コケイン症候群（CS）の集い2010

10月30日（土曜）；医療従事者向け（医師、看護師、介護師、理学療法士など）

司会；森脇　真一（大阪医科大学感覚器機能形態医学講座皮膚科学）

13時　日本ロレアル挨拶

13時5分　CS ワークショック開始（座長；森脇、林先生）　発表時間は討論込み

13時5分～13時10分　挨拶、「CS オーバービュー」

　森脇　真一　大阪医科大学感覚器機能形態医学講座皮膚科学

13時10分～13時40分　「CS の疫学と臨床」

　星野　英紀　先生　国立成育医療研究センター神経内科

13時40分～14時10分　「CS の遺伝と分子細胞診断」

　森脇　真一　大阪医科大学感覚器機能形態医学講座皮膚科学

14時10分～14時40分　「CS 研究　最近の進歩」

　林　雅晴　先生　東京都神経科学総合研究所神経発達・再生（臨床神経病理）

14時40分～15時10分　「CS モデルマウスの解析」

　中根　裕信先生　鳥取大学医学部機能形態統御学講座ゲノム形態学分野

（コーヒーブレイク）

司会；森脇

15時30分～16時　「CS 家族会の活動と歩み」

　CS家族会　土屋代表

16時～16時15分　「CS 遮光対策と本セミナー総括」

　森脇　真一　大阪医科大学感覚器機能形態医学講座皮膚科学

16時15分　日本ロレアル挨拶

16時20分～　18時　ケイタリングでの簡易懇親会

　当日参加医師、CS 患者家族、日本ロレアル社員様、関係者など

10月31日(日曜);一般市民向け

司会;西谷　かつ江(大阪医科大学感覚器機能形態医学講座皮膚科学、医療コーディネーター)

10時　日本ロレアル挨拶

10時5分～10時30分 「CS 患者さんとともに20年」(患者 DVD 上映含む)

森脇　真一　大阪医科大学感覚器機能形態医学講座皮膚科学

10時30分～10時55分 「CS 患者さんのケアと他国 CS 家族会の現況」

杉田　克生　先生　千葉大学教育学部養護教育学基礎医科学部門

(コーヒーブレイク)

11時10分～11時30分 「日焼け止めの使い方」(できればデモ付きで)

日本ロレアルのご担当者様

11時30分～11時50分 「CS 家族会の活動と歩み」

土屋代表

11時50分～12時30分 「CS 患児とともに」「患児の思い出」(仮題)

CS 患者保護者　数名

12時30分～　　簡易懇親会(サンドイッチ、ジュース、お茶など)

パンフレット配布、署名活動(ブース)

日本ロレアル　日焼け止め展示(ブース)

CS 患者想い出の品・写真など展示閲覧、ポスター展示(家族会の歩みなど)

CS 患者さんと市民との触れ合い

CS 患者;ボランティアが面倒みる

患児母親;エステ体験、化粧体験など(13時～14時30分)

15時　終了

懇親会場にて挨拶　(森脇、日本ロレアルご担当者様)

[V] 班員名簿

コケイン症候群の病態解明および治療とケアの指針作成のための研究班

区　　分	氏　　名	所　　属　　等	職　　名
研究代表者	久保田雅也	国立成育医療研究センター神経内科	医長
研究分担者	杉田　克生	千葉大学教育学部養護教育学基礎医科学部門	教授
	林　雅晴	財団法人東京都医学研究機構東京都神経科学総合研究所	副参事研究員
	森脇　真一	大阪医科大学感覚器機能形態医学講座皮膚科学	教授
	中根　裕信	鳥取大学医学部機能形態統御学講座ゲノム形態学分野	助教
	立石　智	熊本大学 発生医学研究所 発生制御部門	講師
	田沼　直之	都立府中療育センター小児科	医長
	熊田　聡子	都立神経病院神経小児科	医長
	星野　英紀	国立成育医療研究センター神経内科	医員
研究協力者	柏井　洋文	国立成育医療研究センター神経内科	フェロー
	奥村さやか	国立成育医療研究センター神経内科	フェロー
	寺嶋　宙	国立成育医療研究センター神経内科	フェロー
	小俣　卓	千葉県子ども病院神経科	医長
	杉田記代子	東京歯科大学市川総合病院小児科	非常勤医師
	喜多　和子	千葉大学大学院医学研究院環境生化学講座	講師

疾患における発達

Cockayne 症候群の運動発達

古山晶子，久保田雅也

- Cockayne 症候群（CS）は常染色体劣性遺伝疾患であり，頻度は 100 万人に 1 人程度とまれな疾患である．
- 近年，DNA 修復機構の一つであるヌクレオチド除去修復異常，とくに転写と共役した DNA 修復の異常であることが明らかになっている．
- 臨床的には早老，光線過敏，低身長，精神運動発達遅滞，小脳失調，網膜色素変性など多彩な症状を呈し，精神運動発達遅滞は必発である．
- 従来，発症年齢と症状の重篤度により，古典型（1 型）と重症型（2 型）とに分けられていたが，近年，より軽症で発症年齢の遅い 3 型の報告がなされている[1,2]．

CS 病型

- CS の 3 病型を ❶ にまとめる．
- 運動発達の経過でみると CS の 3 病型の違いは明らかである．
- 大脳白質および皮質，基底核，小脳，末梢神経が多系統に障害される CS の運動発達を単一の系統の病態で説明することは不可能である．
- 錐体路を含む大脳白質の障害による痙性，および末梢神経障害による筋萎縮，筋力低下，変形拘縮，感覚性失調が複合して歩行機能が低下，さらに小脳病変が関与した運動のタイミングの拙劣さ，基底核が関与した筋緊張制御の異常がこれを修飾悪化させると考えられる．
- 小脳萎縮や基底核の石灰化は高度であるが病型による差はほとんどない．
- CS の経過は停滞ではなく，まさに退行である．
- 錐体路を含む大脳白質および末梢神経障害の強度と分布，経時的変化の速度が 3 病型を決定するのであろう．

CS1 型の運動発達

- 新生児期，乳児期早期には特記すべき発達遅滞を認めない*1．

*1
初期には特異的な顔貌や症状が明らかでなく，脳性麻痺や原因不明の発達遅滞と診断されている例もある．

*2
歩行は前述した歩容異常が徐々に増悪し，これに足の変形が加わり，歩行困難となる．

*3
平成 21 年度厚生労働科学研究費補助金（難治性疾患克服研究事業）「コケイン症候群の実態把握および治療とケアの指針作成のための研究」の全国実態調査中間集計．

❶ CS の病型

1 型	2 型	3 型
• CS 全体の 80％を占める．出生前の成長は正常で，生下時体重，身長，頭囲は正常である． • 2 歳までに発達遅滞が明らかになり，体重，身長，頭囲は正常の 5 パーセンタイルをはるかに下回る． • 視力，聴力といった中枢神経性の障害とともに末梢神経の障害や小脳症状も引き起こす． • 多くは 10～20 歳代で死亡する．	• 先天性 CS とも呼ばれる． • 出生時にすでに成長障害を認めており，生下時より低身長，小頭を呈する． • 先天性の白内障や眼の構造異常を認め，早期より脊柱側彎，関節拘縮を認める． • 生後の神経学的発達がほとんどなく，多くは 7 歳ころまでに死亡する．	• 上記 2 型に比して発症年齢は遅く，臨床症状も軽度であることが多い． • 成人例の報告もあるが，症例数は少ない．

- 遅いながらも独歩までは獲得するが，その後乳児期に発達の遅れを指摘されて受診に至ることが多い．
- 6 か月ころより小頭が明らかとなり，1～2 歳ころより痙性歩行，失調性歩行，歩行耐久性低下，転倒の増加が認められ，以降は進行性の運動発達遅滞を呈する．
- 二次運動ニューロン障害に基づく症状が前面に出ている時期は四肢末梢部に拘縮が見られる．
- やがて四肢体幹の痙性を背景に上下肢の屈筋群の拘縮が，体幹では一側の側屈拘縮や骨盤の回旋拘縮などが出現する．
- 運動は，走行，歩行[*2] など高度な動作から徐々に退行していく．

