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SMARCAL1 Deficiency Predisposes to Non-Hodgkin Lymphoma and Hypersensitivity to Genotoxic Agents *In vivo*

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Abstract

Schimke immuno-osseous dysplasia (SIOD) is a multisystemic disorder with prominent skeletal, renal, immunological, and ectodermal abnormalities. It is caused by mutations of *SMARCAL1* (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a-like 1), which encodes a DNA stress response protein. To determine the relationship of this function to the SIOD phenotype, we profiled the cancer prevalence in SIOD and assessed if defects of nucleotide excision repair (NER) and of nonhomologous end joining (NHEJ), respectively, explained the ectodermal and immunological features of SIOD. Finally, we determined if *Smarcal1*^{del/del} mice had hypersensitivity to irinotecan (CPT-11), etoposide and hydroxyurea (HU) and whether exposure to these agents induced features of SIOD. Among 71 SIOD patients, three had non-Hodgkin lymphoma (NHL) and one had osteosarcoma. We did not find evidence of defective NER or NHEJ; however, Smarcal1-deficient mice were hypersensitive to several genotoxic agents. Also, CPT-11, etoposide and HU caused decreased growth and loss of growth plate chondrocytes. These data, which identify an increased prevalence of NHL in SIOD and confirm hypersensitivity to DNA damaging agents *in vivo*, provide guidance for the management of SIOD patients.

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COMPETING INTERESTS No authors have conflicts of interest to report.

Keywords

Schimke immuno-osseous dysplasia; non-Hodgkin lymphoma; T-cell immunodeficiency; genotoxic agents

INTRODUCTION

Schimke immuno-osseous dysplasia (SIOD) is an autosomal recessive multisystem disorder characterized by the prominent features of dysmorphic facies, growth failure from spondyloepiphyseal dysplasia, renal failure from focal segmental glomerulosclerosis (FSGS), and T-cell immunodeficiency [Schimke et al., 1971; Spranger et al., 1991]. Additional less prominent features include hyperpigmented macules, sparse hair, migraine-like headaches, atherosclerosis with cerebral ischemia, deficiency of other blood cell lineages, hypothyroidism, opportunistic infections, dental anomalies, corneal opacities, autoimmune enteropathy, and pulmonary hypertension [Boerkoel et al., 2000; da Fonseca 2000; Kilic et al., 2005; Spranger et al., 1991]. The mean age of death among SIOD patients is 10.8 years (range: 3-28 years).

SIOD is caused by biallelic mutations of *SMARCAL1*, which encodes an enzyme with annealing helicase activity; this enzyme participates in the DNA stress response to genotoxic agents such as hydroxyurea, camptothecin and aphidicolin and in reactivation of stalled replication forks [Bansbach et al., 2009; Ciccia et al., 2009; Postow et al., 2009; Yuan et al., 2009; Yusufzai and Kadonaga 2008; Yusufzai et al., 2009]. To test the potential contribution of defective DNA repair to the phenotype of SIOD, we compared SIOD to other disorders of DNA repair, assessed cancer prevalence, tested for NER and NHEJ defects and measured the sensitivity of Smarcal1-deficient mice to genotoxic agents. We find that SIOD patients have a high frequency of non-Hodgkin lymphoma (NHL) and that Smarcal1-deficient mice are hypersensitive to several genotoxic agents.

MATERIALS AND METHODS

Human subjects

Patients referred to this study gave informed consent. The study was approved by the Institutional Review Boards of Baylor College of Medicine (Houston, TX USA), the Hospital for Sick Children (Toronto, ON, Canada), and the University of British Columbia (Vancouver, BC Canada). Clinical data were obtained from questionnaires completed by the attending physician as well as medical summaries.

Animal subjects

Mice used in this study were housed, bred, and euthanized in accordance with accepted ethical guidelines. These procedures were approved by the Institutional Review Board of Baylor College of Medicine (Houston, TX, USA, IRB protocol: AN-2983) or the University of British Columbia (Vancouver, BC, Canada, Animal Care Certificate: A10-0296).

