases who may benefit from adjuvant therapy.

However, it is disappointing that the traditional doxorubicin-based chemotherapy used for other non-small round cell soft tissue sarcomas has not been effective for patients with CCS.[12–14] Deenik et al. observed that chemotherapy like doxorubicin, doxorubicin combined with ifosfamide, or doxorubicin combined with dacarbazine did not have any appreciably beneficial effect in CCS.[12] Finley et al. reported that multiagent chemotherapy (doxorubicin, vincristine, cyclophosphamide, dacarbazine, and ifosfamide) administered to 6 patients with CCS had no impact on outcome.[13]

There are only isolated reports of CCS responding to chemotherapy. In 1 such report, a patient with advanced CCS showed a marked response to a combination of vincristine and bleomycin.[29] In another report, a patient with recurrent CCS achieved complete remission that lasted for 17 months after intralesional administration of interferon-α.[30] Although these cases represented a unique and marked response of CCS to these agents, few studies have demonstrated similar results.

In the current series, we observed an objective tumor response to chemotherapy in 23% (7 of 30) of patients with advanced disease. Moreover, patients who received adjuvant chemotherapy appeared to have a better outcome than patients who did not receive chemotherapy. Although the treatment regimens were too variable for statistical analysis, these findings indicate that CCS has a certain degree of chemosensitivity.

It is noteworthy that all chemotherapeutic regimens that elicited a response included cisplatin. Cisplatin has significant activity against pediatric and bone sarcomas, but its reported single-agent response rate in adult soft tissue sarcomas was <20%.[31–34] There is no information available in the literature about the effect of cisplatin on CCS. It also is noteworthy that patients who received caffeine-assisted chemotherapy with doxorubicin and cisplatin showed a marked tumor response. Three of 4 patients with measurable CCS who had received caffeine-assisted chemotherapy showed objective regression of the tumor. Caffeine, which has an inhibitory effect on DNA repair, enhances the cytocidal effects of anticancer drugs and radiation. Tsuchiya et al recently reported an excellent local response to caffeine-assisted chemotherapy in various kinds of musculoskeletal sarcomas.[25,26]

Although our favorable results still require confirmation, the use of cisplatin with or without caffeine warrants further consideration in CCS, especially in patients with metastatic disease or in patients with tumors >5 cm, who have a high likelihood of developing metastases and a poor outcome. Prospective cooperative studies are needed to define the role of cisplatin and caffeine in the treatment of CCS and to explore new active agents for this rare malignant disease.

In conclusion, the overall prognosis for patients with CCS is poor mainly because of its high propensity for distant metastasis. Our current results indicate that tumor size is the most important predictive factor for both distant metastasis and survival. CCS tumors >5 cm are associated with a high metastatic rate; and, once metastasis has occurred, the prognosis is dismal. Early recognition of this disease and prompt wide excision of small tumors are essential for a favorable outcome. To improve the prognosis of patients who have CCS tumors >5 cm, the establishment of an effective chemotherapy regimen is necessary. In this regard, our finding that cisplatin and caffeine may exert an antineoplastic effect against CCS is of considerable interest. Prospective cooperative studies are warranted to define the role of such treatment and to establish the best therapeutic strategies for CCS.

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Dysadherin Expression as a Significant Prognostic Factor and as a Determinant of Histologic Features in Synovial Sarcoma: Special Reference to its Inverse Relationship With E-cadherin Expression