診断

- 診断基準を ❷ に示す．
- 多くは症状や顔貌で診断される．

❷ Cockayne 症候群　診断基準

大症状
低身長
精神発達遅滞
小頭症
小脳失調
網膜色素変性
感音性難聴
早発老化徴候
脳内石灰化
小症状
日光過敏性
亀背
関節拘縮
視神経萎縮
う歯
大きく冷たい手足
性腺機能低下

症例 1　Cockayne 症候群 1 型

11 歳，女児．9 歳の妹が同疾患と診断されている．満期産，3,438 g で出生．定頸 3 か月，坐位保持 8 か月，つかまり立ち 1 歳 2 か月，独歩 2 歳 10 か月，有意語 1 歳 5 か月と運動発達遅滞を認めた．10 m 程度の歩行は可能であったが，痙性が強く，失調様であった．3 歳で独歩不可能となり，ものをつかむときなどに四肢振戦が出現した．運動神経伝導速度（MCV）の上下肢での低下，CT での基底核石灰化を認め，CS と診断された．5 歳時に当院に紹介された．初診時，四肢は痙性で独歩不可能，上肢の運動失調あり．手をひかれての歩行は可能．視力，聴力障害はなし．顔貌は老人様ではなかった．身長 94 cm（−2.7 SD），体重 11.7 kg（−2.4 SD），頭囲 44 cm（−3.4 SD）．6 歳時下肢尖足，深部反射亢進，Babinski 反射陽性．経過中に難聴の進行あり，10 歳時より補聴器を使用．同時期に眼科では網膜色素変性，白内障の進行を指摘されている．疲れで経口摂取量が容易に減少するため，年に数回，入院や外来での点滴を要する．CS に特徴的な老人様顔貌も年齢とともに明らかになった．現在，有意語は消失し，眼振なし，下肢尖足拘縮で歩行不可，深部反射消失．

考察　1 型はいったん自力歩行可能な時期もしくは介助歩行可能な時期があり，その後徐々に退行していく．

最近の調査[*3] によると，CS1 型生存例 9 名（平均 18.3 歳，6～38 歳）中 8 名が歩行可能であった．そのうち 6 名が下肢深部反射亢進，3 名が足クローヌス陽性，2 名が Babinski 反射陽性，下肢深部反射が低下―消失した 1 名も足クローヌス，Babinski 反射は陽性であった．全例に下肢関節拘縮，尖足を認めた．側彎は 1 例のみ，ジストニア，ミオクローヌスなど不随意運動は全例で認めず．手指振戦は 3 例に認めた．

また CS1 型死亡例 12 名（平均 18.8 歳，14～26 歳）中 8 名が歩行可能であったが徐々に退行し，移動不能になる例が多かった．そのうち 6 名が下肢深部反射亢進，1 名が足クローヌス陽性，3 名が Babinski 反射陽性，関節拘縮は 11 例，側彎は 3 例に認めた．

より下流がすでに機能不全の場合，小脳や基底核の病態への関与の評価は困難であるが，少なくとも CS1 型は基底核の石灰化が強い段階でも歩行可能であり，錐体路を含む大脳白質および末梢神経障害が歩行障害の主要因であろう．

ABR の経時的変化でも中枢から末梢への病理（上部脳幹から聴神経）の進行が報告されている[3]．

MCV：motor nerve conduction velocity，CT：compucted tomography.

症例 2 Cockayne 症候群 2 型

在胎 41 週，出生時体重 2,472 g，身長 44 cm，頭囲 31 cm，頭位自然分娩にて出生．仮死なし．哺乳障害なし．定頸 4 か月，寝返り 5 か月，追視，音への反応あり．下肢，とくに足関節の緊張はやや亢進していた．10 か月時，寝返りで移動できた．深部反射は全般に低下．坐位保持不可．日ざしの強い日には眼の周囲，頬部に発赤ができた．

11 か月時，身長 61 cm（−4 SD），体重 5,695 g（−3.5 SD），頭囲 36 cm（−4 SD）．特徴的な顔貌，運動発達遅滞，光線過敏（頬部），難聴（ABR は両側Ⅲ波以降導出不良），末梢神経障害（正中神経伝導速度：MCV 32.1 m/s，SCV 26.9 m/s）から CS を強く疑われた．頭部 CT 上の石灰化は認めず（❷）．1 歳〜1 歳 6 か月時，手で支えての坐位，寝返りでの移動がスムーズになる．上肢の振戦あり．音に対する反応は遅い．下肢の rigidity が目立つ．

2 歳時両上肢を腹臥位でずって移動可能になる．呼ぶと振り向く．足関節の拘縮を認める．

2 歳 6 か月時，音への反応が悪くなる．60 dB の聴力損失．津守式で DQ37．バイバイがわかり，よく笑う．ABR 上Ⅱ波まで確認（❸），補聴器装用で音への反応が改善した．

3 歳時，体重 6,270 g，身長 68.2 cm，頭囲 38 cm．坐位の姿勢で前方へ移動，つかまり立ち可能となる．尖足が目立ち，しゃがみ込むことはできず．やや発声が増えるも単語は出ず．う歯多数．頭部 CT 上基底核に石灰化を認め（❷），CS と確定．

4 歳時，頭囲 38 cm と変わらず．体重 5,760 g．はいはいはできず．眼振はないが，上肢の振戦あり．聴力損失 85〜95 dB．補聴器装用で 50〜70 dB．高音域の損失が大きい．名前を呼ぶと笑い右手を上げる．発声が増える．

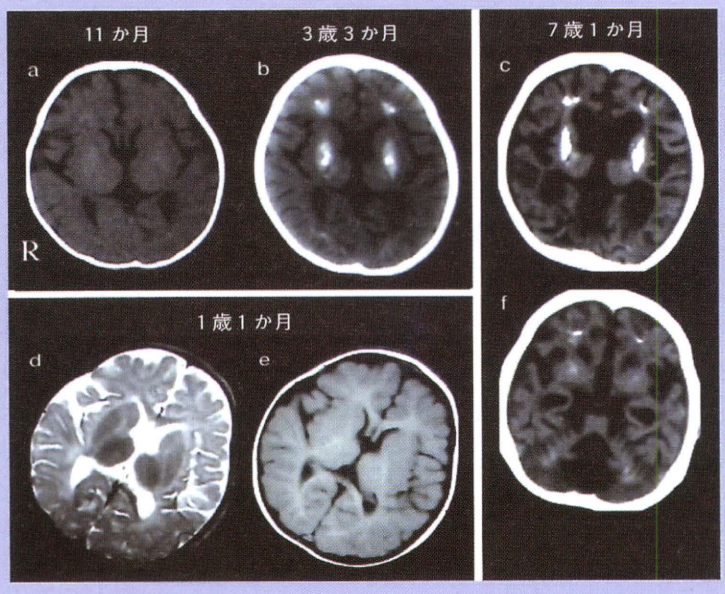
5 歳時，体重 5,345 g．腹臥位で下肢を伸展し，肘を使ってずりばいをする．足関節，股関節の拘縮が目立つ．単語は出ないが，パ行，バ行の表出，TV を見ての模倣，バイバイの模倣が可能となる．「ちょうだい」「食べたい」などをジェスチャーで表す．活発で表情もよい．右手をよく使うようになる．粗大運動の低下に比べると微細な運動はまだ保たれている．睡眠覚醒リズムは規則的である．

6 歳時，視力 0.1．体重 4,980 g．食事摂取量が減ってくる．皮膚がたるみやせが目立つようになる．腎機能悪化，BUN 35.8 mg/dL，Cr 0.78 mg/dL．