Immunoglobulin switch junction amplification and sequencing

Genomic DNA was extracted from peripheral blood cells of 7 SIOD patients and 4 unaffected controls. Amplification, sequencing and analysis of the switch $S\mu$ - $S\alpha 1$ and $S\mu$ - $S\gamma 1$ junctions were performed as previously described with minor modifications [Pan-Hammarstrom et al., 2005]. In brief, nested polymerase chain reaction (PCR) strategy was used to amplify $S\mu$ - $S\alpha 1$ and $S\mu$ - $S\gamma 1$ junctions. In the first round of PCR reactions, 50 ng or 150 ng of genomic DNA from each individual was amplified in 8 parallel reactions using

either S μ 1 and S α -common-1 primers for a total of 35 cycles of 94°C for 1 minute, 60°C for 1 minute, and 72°C for 90 seconds, or S μ 1 and S γ -common primers for a total of 35 cycles of 94°C for 1 minute, 66°C for 1 minute, and 72°C for 30 seconds, respectively. In order to amplify S μ -S α 1 junctions, 1 μ l of each S μ 1-S α -common-1 reaction was amplified using S μ 5 and S α 1-specific primers in reactions consisting of 35 cycles of 94°C for 1 minute, 65°C for 1 minute, and 72°C for 1 minute. Also, in order to amplify S μ -S γ 1 junctions, 3 μ l of each S μ 1-S γ -common reaction was amplified using S μ 5 and S γ 1-specific primers in reactions consisting of 40 cycles of 94°C for 1 minute, 66°C for 1 minute, and 72°C for 1 minute. PCR products from the second round of reactions were gel purified with QIAEX II Gel Extraction Kit (Qiagen, Toronto, ON, Canada) and sequenced using the S μ 5, S α 1-specific, and S γ 1-specific primers. The switch fragment sequences were aligned with S μ (X54713) /S α 1 (L19121) or S μ /S γ 1 (U39737) reference sequences and microhomology at each switch junction was defined as the longest region of identity with both the S μ and S α 1 or S μ and S γ 1 reference sequences. The primers are listed in Supplementary eTable S1 (See Supporting Information online).

***Smarcal1*^{del/del} mice and treatment with CPT-11, etoposide, and HU**

The *Smarcal1*^{del/del} mice have a deletion of the first two coding exons of *Smarcal1* (NM_018817.2:c.172_989del), which includes the replication protein A (RPA) binding site, nuclear localization signal and the first HARP domain. They were generated and analyzed as described elsewhere [Baradaran-Heravi et al., 2012]. *Smarcal1*-deficient (*Smarcal1*^{del/del}) and wild-type (*Smarcal1*^{+/+}) male mice were divided into 4 groups (3-5 mice in each group) at the age of 4-weeks. Mice in each group were given intra-peritoneal (ip) injections of CPT-11 (40 mg/kg, Sandoz, Boucherville, QC, Canada), etoposide (20 mg/kg, Sigma, Oakville, ON, Canada), HU (100 mg/kg, Sigma, Oakville, ON, Canada), or carrier (PBS, Gibco, Burlington, ON, Canada). After 5 consecutive daily injections, the mice were followed for 3 days and then their spleens were harvested. To analyze the long-term effect of these drugs, mice were injected daily for 8 weeks. To assess the recovery of growth retardation, CPT-11-injected mice were observed for another 12 weeks without further injections. At the end of the treatment, radiography of mice was performed using the Faxitron X-ray cabinet. The density of the distal femur was measured using a Scanco μ CT35 scanner (Scanco Medical, Bassersdorf, Switzerland) as previously described [Arama and Steller 2006].

To compare the toxicity of each drug among *Smarcal1*^{del/del} and *Smarcal1*^{+/+} mice, 3-4 mice of each genotype were given daily ip injections with CPT-11 (80 mg/kg), etoposide (40 mg/kg), HU (500 mg/kg) or PBS for 5 consecutive days and followed for two weeks unless sacrificed earlier.

Urinary creatinine and albumin assays

Spot urine was collected from *Smarcal1*^{del/del} and *Smarcal1*^{+/+} mice injected with PBS, CPT-11, etoposide or HU. Urinary creatinine was measured using the creatinine assay kit from Cayman Chemical Company (Ann Arbor, MI, USA) following the manufacturer's instructions. Urinary albumin was measured using the mouse albumin ELISA kit from Immunology Consultants Laboratory (Portland, OR, USA) following the manufacturer's instructions. Urinary albumin excretion was normalized to urinary creatinine for each sample.