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Abstract: Dysadherin is a cancer-associated cell membrane glycoprotein, which down-regulates E-cadherin and promotes metastasis. Synovial sarcoma is a very rare mesenchymal tumor that exhibits an epithelial profile. To confirm the diagnosis of synovial sarcoma, we evaluated several immunohistochemical markers, or detected SYT-SSX fusion gene transcript. We studied the clinicopathologic features in 92 synovial sarcoma patients and also assessed the immunohistochemical expression of dysadherin and E-cadherin to examine their possible association with histologic subtype and biologic behavior. Moreover, among 30 patients, for whom frozen materials were available, *dysadherin* mRNA expression was examined by reverse transcription-polymerase chain reaction and real-time quantitative reverse transcription-polymerase chain reaction analysis. Dysadherin-positive expression was significantly correlated with E-cadherin–reduced expression ($P = 0.0004$). Dysadherin-positive immunostaining was diffusely observed in the membranes of tumor cells in 30/68 (44%) patients with monophasic fibrous type and in 1/2 (50%) patients with poorly differentiated type. However, in biphasic tumors, dysadherin expression in the fibrous component was not diffusely observed, but often sporadically or focally observed [20/22 (91%) patients]. In addition, *dysadherin* mRNA expression in monophasic fibrous type was significantly higher than in biphasic type ($P = 0.0079$). Synovial sarcoma patients with dysadherin expression survived for a significantly shorter time than those without dysadherin expression ($P = 0.0006$). Patients with combined dysadherin-positive expression and E-cadherin–reduced expression had a significantly worse prognosis than those with other combinations of dysadherin and E-cadherin expression ($P = 0.0007$). SYT-SSX fusion gene transcript was detected in 39 patients. In our series, SYT-SSX fusion type was found to have no correlation with histologic subtype, prognosis, or dysadherin expression. In multivariate analysis, dysadherin immunopositivity ($P = 0.0411$) was an independent adverse prognostic factor, in addition to a high MIB-1 labeling index ($\geq 10\%$). We conclude that E-cadherin dysfunction by dysadherin is associated with reduced E-cadherin expression and morphologic change from epithelioid to spindle phenotype. Dysadherin expression is considered to be one of the determinants of histologic subtype in synovial sarcoma. Moreover, dysadherin expression is an excellent and independent prognostic indicator.

Key Words: synovial sarcoma, dysadherin, E-cadherin, prognosis, histologic features

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Synovial sarcomas are known to have 2 major forms, monophasic fibrous type, in which the tumors are composed of spindle cells, and biphasic type, in which the tumors contain both epithelial cells arranged in a glandular structure and spindle cells. In an effort to find the possible determinants of the histologic subtype of synovial sarcoma, studies have focused on the chimeric fusion transcripts, SYT-SSX1 and SSX2, that are caused by a characteristic chromosomal translocation, t(X;18)(p11;q11).[16] It has been demonstrated that there is a significant relationship between histologic subtype and the type of fusion transcript, although the mechanisms involved in epithelial differentiation in synovial

sarcoma remain unclear.[1,16,19] Dysadherin is a cancer-associated cell membrane glycoprotein, which down-regulates E-cadherin by a posttranscriptional mechanism and promotes metastasis.[15] In the present study, we assessed the immunohistochemical expression of dysadherin and E-cadherin in synovial sarcoma, and we examined their possible association with histologic subtype and biologic behavior. In addition, we also examined *dysadherin* mRNA expression in synovial sarcoma, and compared its expression with dysadherin immunohistochemical expression and histologic features. The aim of this study was to evaluate the influence of dysadherin on the progression of synovial sarcoma, with special emphasis on its histologic subtype, and to clarify the importance of dysadherin in the biologic behavior of synovial sarcoma.