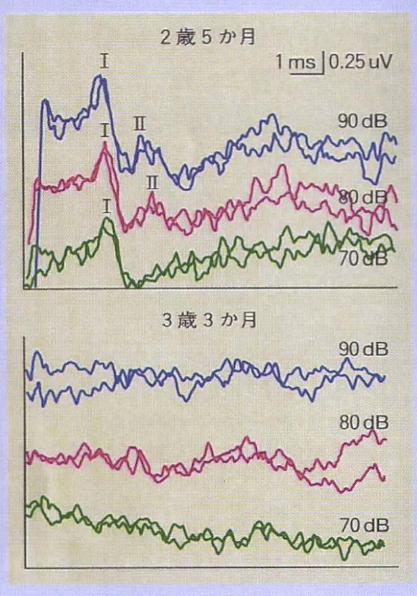
7 歳時，臥床状態で下痢が続き，経口摂取量も減少傾向にあった．誤嚥性肺炎で入院．腎不全悪化．一日尿蛋白 549 mg，収縮期血圧は 90〜150 mmHg の間を変動，呼吸不全となり死亡．

考察 2 型の移動はずりばいまでで，つかまり

❷ 症例 2（Cockayne 2 型）の CT，MRI の経年齢による変化



❸ 症例 2（Cockayne 2 型）の ABR の経年齢による変化



立ちができても立位での移動は不可能である．

CS2 型死亡例 2 例において 1 例は眼振，手指振戦を有し，下肢深部反射は亢進，Babinski 反射陽性であったが，他の 1 例は眼振を認めず，手指振戦を有し，下肢深部反射は消失し，Babinski 反射陰性であった．いずれも関節拘縮，尖足を認めた．この 2 例は同様の末梢神経伝導速度の低下を認めた．

CS2 型の初期乳児期の発達は決して悪くなく（上記），1 歳 1 か月の MRI 上髄鞘化にとくに問題はなかった（❷）．

ABR：auditory brainstem response（聴性脳幹反応），SCV：sensory nerve conduction velocity，DQ：developmental quotient（発達指数），BUN：blood urea nitrogen，Cr：creatinine，MRI：magnetic resonance imaging.

症例 3　Cockayne 症候群 3 型

30 歳，女性．双子の妹も同疾患と診断されている．独歩はやや遅かったが明らかな精神運動発達の遅れはなく，小学校低学年ころまでは普通に運動できていた．小学校高学年ころより運動が苦手になり，学業成績も落ちてきた．18 歳時，歩行のふらつきが明らかになり，病院受診するも診断には至らなかった．24 歳，紫外線感受性試験で CS1 型と正常の中間の感受性であり，CS3 型と診断された．手指振戦が出現，徐々に下肢関節拘縮が進行して歩行困難となり，28 歳で車椅子使用となった．現在，坐位の保持は困難である．食事は全量経口摂取可能であるが摂取量は落ちてきている．

考察　3 型は自力歩行可能で通常の運動能力を示す時期が少なくとも 10 歳代中盤まで続き，その後ゆっくりとした退行を認める．

CS3 型 2 例（双胎）では歩行障害の顕在化した 20 歳前後では筋トーヌスは上下肢でやや低下，深部反射は上下肢で低下，Babinski 反射陰性，上下肢深部感覚障害あり，関節拘縮あり（尖足，肘外反），28 歳での所見は自力での立位保持不可，四肢の筋緊張亢進あり．rigidity あり．関節拘縮，変形あり．振戦あり．深部反射は消失，Babinski 反射は陽性であった．

末梢神経伝導速度は遅延，針筋電図では脱神経所見，末梢神経生検では有髄線維減少，硝子化様の線維化，変性を認めた．

- 皮膚線維芽細胞の紫外線感受性試験で高感受性を認める．
- 特徴的な神経所見を ❹ にまとめる．

遺伝子診断

- CS の 75％が *ERCC6* の変異であり，残り 25％が *ERCC8* の変異である．

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❹ 特徴的な神経所見

中枢神経障害
感音性難聴 視神経萎縮 大脳基底核の石灰化
末梢神経障害
筋電図の神経原性変化 神経伝導速度の遅延 神経生検で脱髄性神経所見
小脳障害
失調歩行 振戦 協調運動障害 構音障害

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Disorders of DNA repair

10.4

Shinichi Moriwaki and Kenneth H. Kraemer

Key Features

- Nucleotide excision repair (NER) is an essential system for correcting ultraviolet (UV) – induced DNA damages.
- Lesions remaining in DNA due to reduced capacity of NER may result in cellular death, premature aging, mutagenesis, and carcinogenesis of the skin. NER is an important protection against these changes.
- There are three rare genodermatoses resulting from defects in NER: xeroderma pigmentosum (XP), Cockayne syndrome (CS), and trichothiodystrophy (TTD).
- At present, there is no cure for any human genetic disorder. Early diagnosis and symptomatic treatment of neurological, ocular and dermatological abnormalities are the mainstays of therapy
- Especially for XP, early diagnosis, rigorous sun protection, and early detection and treatment of cancers may reduce the more serious skin and eye problems and prolong life.

10.4.1 What is DNA repair? [1, 2]

DNA repair is a very important function for all living organisms exposed to sunlight. Efficient removal of UV-induced DNA lesions is vital for survival. The nucleotide excision repair (NER) system is mainly responsible for correcting UV-induced damage in DNA; these are predominantly cyclobutane pyrimidine dimers (CPD) and 6-4 pyrimidine-pyrimidone photoproducts (6-4PP) (Fig. 10.4.1). More than 25 proteins are involved in this system, several of which are also related to the transcription of mRNA. Photoproducts are not randomly repaired by NER throughout the genome. Lesions in DNA that are actively transcribed by RNA polymerase II are repaired more rapidly (transcription-coupled repair (or TCR)) than those in the global genome or in the non-transcribed strand of active genes (global genome repair (or GGR)). The first recognition of DNA damage involves CSA and CSB proteins in TCR and DNA damage binding protein (DDB) plus XPC-hHR23B-centrin 2 in GGR. XPA, transcription factor II H (TFIIH), and replication protein A (RPA) are involved both in TCR and GGR. TFIIH, a basal transcription factor, contains two kinds of DNA helicase activity; XPB and XPD with opposite direction (3'->5', 5'->3', respectively), which play an important role in DNA unwinding of the double strand DNA. After the recognition of the DNA damage, two endonucleases XPF-ERCC1 and XPG, make incisions 5'- and 3' to the lesion releasing a 25–27 nucleotide DNA fragment containing the photoproduct. The resulting gap can be filled by a process involving DNA polymerases, ε/δ, proliferating cell nuclear antigen (PCNA), and replication factor C (RFC), followed by sealing with DNA ligase I. This NER pathway removes the lesion containing the damaged DNA and restores the normal DNA sequence using the opposite strand as a template.