Histopathology and TUNEL assay

Mouse tissues for histopathology and TUNEL assay were fixed in 4% paraformaldehyde (PFA) in PBS, processed, paraffin embedded, and cut into 5 μ m sections according to standard protocols as previously described [Hirano et al., 2007]. TUNEL detection of

apoptotic cells in the spleens was carried out using the ApopTag Peroxidase In Situ Apoptosis Detection Kit (S7101, Chemicon, Billerica, MA, USA) and the tissue was counterstained with Mayer's hematoxylin. Harris hematoxylin and eosin (H&E) staining of the femurs and Masson trichrome staining (Sigma, Oakville, ON, Canada) of the kidneys were performed following the manufacturer's instructions. Images of the processed tissues were captured using a Zeiss Axiovert 200 microscope. Chondrocytes within the proliferative and hypertrophic zones of the distal femoral growth plates were counted as previously described [Baradaran-Heravi et al., 2012].

Cell culture

The isolation and culturing of human dermal fibroblast cell lines were performed as described by Clewing *et al.* [Clewing et al., 2007b]. The SIOD patient fibroblasts (N859) were derived from patient SD31, who had a homozygous deletion of the first 5 exons of *SMARCAL1* (NT_005403.17:g.[67482574_67497178del]+[67482574_67497178del]), and cultured in DMEM (Gibco, Burlington, ON, Canada) containing 15% fetal bovine serum (FBS; Gibco, Burlington, ON, Canada) and 1% antibiotic/antimycotic (penicillin, streptomycin and amphotericin B; Gibco, Burlington, ON, Canada) at 37°C and 5% CO₂. Other cell lines including normal human fibroblasts (C5RO) and Cockayne syndrome B fibroblasts (CS188TOR and CS163TOR) were also cultured in DMEM containing 15% FBS and 1% antibiotic/antimycotic at 37°C and 5% CO₂.

Cell survival assays

Cell survival following UV or illudin S treatment was determined using a modified assay based on tritiated thymidine incorporation [Jaspers et al., 2002]. In brief, triplicate sparse cultures (5×10^3 to 1×10^4 cells/6-cm dish) of primary C5RO, N859, CS188TOR, and CS163TOR fibroblasts were grown for 48 hours and exposed to illudin S (0, 0.2, 0.4, 0.6, 0.8, and 1ng/ml) or UV (0, 5, 10, and 15 J/m²). After 3–5 days additional culture, the cells were pulse-labelled with tritiated thymidine (40–60 Ci/mmol, 5 μCi/ml) for 3 hours in presence of 20 mM HEPES pH 7.3. Following 30 minutes incubation in unlabelled medium, the cells were harvested and the incorporated radioactivity was measured in a liquid scintillation counter.

Unscheduled DNA synthesis and recovery following UV-induced transcription inhibition

Global-genome and transcription-coupled NER activities were determined by the Unscheduled DNA Synthesis (UDS) assay and recovery following UV-induced transcription inhibition as described by Jaspers *et al.* [Jaspers et al., 2007]. For measurement of UDS, C5RO and N859 fibroblasts were cultured on coverslips and exposed to 16 J/m² of UV light and subsequently incubated for 3 hours in media containing 10 μCi/mL [3H-1',2']-thymidine (120 Ci/mmol). Cells were fixed and processed for autoradiography by dipping in Ilford K2 photographic emulsion. After 3 days slides were developed, Giemsa staining was performed and nuclear silver grains were counted in 50 non-S-phase cells. To assess the ability to recover from UV-induced inhibition of transcription, C5RO and N859 primary fibroblasts were exposed to 16 J/m² of UV for 16 hours, and then were cultured for 1 h in medium containing [3H-5',6']-uridine (40 Ci/mmol). Cells were fixed and processed for autoradiography as described above.