MATERIALS AND METHODS

Cases were selected from among more than 12,000 cases of bone and soft-tissue tumors registered in the Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan between 1955 and 2004. Because written informed consent had not been obtained, identifying information for all patients was removed before analysis for the purpose of strict privacy protection. There were 92 synovial sarcoma patients, suitable for immunohistochemical study. Histologic subtypes comprised 68 patients with monophasic fibrous type, 22 patients with biphasic type, and 2 patients with poorly differentiated synovial sarcoma. The monophasic fibrous type synovial sarcoma may resemble a number of other spindle cell neoplasms such as fibrosarcoma, leiomyosarcoma, malignant peripheral nerve sheath tumor, hemangiopericytoma, and spindle cell carcinoma. To confirm the diagnosis of synovial sarcoma, we evaluated several useful immunohistochemical markers such as cytokeratins (cytokeratin 7, cytokeratin 19, etc.), epithelial membrane antigen, S-100 protein, myogenic markers, bcl-2 protein and CD34,[26,27] or examined SYT-SSX fusion gene transcript. Frozen materials were available in 30 patients to detect SYT-SSX fusion gene transcript. Moreover, formalin-fixed paraffin-embedded materials were available to try detecting SYT-SSX fusion gene transcript in additional 43 patients. Clinical data for these 92 patients were collected from the medical records. Survival data were available for all 92 patients. Follow-up ranged from 1 to 278 months (mean, 56.2 mo). To assess the correlation between clinicopathologic factors and dysadherin expression, age, sex, the anatomic location of the tumor, tumor depth, size, glandularity (histologic subtype), mitotic rate, tumor necrosis, the presence of rhabdoid cells, SYT-SSX fusion type, FNCLCC (French Fédération Nationale des Centres de Lutte Contre le Cancer) grade and AJCC (American Joint Committee on Cancer) stage, were analyzed. The influence of these factors on prognosis has been previously reported.[26] As for histologic subtype, "presence of glandularity" implies biphasic type, whereas "absence of glandularity" includes monophasic fibrous type and poorly differentiated type. As for tumor depth, "superficial" tumor is located exclusively above the superficial fascia without invasion of the fascia, whereas "deep" tumor is located either exclusively beneath the superficial fascia, or superficial to the fascia with invasion of or through the fascia. As for tumor location, "distal" tumor occurs in the distal extremities (below knee or below elbow), whereas "proximal" tumor occurs in the proximal region of the extremities and in the trunk.

Immunohistochemistry

Immunohistochemical study was performed using mouse IgG monoclonal antibodies against dysadherin[15] (NCC-M53, 1:1000, National Cancer Center, Tokyo), E-cadherin (1:1000, BD Transduction Laboratories, San Jose, CA), and Ki-67 (MIB-1, 1:100, Dako, Grostrup, Denmark). Specimens are pretreated in an autoclave for dysadherin (20 min, 120°C) or in a microwave oven for E-cadherin and MIB-1 (20 min for both antibodies). Sections were incubated with the primary antibodies at 4°C overnight, followed by staining with a streptavidin-biotin-peroxidase kit (Nichirei, Tokyo, Japan). Staining for dysadherin and E-cadherin was localized within the cell membranes. The pattern of dysadherin immunostaining in tumors was compared with that observed in the basal cells of normal skin tissue, endothelial cells and lymphocytes. Dysadherin immunoreactivity was evaluated in the fibrous component that was common to the 3 histologic subtypes, even in the biphasic type containing glandular components. Tumors were classified as dysadherin-positive when more than 50% of the cells in the fibrous component were stained.[2,24,34,35] Moreover, the proportion of dysadherin positive cells was evaluated semiquantitatively and classified into 4 groups: 0% to 9% (1+; sporadic), 10% to 29% (2+; focal), 30% to 49% (3+), and ≥ 50% (4+; diffuse). E-cadherin immunoreactivity has been described as preserved (positive) when more than 90% of the cells were stained within the membrane of the tumor cells.[29] The proportion of E-cadherin positive cells was also evaluated semiquantitatively and classified into 4 groups: 0% to 49% (1+), 50% to 69% (2+), 70% to 89% (3+), and ≥ 90% (4+). In biphasic types of synovial sarcoma, E-cadherin immunoreactivities were evaluated in both the fibrous and the glandular components, and if at least one component was described as preserved (positive), then that case was described as preserved (positive).[29] The MIB-1–labeling index was estimated as the percentage of Ki-67–positive cells based on a count of 1000 tumor cells within the tumor. Dysadherin and E-cadherin expression were evaluated independently by 3 of the authors (T.I., Y.O., and T.H.) without any knowledge of the clinical features in each case.