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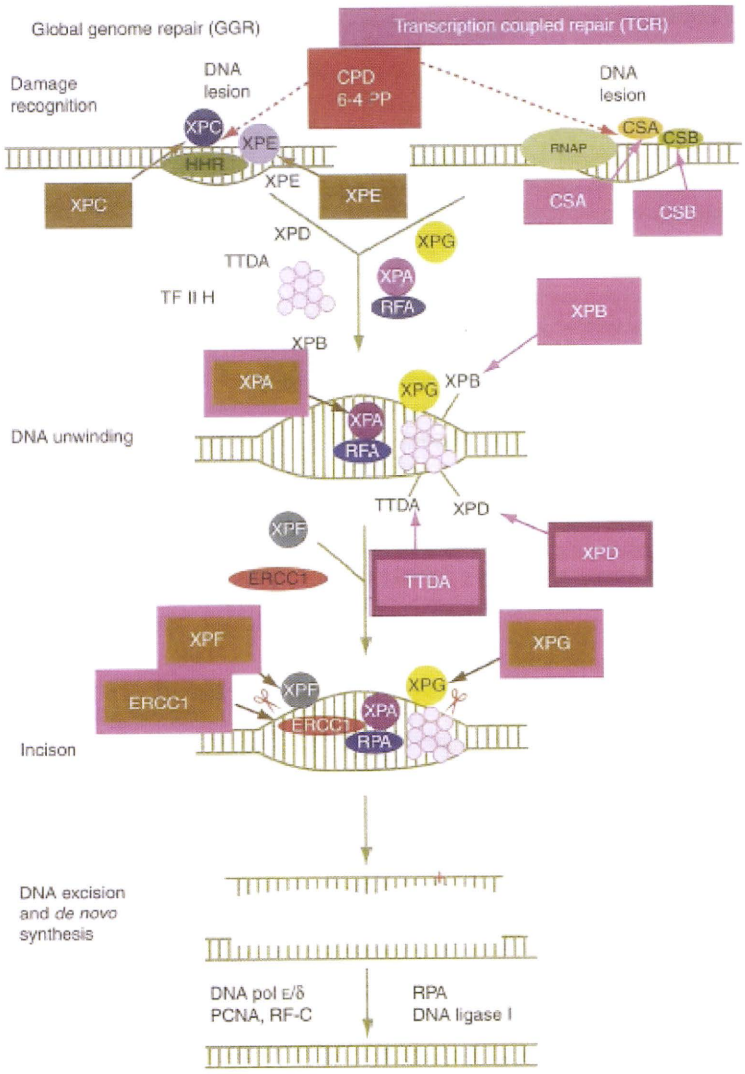

Fig. 10.4.1 The pathway of nucleotide excision repair [27]

10.4.2 Clinical Characteristics and Diagnosis of Disorders Related to DNA Repair Deficiency

10.4.2.1 XP

XP is a rare autosomal recessively transmitted cancer-prone genodermatosis involving impaired repair of UV-induced DNA damage. XP patients have severe sensitivity to sunlight and a high incidence (more than 1,000-fold increase compared to the normal population) of skin cancers: basal cell cancers (BCC), squamous cell cancers, and melanoma [2, 3]. There is a smaller increase in internal neoplasms including cancer of the central nervous system. About 20–30% of XP patients have neurological disease such as mental retardation, sensorineural

deafness, and abnormal motor function (spasticity) in association with a primary progressive neuronal degeneration [4]. MRI in XP may show progressive cerebral atrophy with enlargement of the ventricles. While multiple infections are not common in XP, there are reports of impaired immune response such as impaired production of interferon-gamma, interferon-alpha, IL1-beta, and IL-6 [5, 6] The molecular basis of XP neurological abnormalities and impaired immune function still remain to be elucidated. Cultured cells derived from most XP patients show hypersensitivity to killing by UV irradiation, reduced DNA repair capacity, and high frequency of UV-induced mutations. There are seven genetically different complementation groups (defective genes), XPA through XPG, with defective NER and one excision repair proficient form with deficient DNA polymerase eta (XP variant). XP group A, a severe form of XP with marked neurological degeneration, is more common in Japan (about 55% of Japanese XPA patients) than in US and Europe where 40% of XP cases are XP group C [7, 8]. Previously the diagnosis of XP was made by DNA repair tests such as the measurement of post-UV unscheduled DNA synthesis (UDS), UV survival by colony formation or complementation assay by somatic cell fusion technique. Now the assignment of complementation group of XP has mainly been made by plasmid host cell reactivation (HCR) assay and gene analysis. The recent identification and characterization of the genes responsible for XP (group A through G and variant) permit the use of molecular biological techniques for the sensitive diagnosis of XP. These techniques include polymerase chain reaction (PCR) –RFLP analysis, DNA sequencing, and plasmid HCR assay with cloned XP genes. Plasmid HCR assay is an easier, more rapid and more sensitive laboratory assay for the diagnosis of XP than the classical method; cell fusion assay [7]. HCR utilizes an ultraviolet (UV)-treated plasmid containing the sequence of a reporter gene such as luciferase in addition to cloned expression vector of each XP group. The disadvantage of the assay is that XP group E and XP variant cannot be determined. Most of Japanese XP patients are homozygous or heterozygous for a founder mutation: a G to C transversion at the 3' splice acceptor site of intron 3 in the XPA gene. This mutation is conveniently identified with a restriction enzyme, *AlwNI*. PCR-RFLP method permits rapid diagnosis, carrier detection, and prenatal diagnosis of XP, especially in Japanese XPA families [7].

10.4.2.2 CS

CS is a rare autosomal recessive disease with features of sun sensitivity, short stature, pigmentary retinal degeneration, hearing loss, and progressive neurological degeneration [9]. MRI in CS may show absence of myelin in the cerebrum. CT scan in CS may show calcification of the basal ganglia. Unlike XP, CS is not associated with an increased frequency of skin cancers. CS patients have a deficiency of transcription-coupled NER of UV-induced DNA damage. Cells from CS patients are hypersensitive to killing by UV, have decreased post-UV RNA synthesis, and normal level of post-UV UDS [7]. The genes for CS, CSA, and CSB have been cloned. To define the complementation group, HCR is useful as in cases of XP. Alterations of XPB, XPD and XPG gene can sometimes cause CS phenotype in addition to XP lesions such as freckles and skin neoplasms and these cases are diagnosed to be XP/CS complex [4, 10, 11].

10.4.2.3 TTD

TTD is an autosomal recessive disorder with features of sulfur-deficient brittle hair (with characteristic "tiger tail" banding with polarized microscopy), ichthyosis, short stature, nail dystrophy, cataracts, multiple infections, and neurological abnormalities [12]. MRI in TTD may show decreased or absent myelin in the cerebrum. About 50% of TTD patients have sun sensitivity. There is no increase in skin cancer [13]. Cells from photosensitive TTD patients have defects in XPD, XPB, and TTDA genes, all of which are components of TFIIH [14]. Cells from photosensitive TTD cases are hypersensitive to UV and have reduced level of UDS. Cells from some TTD patients without sun sensitivity have normal UV survival and normal UDS and a defect in the TTDN1 gene. To determine the genetic group of photosensitive form of TTD, HCR assay is useful as in XP and CS.

10.4.3 Management of Patients with XP, CS or TTD and Therapeutic Outline

XP patients may present with many cutaneous malignancies and continue to form skin cancer at a high rate. As there is no cure for genetic disorders, life-long

protection of XP patients from UV radiation and early recognition and prompt treatment of skin cancers are essential. CS and TTD patients do not have an increased risk of skin cancer.

Clinical management of XP consists of early diagnosis followed by a rigorous program of sun protection including avoidance of unnecessary UV exposures, wearing UV blocking clothing, (Fig. 10.4.2) and use of sun blocks on the skin. Sun protection with sunscreen should be done as completely as possible to prevent photodamage of the skin. Exposure to cigarette smoke should be avoided since it contains carcinogens that damage DNA in a manner similar to UV.

A series of baseline skin photographs is often helpful in early detection of skin cancers. Suspicious lesions should be biopsied readily. Premalignant lesions such as actinic keratoses can be treated by freezing with liquid nitrogen or by 5-fluoruracil ointment. Imiquimod, an immune response modifier through the induction of IFN-alpha, INF-gamma, TNF-alpha, IL-6, and IL-12 appears to be safe for BCC in XP patients. [15]. Clearing of multiple melanoma in situ lesions by local injection of interferon alpha has been reported in one patient [16].

Surgical resection of skin tumors utilizing standard methods, including Moh's microsurgery, can be used. This approach should attempt to preserve surrounding undamaged tissue for possible further procedures. Clear margins of pigmented lesions may be difficult to achieve because of the widespread pigmentation of surrounding skin. Larger areas have been treated with therapeutic dermatome shaving or dermabrasion to remove the damaged superficial epidermal layers. This procedure permits repopulation by relatively UV-shielded cells from the follicles and glands. Standard doses of radiotherapy have been used for treatment of unresectable cancers of the skin or central nervous system. Cells from XP patients are not hypersensitive to killing by X-rays. On the other hand, there is a recent report of worsening of an eye tumor following treatment with photodynamic therapy (PDT); so this treatment cannot be recommended [17].