RESULTS

SIOD patients have features observed in other disorders of DNA repair

Comparison of SIOD patients to individuals with Bloom syndrome, Fanconi anemia, Cockayne syndrome, ataxia telangiectasia, Nijmegen breakage syndrome, and xeroderma

pigmentosum showed that SIOD shares many features with these disorders including poor postnatal growth, immune defects, and premature atherosclerosis (Table I) [Boerkoel et al., 2000; Clewing et al., 2007a]. Unlike these disorders, however, SIOD is not reported to predispose to cancer although there are anecdotal reports of patients with non-Hodgkin lymphoma (NHL) [Basiratnia et al., 2011; Boerkoel et al., 2000; Taha et al., 2004]. To ascertain better the prevalence of cancer in SIOD, we reviewed the medical records of 71 SIOD patients with identified biallelic *SMARCAL1* mutations. Three individuals developed B-cell non-Hodgkin lymphoma (NHL) in the first ten years of their life and two had EBV positive tumors. One individual developed osteosarcoma. No other cancers were reported for this cohort.

Ectodermal features in SIOD do not arise from a defect of NER

Reminiscent of disorders of NER such as xeroderma pigmentosum and Cockayne syndrome, prominent ectodermal features of SIOD include hyperpigmented macules as well as fine sparse hair. Therefore, to test if a defect of NER might contribute to SIOD, we measured hypersensitivity of dermal fibroblasts from a patient with a homozygous deletion of *SMARCAL1* promoter (SD31) [Elizondo et al., 2009] to ultraviolet light (UV) and to the anti-tumor antibiotic illudin S. The latter agent causes damage that is repaired exclusively by transcription coupled (TC)-NER. In contrast to Cockayne syndrome (CS) cells, which have a defect of TC-NER [Jaspers et al., 2002], *SMARCAL1*-deficient fibroblasts were not hypersensitive to either UV or to illudin S treatment (Fig. 1a,b). Also, both UV-induced unscheduled DNA synthesis, a measure of global genomic (GG)-NER activity, and recovery of RNA synthesis inhibition, a measure of TC-NER activity, were unaffected in the SIOD cells (data not shown).

Immunological defects in SIOD do not arise from a defect of NHEJ

The efficient generation of antibodies and T-cell receptors requires competency for double strand break repair, particularly NHEJ [Gellert 2002; Schatz and Ji 2011]. Therefore, to test if SIOD patients had defects of NHEJ that could account for their prominent T-cell immunodeficiency and occasional immunoglobulin deficits [Boerkoel et al., 2000], we checked for a NHEJ defect altering immunoglobulin switching in SIOD patients. We analyzed 25 S μ -S α 1 and 22 S μ -S γ 1 immunoglobulin switch recombination junctions amplified from the peripheral blood DNA of 7 SIOD patients including one who died of NHL and had hypersensitivity to chemotherapy [Basiratnia et al., 2011]. Comparison of the junctions from these patients to those from 4 control individuals did not identify abnormalities such as increased switch junction homology or breakpoint mutations (Supplementary eFigures. S1 and S2 – See Supporting Information online).

Smarcal1-deficient mice are hypersensitive to genotoxic agents

Recent findings have indicated *SMARCAL1* is a component of the DNA stress response and that *SMARCAL1*-deficient cells are hypersensitive to genotoxic agents hydroxyurea and camptothecin [Bansbach et al., 2009; Ciccia et al., 2009; Postow et al., 2009; Yuan et al., 2009; Yusufzai et al., 2009]. Nonetheless, with one exception [Basiratnia et al., 2011], SIOD patients treated for cancer or bone marrow ablation have been given doses of genotoxic agents standard for individuals with immunodeficiency without reports of increased mortality [CFB, unpublished data; Petty et al. 2000]. Therefore to clarify this issue better, we asked if *Smarcal1*^{del/del} mice were hypersensitive to genotoxic agents (Table II). Using growth and splenic cell apoptosis as outcome measures, *Smarcal1*^{del/del} mice were more sensitive to CPT-11, etoposide, or HU than were *Smarcal1*^{+/+} mice (Fig. 2a-l and Fig. 3a-j).