RT-PCR and Real-time Quantitative RT-PCR to Detect *Dysadherin* mRNA

Frozen materials were also available for 30 patients with synovial sarcoma (20 patients with monophasic

fibrous type, 8 patients with biphasic type, and 2 patients with poorly differentiated type). Two synovial sarcoma cell lines (HS-SY-II,[39] and SYO-1[17]) were generously provided by the coauthors for this study. HS-SY-II was established from monophasic fibrous type, whereas SYO-1 was established from biphasic type synovial sarcoma. Total RNA was prepared, using Trizol Reagent (Invitrogen Corp, Carlsbad, CA) according to the manufacturer's protocol. Five micrograms of RNA in each sample were used for the subsequent reverse transcription. Sequences of specific pairs of primers were as follows: *dysadherin* (upper primer: 5'-AGA GCA CCA AAG CAG CTC AT-3'; lower primer: 5'-GGG TCT GTC TGG ACG TCT GT-3'; product size, 85 bp). The PCR products were electrophoresed in 2.0% agarose gel and visualized with ethidium bromide. cDNA from the Li-7 human hepatoma cell line was used as a positive control for *dysadherin* expression, and PLC/PRF/5 was used as a negative control.[15]

Real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR) (TaqMan PCR) for *dysadherin* was performed using an ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA) and predeveloped TaqMan assay reagents of human *dysadherin* and *GAPDH*. The PCR reaction was carried out according to the manufacturer's protocol.[13] The standard curve was constructed with serial dilutions of the cDNA samples of Li-7. All reactions of the samples were triplicated and the data were averaged from the values obtained in each reaction. To determine the mRNA levels of *dysadherin*, we used an mRNA expression index (EI), which is a relative mRNA expression level standardized by using the internal housekeeping gene, *GAPDH*. The *dysadherin* mRNA EI was calculated as follows [in arbitrary units (AU)]: mRNA EI = (copy numbers of *dysadherin* mRNA/copy numbers of *GAPDH* mRNA) × 1000 AU.

Statistical Analysis

The survival curves were analyzed by the Kaplan-Meier method and the outcome of the different groups of patients was compared by the log-rank test. The Cox proportional hazards regression with the stepwise procedure was used in multivariate analysis of survival data. The correlations between dysadherin and E-cadherin immunoreactivity and glandularity were evaluated using the Mann-Whitney *U* test. The correlation between dysadherin and E-cadherin expression was evaluated using Kendall rank correlation coefficient. Fisher exact test was used to evaluate the association between 2 dichotomous variables. The correlations between each group and *dysadherin* mRNA expression were determined by using the Mann-Whitney *U* test. Probability values of less than 0.05 were considered as significant.

RESULTS

The clinicopathologic findings of 92 patients with synovial sarcoma are summarized in Table 1. Sixty patients were affected at the proximal extremities or trunk, whereas 32 patients had a tumor at the distal extremities. Eighty-one tumors were deeply situated, and 11 were superficially situated. Histologically, 22 patients were categorized as presence of glandularity (biphasic type) and 70 patients as absence of glandularity (monophasic fibrous type and poorly differentiated type). Rhabdoid cells were detected in 9 patients, but were absent in 83 patients. Among the 30 patients, for whom frozen materials were available, 20 patients demonstrated SYT-SSX1 fusion type, whereas 10 patients demonstrated SYT-SSX2 fusion type. Among the 43 patients, for whom only formalin-fixed paraffin-embedded materials were available, high quality and quantity of total RNA suitable for RT-PCR analysis could be extracted in 15 patients. Fusion type was detected in 9 out of these 15 patients. Eight patients demonstrated SYT-SSX1 fusion type, whereas 1 patient demonstrated SYT-SSX2 fusion type.