Oral isotretinoin (13-cis retinoic acid), a vitamin A derivative, has been shown to prevent the appearance of new skin neoplasms in XP patients; however, this treatment has many side effects [18]. The topical use of T4 endonuclease V, a prokaryotic DNA repair enzyme which initiates repair of UV-induced CPD in DNA may be a useful tool to prevent new appearance of actinic keratosis or other skin tumors in XP patients [19]. This agent has not yet been approved by the US Food and Drug Administration.

XP patients often have problems with their eyes such as conjunctivitis, corneal clouding, cataract, and ocular neoplasms at a much younger age than in the general population. Dry eyes are common and should be treated with lubrication. To avoid these ocular symptoms, patients should use sunglasses which block UV light. CS patients often develop a retinal degeneration. Ophthalmologic involvement in TTD commonly includes bilateral cataracts (which may be congenital and require early surgical treatment), nystagmus, and errors of refraction.

Some XP patients and most CS patients have neurological symptoms which progress gradually. Care (rehabilitation) for neurological abnormalities including use of hearing aids for patients with hearing loss is essential. Progressive neurological symptoms frequently seen in patients with defects in the XPA, XPB, XPD or XPG genes may be difficult to manage. Patients with XP neurodegeneration may have problems with walking, talking, and caring for themselves. They may experience progressive loss of intellectual functioning. Rehabilitation therapy may be beneficial. Patients should have periodic evaluations by experts in ophthalmology, orthopedics, neurology, and dentistry as well as by dermatologists.

TTD patients may have developmental delay and intellectual impairment. They may benefit from neurologic and developmental assessment. Some TTD patients have recurrent infections which have been managed with prophylactic antibiotics or intravenous immunoglobulin G (IVIG). TTD patients with skeletal abnormalities may benefit from rehabilitation medicine evaluation and support.

10.4.4 Experimental Approaches

Recently a gene therapy of XP cells or XP mice has been reported [20]. When DNA containing an XPA complementing gene was applied to XPA mice skin using liposomes, the level of UDS after UV exposure

Fig. 10.4.2 Sun protective clothing. *Left*. This 10-year-old boy with xeroderma pigmentosum wears a long sleeved undershirt, long leggings and socks made of lightweight UV absorbing cloth. *Right*. When going outdoors for short periods of time,in addition, he wears a long sleeved shirt, tightly woven denim pants, gloves, and shoes with high tops. His head is covered with a hood made of the same lightweight material as his undergarments. The hood is attached to UV absorbing goggles

was increased and the DNA damage was repaired almost normally (personal communication). Functional lentiviral, adenovirus or retrovirus vectors have been reported to restore DNA repair ability of XP cells or UV-irradiated mice [21–25]. Topical application of epigallocatechin, the major polyphenol of green tea has been reported to prevent photocarcinogenesis in mice through IL12-dependent DNA repair mechanism [26]. These research methods offer hope for the future.

Take Home Messages

› Disorders of post-UV DNA repair deficiency, such as XP, CS or TTD are very rare photosensitive genodermatoses. When physicians see cases with sun sensitivity in clinic, they should consider these rare diseases in the differential diagnosis. Early diagnosis and protection from sun exposure can improve the patients' prognosis.

› Patient support groups have been established in several countries that provide information and resources for patients and their family; several have established summer camps with a UV protected environment. The Xeroderma Pigmentosum Society is an educational, advocacy, and support organization in New York: http://www.xps.org. The XP Family Support Group is located in California http://www.xpfamilysupport.org/ The XP Support Group in the UK has a website: http://xpsupportgroup.org.uk/ A group in Germany has a website : http://www.xerodermapigmentosum.de/ A Japanese group of XP families has a website: http://www.xp-japan.net/ Enfants de la lune (Children of the moon) in France: http://www.orpha.net/uestasso/AXP/ A web site listing disease-causing mutations in XP and CS genes has been established: http://xpmutations.org/.

› The booklet "Understanding Xeroderma Pigmentosum," prepared by the National Institutes of Health, is available on the Internet at http://www.cc.nih.gov/ccc/patient_education/pepubs/xp5_18.pdf This booklet provides information about XP for patients, their families, educators, students, health professionals, media inquiries, and others interested in learning more about XP.

› There is an educational, advocacy, and support organization for helping patients with CS and their families: The Share and Care Cockayne Syndrome Network in Texas : http://www.cockayne-syndrome.org The Japanese Cockayne Syndrome Network.

10.4.5 Global Variations

Oral isotretinoin has been shown to be effective in preventing new skin cancers in XP patients in a controlled study [18]. Topical Imiquimod can be used in Europe and the US but this agent has not been shown to be beneficial in a controlled study. Topical T4 endonuclease V was reported to be beneficial in a small study with XP patients [19] but has not yet been approved for use by the US Food and Drug Administration. XP patients in Japan tend to have mutations that lead to progressive neurological degeneration while those in Europe and the US tend to have predominantly skin and anterior eye involvement.

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The Roles of HSP27 and Annexin II in Resistance to UVC-Induced Cell Death: Comparative Studies of the Human UVC-Sensitive and -Resistant Cell Lines RSa and AP^r-1

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We have reported that heat shock protein 27 (HSP27) and annexin II are involved in the protection of human cells against UVC-induced cell death. In this study we tried to confirm the combined roles of HSP27 and annexin II in cell death after UVC irradiation. In RSa cells with sensitivity to UVC, expression of annexin II decreased after UVC irradiation, but not in AP^r-1 cells with increased resistance to UVC. HSP27 siRNA-transfected AP^r-1 cells were sensitized to UVC lethality and showed decreased annexin II expression after UVC irradiation. In contrast, transfection of RSa cells with *HSP27* cDNA increased their resistance to UVC lethality and caused increased annexin II expression. Furthermore, over-production of annexin II in RSa cells resulted in increased resistance to UVC lethality. This study indicates the involvement of cellular HSP27 expression in the UVC susceptibility of human cells, which occurs in association with regulation of annexin II expression.

Key words: HSP27; annexin II; human cells; UVC

Heat shock proteins (HSPs) are expressed in response to a wide variety of physically and chemically induced stress conditions in prokaryotes and eukaryotes, and the increased expression of HSPs is involved in protection against stress-induced cellular injury.[1–3] We have found that one HSP, HSP27, may be involved in the resistance of human cells to UVC-induced cell death.[4] This finding was based on a comparison of gene expression profiles using a cDNA array between human RSa cells and their variant AP^r-1 cells. RSa cells were established from human embryo-derived fibroblastic cells by double infection with Simian virus 40 and Rous sarcoma virus.[5] The high sensitivity of RSa cells to UVC-induced cell death (at a principal wavelength of 254 nm) has been reported.[6] AP^r-1 cells, which are more resistant to UVC-induced cell death, were established from RSa cells by mutagenesis with ethyl methanesulfonate followed by UVC irradiation.[7] The expression of HSP27 was found to be lower in the RSa cells than in the AP^r-1 cells.[4] RSa cells transfected with *HSP27* cDNA showed slightly lower sensitivity to UVC-induced cell death than the control cells transfected with empty vector alone, and much lower sensitivity than RSa cells transfected with antisense *HSP27* cDNA.[4]

An important function of HSP27 is to act as a molecular chaperone. Under stressful conditions, HSP27 proteins form complexes with a variety of cellular proteins, such as Akt,[8] cytochrome *c*,[9] actin microfilament,[10] and eIF4G,[11] resulting in inhibition of apoptosis induction,[8,9] cytoskeleton disruption,[10] and translation initiation[11] respectively. Hence, we next evaluated the potential roles of HSP27 in the UVC response by determining which proteins interact with HSP27 and are involved in the UVC resistance of AP^r-1 cells.[12] Annexin II was identified as one of HSP27-interacted proteins from AP^r-1 cell lysates by pull-down with GST-fused HSP27 proteins and subsequent molecular mass analysis.[12] Moreover, AP^r-1 cells transfected with annexin II siRNA were found to have increased susceptibility to UVC-induced cell death.[12]

Annexin II is a phospholipid-binding protein implicated in several membrane related-events, including regulation of the exocytotic and endocytotic pathways, and it functions as a plasminogen receptor.[13] Increased expression levels of annexin II have been reported in some cancer cell lines,[14] but the relationship between HSP27 and the functions of annexin II in the susceptibility of human cells to UVC-induced cell death remains unknown.