Immunohistochemical Findings

The results of immunohistochemical study for dysadherin and E-cadherin are summarized in Table 2. As for glandularity, there was a strong relationship between higher dysadherin expression and absence of glandularity ($P = 0.0002$), and a strong relationship between higher E-cadherin expression and presence of glandularity ($P < 0.0001$). Dysadherin-positive staining was diffusely ($\geq 50\%$) and strongly observed in the membranes of tumor cells in 30/68 (44%) patients with monophasic fibrous type (Fig. 1B) and in 1/2 (50%) patients with poorly differentiated type. E-cadherin membranous expression was reduced (negative) in 56/68 (82%) patients with monophasic fibrous type (Fig. 1C) or in 2/2 (100%) patients with poorly differentiated type. In biphasic tumors, dysadherin expression in the fibrous component was not diffusely ($\geq 50\%$) observed, but often sporadically (0% to 9%) or focally (10% to 29%) observed [20/22 (91%) patients]

TABLE 1. Clinicopathologic Parameters in 92 Cases of Synovial Sarcoma

Age (y)		Sex		Location		Depth		Size (cm)		Glandularity	
≤20	19	Male	35	Proximal	60	Deep	81	≤5	35	Present	22
>20	73	Female	57	Distal	32	Superficial	11	>5	57	Absent	70
Mitotic Rate (per 10 HPFs)		**Tumor Necrosis (%)**		**Rhabdoid Cells**		**Chimera Gene (39 Patients)**		**FNCLCC Grade**		**AJCC Stage**	
≤15	59	<50	74	(+)	9	SYT-SSX 1	28	Grade 2	41	Stage II	37
>15	33	≥50	18	(−)	83	SYT-SSX 2	11	Grade 3	51	Stage III	42
										Stage IV	13

TABLE 2. Immunohistochemical Results for Dysadherin and E-cadherin

	Histologic Subtype	
	Glandularity (Absent) (n = 70)	Glandularity (Present) (n = 22)
	Monophasic (n = 68) and Poorly (n = 2)	Biphasic (n = 22)
Dysadherin		
≥ 50% (4+) (diffuse)	31 (1)*	0
30%-49% (3+)	7	2
10%-29% (2+) (focal)	14 (1)*	8
0%-9% (1+) (sporadic)	18	12
E-cadherin		
≥ 90% (4+)	12	20
70%-89% (3+)	4	1
50%-69% (2+)	12	0
0%-49% (1+)	42 (2)*	1

$P = 0.0002$ by Mann-Whitney U test [between dysadherin expression and glandularity (histologic subtype)].
$P < 0.0001$ by Mann-Whitney U test [between E-cadherin expression and glandularity (histologic subtype)].
*Poorly differentiated type.

(Fig. 1E) (Table 2). However, in the glandular component, dysadherin and E-cadherin were frequently coexpressed in 20/22 (91%) patients (Figs. 1E, F). The correlations between dysadherin immunoreactivity and E-cadherin immunoreactivity (inverse relationship) are summarized in Table 3. Kendall τ value was calculated to be −0.339 ($P < 0.0001$, by Kendall rank correlation coefficient), indicating a significant negative correlation between dysadherin expression and E-cadherin expression. The correlations between dysadherin and E-cadherin immunoreactivity and clinicopathologic parameters are summarized in Table 4. There was a strong relationship between absence of glandularity and dysadherin-positive expression ($P < 0.0001$), and a strong relationship between presence of glandularity and E-cadherin—preserved (positive) expression ($P < 0.0001$). Dysadherin-positive expression was also significantly correlated with a high age (> 20 y), a large tumor size (> 5 cm), and a high MIB-1 labeling index (≥ 10%). E-cadherin–preserved (positive) expression was significantly correlated with a low mitotic rate (≤ 15 per 10 HPFs), a low MIB-1 labeling index (< 10%) and a low FNCLCC grade (grade 2). Other parameters including the chimera gene (SYT-SSX fusion type) had no statistically significant correlation with dysadherin or E-cadherin expression.

Dysadherin mRNA Expression by RT-PCR and Real-time Quantitative RT-PCR

The specificity of RT-PCR was confirmed and visualized by the presence of *dysadherin* mRNA in the Li-7 cell line and the absence of *dysadherin* mRNA in the PLC/PRF/5 cell line (Fig. 2). *Dysadherin* mRNA expression by RT-PCR in synovial sarcoma was observed in all 3 histologic subtypes, varying in density (Fig. 2). Monophasic fibrous type tumors showed a higher *dysadherin* mRNA expression compared with biphasic type tumors.

The comparison between histologic subtypes and *dysadherin* mRNA EIs by real-time quantitative RT-PCR is summarized in Figure 3. *Dysadherin* mRNA EIs in monophasic fibrous type (median, 568.25 AU) were significantly higher than those in biphasic type (median, 56.07 AU) ($P = 0.0079$). In addition, the HS-SY-II cell line established from monophasic fibrous type revealed a higher *dysadherin* mRNA EI (286.10 AU) compared with the SYO-1 cell line established from biphasic type (60.86 AU). A comparison between dysadherin immunoreactivity and *dysadherin* mRNA EIs is summarized in Figure 4. Parallel to the immunohistochemical results, dysadherin-positive patients showed statistically significant higher *dysadherin* mRNA EIs (median, 912.32 AU) compared with those seen in dysadherin-negative cases (median, 81.66 AU) ($P = 0.0001$).

Prognostic Value of Dysadherin Expression

Patients with dysadherin immunoreactivity survived for a significantly shorter time than those without dysadherin immunoreactivity ($P = 0.0006$; Fig. 5). Patients with diffuse dysadherin immunoreactivity (4+; ≥ 50%) had the worst survival, whereas patients with sporadic dysadherin immunoreactivity (1+; 0% to 9%) had the best survival ($P = 0.0068$; Fig. 6). The greater the proportion of dysadherin positive cells, the worse the prognosis turned out to be. Patients with combined dysadherin-positive expression and E-cadherin–reduced (negative) expression had a significantly worse prognosis than those with other combinations of dysadherin and E-cadherin expression ($P = 0.0007$; Fig. 7). In univariate analysis, dysadherin-positive expression, E-cadherin–reduced (negative) expression, large tumor size (> 5 cm), absence of glandularity, massive tumor necrosis (≥ 50%), the presence of rhabdoid cells, a high MIB-1 labeling index (≥ 10%), a high FNCLCC grade (grade 3), and a high AJCC stage (stages III and IV) were all significantly correlated to a worse overall survival rate (Table 5). Multivariate analysis revealed that dysadherin immunopositivity ($P = 0.0411$) and a high MIB-1 labeling index (≥ 10%) ($P < 0.0001$) were independent poor prognostic