In the present study, we tried to confirm the involvement of both proteins in the difference in resistance to UVC-induced cell death between RSa and AP^r-1 cells by transfecting RSa cells with *HSP27* cDNA and *annexin II* cDNA.

Materials and Methods

Cell culture conditions and UVC irradiation. Cells were cultured in Eagle's MEM (EMEM) (Nissui, Tokyo) medium supplemented with 10% (v/v) calf serum (CS) (Invitrogen, Carlsbad, CA) at 37 °C in a humidified atmosphere containing 5% CO_2. UVC was generated by a 6-W National germicidal lamp (Panasonic, Osaka, Japan). The

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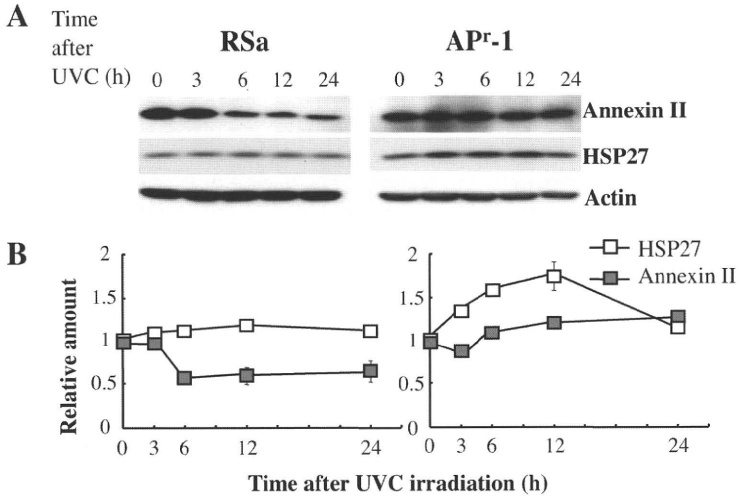

Fig. 1. Annexin II and HSP27 Expression Levels after UVC Irradiation in RSa Cells and APr-1 Cells.

A, Cells were irradiated with UVC (8 J/m^2) and harvested at the indicated times after UVC irradiation. Whole cell lysates were separated in 12% SDS gel and the protein levels were examined using antibodies against the respective proteins by Western blotting, as described in "Materials and Methods." The protein levels of actin were also analyzed as the loading control. B, The protein levels are expressed as levels relative to those at time 0 (immediately after UVC irradiation). Data are the means ± SD for three experiments.

intensity of UVC was 1 J/m^2/s, measured with a UV radiometer, UVR-254 (Tokyo Kogaku Kikai, Tokyo). Cells were irradiated with UVC as previously described,[7] and mock-irradiated cells were treated in the same manner but without UVC irradiation.

Western blotting. Immunoblotting analysis was carried out as described previously.[4] In brief, cells were washed twice with phosphate-buffered saline and whole cells were lysed with SDS sampling buffer and boiled for 5 min. Whole cell lysates were separated by 12% SDS gel and transferred onto a Millipore membrane. HSP27 protein was detected using mouse anti-HSP27 monoclonal antibody (mH3)[15,16] (1:1,000 dilution) and annexin II protein was detected using rabbit anti-annexin II antibody (sc-9061) (Santa Cruz Biotechnology, Santa Cruz, CA) (1:1,000 dilution). The antigen-antibody complexes were detected using horseradish peroxidase-conjugated anti-mouse IgG antibody (Amersham Biosciences, Buckinghamshire, UK) and anti-rabbit IgG antibody (GE Healthcare, Bucks) following the ECL system (Amersham Biosciences). The protein levels of actin were also analyzed using mouse anti-actin antibody (ICN Biomedicals, Costa Mesa, CA) (1:10,000 dilution) as the loading control. The intensity of the protein signals was quantified using Multi Gauge Ver2.2 image analyzing software (Fuji Foto Film, Tokyo) and were expressed as values relative to that of actin.

Construction of expression vectors. Full-length human *HSP27* cDNA was prepared as described previously.[4] The cDNA was ligated into pQE-30 plasmid (Qiagen, Valencia, CA) using Sac I and Pst I restriction sites. The construction was confirmed by sequencing and was then digested with EcoR I and Hind III. The digested fragment was gel-purified and then ligated into pcDNA3.1(−) (Invitrogen) using EcoR I and Hind III restriction sites to construct a His-tagged HSP27 (His-HSP27) expression vector (His-HSP27/pcDNA3.1(−)), and the construction was confirmed by sequencing. Human *annexin II* cDNA was prepared by PCR using forward (5′-GCAGGTACCGGATTTCA-CTCTCTACCCGGAG-3′) and reverse (5′-TCACTCGAGTTCAGT-CATCTCCACCACACA-3′) primers from a cDNA library of human SW837 cells, and PCR products were inserted into pcDNA3.1(+) (Invitrogen) using Kpn I and BamH I restriction sites to construct the annexin II expression vector (ANX II/pcDNA3.1(+)). The construction was confirmed by sequencing.

Plasmid transfection. RSa cells grown to 60–80% confluence in 60-mm dishes were transiently transfected with the indicated expression plasmids using FuGENE HD transfection reagent (Roche Applied Science, Grenzach-Wyhlen, Germany) according to the manufacturer's instructions. To obtain cell lines with stable overexpression of annexin II, RSa cells were transfected with ANX II/pcDNA3.1(+) and pcDNA3.1(+) as described above, and cultured in G418-containing medium, followed by cloning the G418-resistant cells. Two cell lines with overexpression of annexin II, ANXII-11, and ANXII-13, and two cell lines with the empty vectors, V1 and V2, were obtained after analysis of annexin II expression by immunoblotting.

Knockdown of HSP27. Duplex small interfering RNA (siRNA) against human HSP27 (HSP27 siRNA) was synthesized based on the nucleotide sequence. The sequences of the duplex were as follows: 5′-GCUGCAAAAUCCGAUGAGACTT-3′/3′-TTCGACGUUUUAGG-CUACUCUG-5′. A negative control for HSP27 siRNA (NC siRNA) with random sequences designed as follows: 5′-GAUCCGACCUGC-AAGAUGAATT-3′/3′-TTCUAGGCUGGACGUUCUACUU-5′. These siRNA duplexes were synthesized by FASMAC (Atsugi, Japan). The siRNAs for HSP27 (100 nM) were transfected into APr-1 cells for 5 h using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Two d after transfection, the cells were irradiated with UVC and harvested at the indicated times after UVC irradiation, and the levels of annexin II and HSP27 proteins were analyzed by immunoblotting. To analyze the susceptibility of the cells transfected with HSP27 siRNA, the cells were replated for colony survival assay 2 d after transfection.

Cell survival assay. The susceptibility of the cells to UVC-induced cell death was measured by colony survival assay and methylthiazole tetrazolium (MTT) metabolic viability assay, as reported previously.[17]

Results and Discussion

To determine the relative roles of HSP27 and annexin II in cell survival after UVC irradiation of human cells, we analyzed the expression of HSP27 and annexin II after UVC irradiation (up to 24 h) in RSa cells and APr-1 cells. The levels of HSP27 immediately after irradiation (time 0) were lower in RSa cells than in APr-1 cells (Fig. 1A). The levels of HSP27 increased in APr-1 cells after UVC irradiation, but hardly increased in RSa cells (Fig. 1A, B). In APr-1 cells, the levels of HSP27 at 12 h were 1.7-fold higher than those at time 0, and the peak levels (at 12 h) decreased at 24 h towards the basal levels (Fig. 1A, B). The reason for the decrease at 24 h remains unclear. HSP27 has been reported to

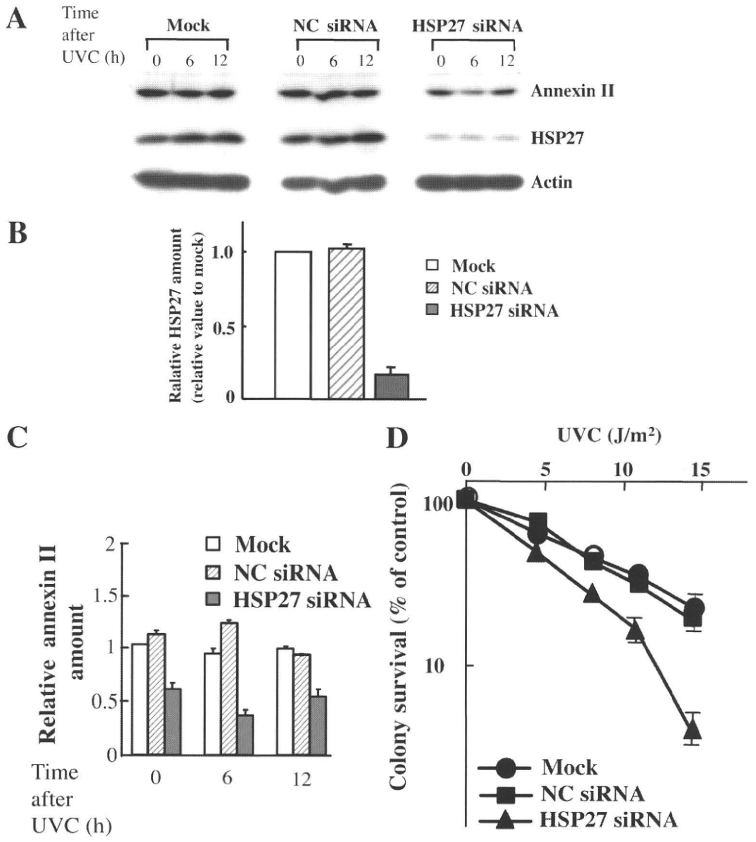

Fig. 2. Reduction of Annexin II after UVC Irradiation and UVC Sensitization in Association with Knockdown of HSP27 by siRNA in AP^r-1 Cells. A, Forty-eight h after transfection with mock, NC siRNA, or HSP27 siRNA, AP^r-1 cells were irradiated with UVC (8 J/m^2) and harvested at the indicated times after UVC irradiation. Whole cell lysates were prepared, and the protein levels of HSP27, annexin II and actin were analyzed using antibodies against the respective proteins by Western blotting, as described in "Materials and Methods." B, The HSP27 protein levels in HSP27 siRNA- and NC siRNA-transfected cells without UVC irradiation are expressed as levels relative to those in the mock-transfected cells. C, The annexin II protein levels in the HSP27 siRNA- and NC siRNA-transfected cells and mock-transfected cells after UVC irradiation are expressed as levels relative to those in the mock-transfected cells at time 0 (immediately after UVC irradiation). D, Colony survival activity after UVC irradiation of HSP27 siRNA-, NC siRNA-, and mock-transfected cells was analyzed by colony formation assay, as described in "Materials and Methods." Data are shown as percentages of colony numbers relative to those of non-irradiated cells. All data (B–D) are means ± SD for three independent experiments.

translocate from the cytoplasm to the nucleus after UVB irradiation in human cells.[18] However, the decrease in HSP27 observed in this analysis might not have been due to translocation of HSP27, because whole cell lysates were used. Oxidative stress also induced HSP27 expression in human cells; the peak protein levels decreased slightly with exposure time, and the change in the protein levels did not always correspond with that in the mRNA levels.[19] Therefore, posttranslational regulatory mechanisms have been involved in the regulation of HSP27 protein levels. Annexin II expression decreased in RSa cells after UVC irradiation, and at 6 h after irradiation the levels were approximately 60% of those at time 0 (Fig. 1A, B). In contrast, annexin II expression did not decrease but increased in AP^r-1 cells after UVC irradiation (Fig. 1A, B).

HSP27 functions as a chaperone by interaction with target proteins under stress conditions and assists protein stabilization, modification, and translocation.[20,21] Taking this together with the results presented in Fig. 1, we hypothesized that HSP27 acts as a molecular chaperone for annexin II in resistance to UVC-induced cell death, thus regulating annexin II expression after UVC irradiation. Hence, we examined the effect of HSP27 expression levels on UVC-induced reduction in annexin II protein levels by knockdown of HSP27 in AP^r-1 cells and overexpression of HSP27 in RSa cells.

In HSP27 siRNA-transfected AP^r-1 cells, the levels of HSP27 protein were lower than in mock- and NC siRNA-transfected AP^r-1 cells without UVC irradiation (Fig. 2A, B). The HSP27 levels increased in mock- and NC siRNA-transfected AP^r-1 cells after UVC irradiation (up to 12 h), but did not increase in HSP27 siRNA-transfected AP^r-1 cells (Fig. 2A). The levels of annexin II protein were also lower in HSP27 siRNA-transfected AP^r-1 cells than in the mock- and NC siRNA-transfected cells without UVC irradiation (Fig. 2A). After UVC irradiation, the levels of annexin II protein in HSP27 siRNA-transfected AP^r-1 cells decreased; the levels at 6 h were approximately 50% compared with those at time 0, and then recovered at 12 h to the basal levels (Fig. 2A, C). In contrast, the levels did not change as much after UVC irradiation in the mock- and the NC-transfected cells (Fig. 2A, C). In addition, the sensitivity of the HSP27 siRNA-transfected cells to UVC-induced cell death was higher than that of the mock- and NC siRNA-transfected cells (Fig. 2D). To induce HSP27 overexpression in RSa cells, we transfected His-HSP27/pcDNA3.1(−) into the cells. The transfectant showed higher expression of His-HSP27 protein than the control

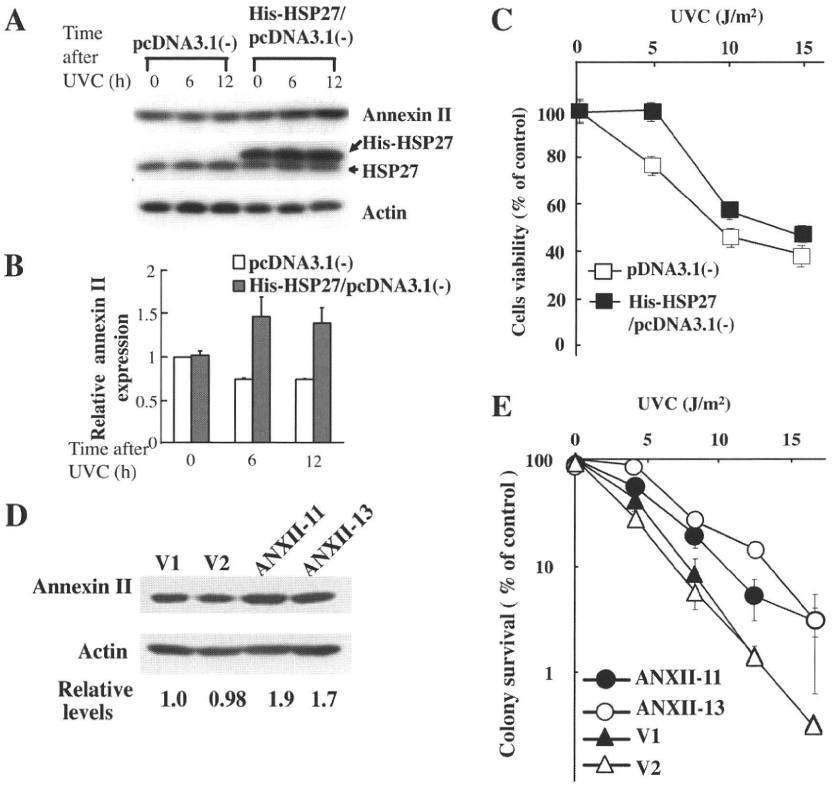

Fig. 3. Suppression of a Decrease in Annexin II Expression in RSa Cells Transfected with *HSP27* cDNA, and Increased Resistance to UVC-Induced Cell Death in Cells with Annexin II Overexpression.