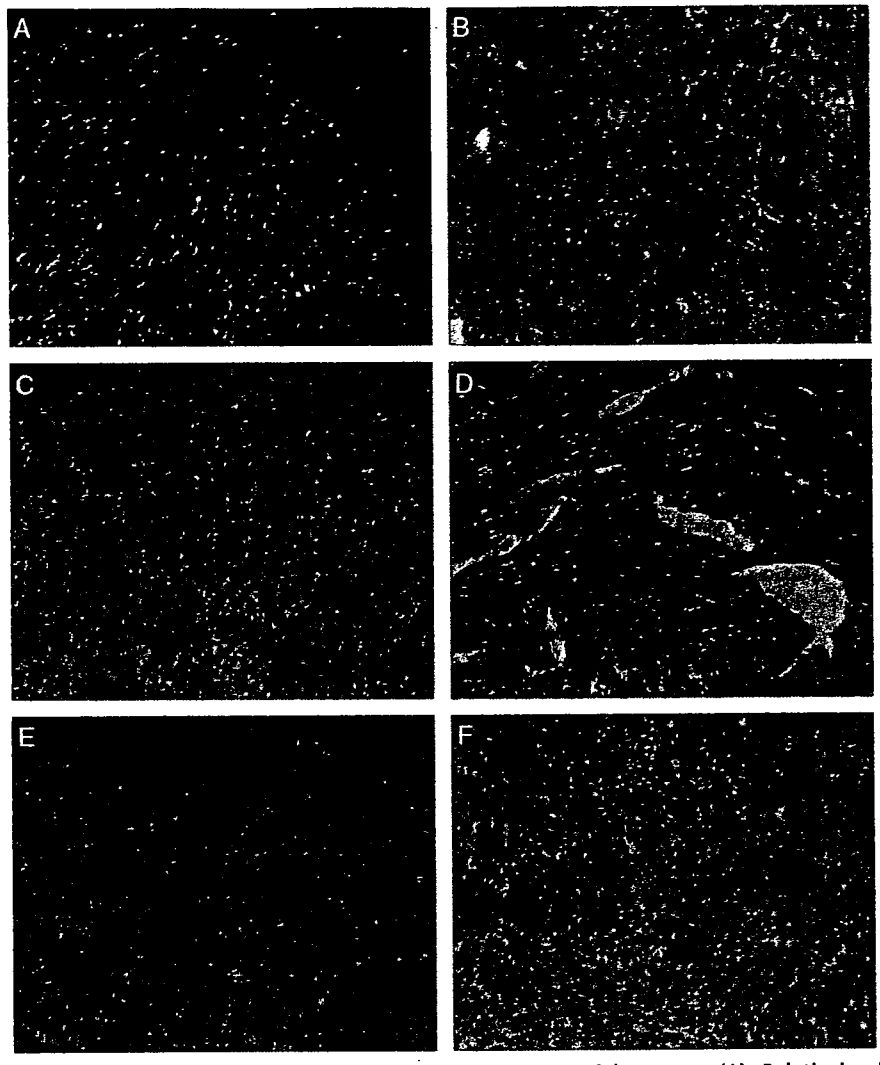

FIGURE 1. The hematoxylin and eosin staining of a monophasic fibrous synovial sarcoma (A). Relatively plump spindle or small ovoid tumor cells arranged in fascicles (A). In monophasic fibrous type, dysadherin-positive staining was diffusely ($\geq$ 50% positive cells) observed in the membranes of tumor cells in 30/68 (44%) patients (B). E-cadherin membranous expression was reduced (negative) in 56/68 (82%) patients and aberrational nuclear staining for E-cadherin was often detected (C). The hematoxylin and eosin staining of a biphasic synovial sarcoma (D). The fibrous component composed by plump spindle or small ovoid cells admixed with variably sized epithelioid glandular component (D). In biphasic type, dysadherin expression in the fibrous component was not diffusely ($\geq$ 50% positive cells) observed [0/22 (0%) patients], but often sporadically (0% to 9% positive cells) or focally (10% to 29% positive cells) observed [20/22 (91%) patients] (E). However, in the glandular component, dysadherin (E) and E-cadherin (F) were frequently coexpressed in 20/22 (91%) patients.

factors (Table 5). Other clinicopathologic prognostic factors were not significant.

DISCUSSION

Dysadherin is a cancer-associated cell membrane glycoprotein, which down-regulates E-cadherin by a posttranscriptional mechanism and promotes metastasis.[15] Dysadherin is expressed in a variety of cancer cells and malignant melanoma cells,[2,15,24,25,34,35] however, it has never been reported in the field of sarcomas.

Synovial sarcoma is a mesenchymal tumor that has an epithelial profile, with cell shapes varying from epithelioid to spindle phenotype. Recent reports have demonstrated E-cadherin expression in sarcomas with epithelioid features, such as synovial sarcoma, especially in the glandular structures of biphasic type.[20,29,32,44] A reduced E-cadherin expression by genetic and epigenetic alterations in the E-cadherin gene has been shown to cause cellular morphologic changes in epithelial cells, from epithelioid features to a more spindle phenotype.[3,6,42] Initially, we identified mutational inactivation