A, Twenty-four h after transfection with His-HSP27/pcDNA3.1(−) or pcDNA3.1(−), RSa cells were irradiated with UVC (8 J/m^2) and were harvested at the indicated times after UVC irradiation. Whole cell lysates were prepared, and the protein levels of His-HSP27 and endogenous HSP27 were analyzed using anti-HSP27 antibody, and those of annexin II and actin were analyzed using the respective antibodies by Western blotting, as described in "Materials and Methods." B, Annexin II protein levels in the transfected cells after UVC irradiation are expressed as levels relative to those in the control cells transfected with the empty vector at time 0 (immediately after UVC irradiation). C, Twenty-four h after transfection with His-HSP27/pcDNA3.1(−) or pcDNA3.1(−), the cells were irradiated with UVC, and susceptibility to UVC irradiation was analyzed by MTT assay as described in "Materials and Methods." Data are shown as percentages of viability relative to those of non-irradiated cells. D, The annexin II protein levels in V1, V2, ANXII-11, and ANXAII-13 cells were analyzed by Western blotting, and are expressed as levels relative to those in the V1 cells. E, Colony survival activity after UVC irradiation of V1, V2, ANXII-11, and ANXAII-13 cells was analyzed by colony formation assay, as described in "Materials and Methods." Data are shown as percentages of colony numbers relative to those of non-irradiated cells. All data (B–E) are means ± SD for three independent experiments.

RSa cells transfected with empty vector (Fig. 3A). Furthermore, the His-HSP27-expressing cells showed no reduction in annexin II protein expression, but showed an increase in expression after UVC irradiation (Fig. 3B). On the other hand, the RSa cells transfected with empty vector showed a decrease in annexin II expression after UVC irradiation (Fig. 3A, B), as shown in Fig. 1. The His-HSP27-expressing cells showed higher resistance to UVC-induced cell death than the control cells transfected with empty vector (Fig. 3C and Supplemental Fig. 1; see *Biosci. Biotechnol. Biochem.* Web site), similarly to the stable transfectants with HSP27 cDNA, as described previously.[4)]

To confirm the role of annexin II expression levels in the resistance to UVC-induced cell death, we established cells with stable overexpression of annexin II protein by transfection of RSa cells with *annexin II* cDNA. The transfected cells, ANXII-11 and ANXAII-13, showed higher levels of annexin II than the control RSa cells transfected with empty vector, V1 and V2 (Fig. 3D). There was little difference in cell proliferation rates among the four cell lines; the doubling time of V1 cells was approximately 24 h and that of the other three cells was approximately 23 h. The ANXII-11 and ANXAII-13 cells showed increased resistance to UVC-induced cell death as compared to V1 and V2 cells (Fig. 3E).

The present findings suggest that up-regulation of HSP27 prevents a decrease in annexin II expression after UVC irradiation (Fig. 2A, C and Fig. 3A, B) and UVC-induced cell death in association with the regulation of annexin II expression. The mechanisms underlying the involvement of annexin II in UVC resistance and by which HSP27 regulates the levels of annexin II protein remain unknown. Recently, HSP27 has been reported to increase the stability of the Her2 protein[20)] and androgen receptor[21)] by affording protection against proteasome-mediated degradation. In this study, the changes in annexin II protein levels were a result of the balance between synthesis and degradation activities. The UVC-induced decrease in the annexin II protein levels in the cells with low HSP27 expression, RSa (Fig. 1A, B), may have been due to enhanced degradation activity, overcoming synthesis activity. On the other hand, the UVC-induced increase in the annexin II protein levels in the cells with high HSP27 expression, AP^r-1 cells and His-HSP27-expressing RSa cells (Fig. 1A, B and Fig. 3A, B), may have been associated with greater synthesis activity than degradation activity. Therefore, HSP27 may regulate annexin II protein levels after UVC irradiation *via* stimulation of synthesis

and/or prevention of degradation. Even in the HSP27 siRNA-transfected AP^r-1 cells, the UVC-induced decrease in annexin II recovered at 12 h (Fig. 2A, C). This recovery might also have been due to the balance between protein synthesis and degradation activities, which appeared to be affected by factors other than HSP27, because HSP27 expression was still suppressed at the time. Our preliminary analysis, using a protein synthesis inhibitor, cycloheximide, suggested that UVC irradiation enhanced the rate of annexin II protein degradation in the RSa cells (data not shown). More detailed analysis of the change in annexin II protein levels after UVC irradiation is underway, using protein synthesis inhibitors and protease inhibitors.

In addition, HSP27 enhances not only the stability but also the shuttling to the nucleus and the transcriptional activity of the androgen receptor in human LNCaP cells.[21] In the AP^r-1 cells, the formation of a complex between HSP27 and annexin II increased in the nuclear fraction after UVC irradiation.[12] Annexin II is reported to function mainly in the cytoplasm,[13] but part of the protein localizes to and functions in the nucleus.[22,23] Thus HSP27 might enhance induction, stability, and translocation into the nucleus of annexin II after UVC irradiation, and might regulate the function of annexin II to protect against UVC-induced DNA damage. To prove these hypotheses, a pair of RSa and its variant AP^r-1 cell lines should be valuable.

Acknowledgments

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CASE REPORT

Autopsy study of cerebellar degeneration in siblings with ataxia-telangiectasia-like disorder

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Abstract Ataxia-telangiectasia-like disorder (ATLD) is caused by mutations of the *MRE11* gene and is characterized by cerebellar ataxia, increased frequency of chromosomal translocations and hypersensitivity to ionizing radiation. ATLD is a rare genetic disease and the associated pathological changes in the brain are unclear. Here, we report the neuropathological findings in the first cases of genetically confirmed ATLD in a pair of Japanese male siblings. Magnetic resonance imaging studies performed during infancy revealed that both subjects had cerebellar atrophy. They died of pulmonary cancer at 9 and 16 years. The siblings had the same compound heterozygous mutations of the *MRE11* gene. Brain autopsy demonstrated mild and severe cerebellar atrophy in the vermis and medial part of the hemispheres, oral to the horizontal fissure, respectively. Nuclear immunoreactivity for MRE11 was absent in neurons of cerebellar cortex, cerebral cortex, basal ganglia and midbrain, whereas being widespread in normal control brains. Immunoreactivity for the DNA oxidative stress marker, 8-hydroxy-2′-deoxyguanosine, was identified in nuclei of granule cells and Bergmann glial cells. The combination of MRE11 deficiency and DNA oxidative injury might have led to selective cerebellar degeneration.

Keywords Ataxia-telangiectasia-like disorder · MRE11 · Cerebellar degeneration · Oxidative stress

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Introduction

Ataxia-telangiectasia-like disorder (ATLD) is characterized by cerebellar ataxia, and dysarthria [22]. The clinical features of ATLD are very similar to those of AT caused by mutations in the AT-mutated gene (*ATM*); however, telangiectasia and severe immunodeficiency, which are the cardinal features in AT, are absent in ATLD [1]. ATLD is classified as a chromosomal breakage syndrome because the patients show spontaneously occurring chromosomal aberrations and increased sensitivity to ionizing radiations. ATLD is caused by mutations in the *MRE11* gene [20]. Nijmegen breakage syndrome (NBS), which is caused by mutations in the *NBS1* gene, has also been defined as a chromosomal breakage syndrome, and the main clinical features of NBS are microcephaly, growth retardation, recurrent infections, and predisposition to tumors, but not cerebellar ataxia [22]. ATM, MRE11, and NBS1 are key components of the signaling network involved in cellular response to DNA damage [13]. Neuropathological changes