CPT-11, etoposide and HU exposure partially recapitulate the bone phenotype of SIOD in *Smarcal1^{del/del}* mice

We have previously shown that *Smarcal1^{del/del}* mice do not recapitulate SIOD without environmental or genetic factors further compromising gene expression [Baradaran-Heravi et al., 2012]. We asked therefore if this exposure to genotoxic agents would partially or fully recapitulate SIOD in the *Smarcal1^{del/del}* mice. Reminiscent to the growth plates of SIOD patients [Clewing et al., 2007a; Spranger et al., 1991], the distal femur growth plates of *Smarcal1^{del/del}* mice treated with genotoxic agents were hypocellular, and chondrocytes in the proliferation and hypertrophic zones formed less organized columns (Fig. 2m-t, Fig. 3k-v, and Supplementary eFigure S3a). Also, like the reduced bone density observed in some SIOD patients [Hunter et al., 2010; Tylki-Szymanska et al., 2003], micro-computed tomography scanning of the distal femurs revealed significantly reduced bone density in *Smarcal1^{del/del}* mice treated with CPT-11 (Fig. 2u); the bone density was not significantly different from controls after treatment with etoposide or HU (Fig. 3w). Additionally, we hypothesized that if CPT-11 treatment moves chondrocytes past a threshold required for expression of skeletal dysplasia in *Smarcal1^{del/del}* mice, then the growth rate of *Smarcal1^{del/del}* mice should not return to normal when CPT exposure is removed but remain reduced. To test this, we monitored CPT-11-injected mice for another 12 weeks after discontinuation of CPT-11 injections; interestingly, the *Smarcal1^{del/del}* and *Smarcal1^{+/+}* mice had the same growth rates (Figs. 2e and k).

CPT-11, etoposide and HU exposure do not recapitulate the renal phenotype of SIOD in *Smarcal1^{del/del}* mice

Besides skeletal dysplasia, renal failure is another cardinal feature of SIOD and one SIOD patient developed renal failure following treatment with genotoxic agents [Petty et al., 2000]. We hypothesized therefore that the *Smarcal1^{del/del}* mice treated with genotoxic agents might also develop renal failure. Measurement of urine albumin excretion as well as Masson trichrome staining of the kidneys after 8 weeks of exposure to CPT-11, etoposide, and HU did not show increased albuminuria in *Smarcal1^{del/del}* mice or signs of sclerosis compared to controls (Supplementary eFigure S3b-c – See Supporting Information online).

DISCUSSION

Despite superficial similarities to classical disorders of DNA repair, SIOD is a distinct disorder. The ectodermal defects cannot be ascribed to an obvious defect of NER as in Cockayne syndrome or xeroderma pigmentosum, and the immune defects cannot be ascribed to molecular defects of NHEJ as in ataxia telangiectasia and Nijmegen breakage syndrome.

SIOD is not a classical cancer predisposition syndrome in which there is increased occurrence of multiple cancers although there is an increased prevalence of NHL and possibly osteosarcoma. The detection of EBV in two of the three NHL tumors suggests that the increased prevalence of NHL is attributable to the immunodeficiency. NHL is a heterogeneous group of malignancies with variable etiologic, morphologic, immune, genetic, and clinical features [Alexander et al., 2007]. Risk factors for NHL include congenital and acquired immunodeficiency, chronic antigen stimulation, autoimmune disorders, environmental factors, and genetic deficiency of NHEJ enzymes [Grulich and Vajdic 2005; Hill et al., 2006]. Since the prevalence of NHL in the general population during the first ten years of life is 0.0026% [Howlader et al., 2012], the relative risk for NHL in our cohort of SIOD patients is 1,625. Although defective DNA repair might in an undefined manner contribute to this, the absence of recurrent cancers during the lifetime of

SIOD patients suggests that the immunodeficiency is likely the major contributor to the increased risk of NHL in SIOD.

Whether the single SIOD patient with osteosarcoma represents an increased risk for osteosarcoma or is a coincidental co-occurrence is unclear. Assuming that this is not coincidental co-occurrence, the relative risk for osteosarcoma in our cohort of SIOD patients is 150 since the prevalence of osteosarcoma in the general population during the first twenty years of life is 0.0093% [Howlader et al., 2012]. An increased risk for osteosarcoma is also observed in the DNA repair disorders Rothmund-Thompson syndrome, RAPADILINO syndrome and Werner syndrome [Goto et al., 1996; Lindor et al., 2000; Siitonen et al., 2003]. Again, the absence of recurrent cancers during the lifetime of SIOD patients questions whether SIOD is a cancer predisposition disorder unless one assumes the patients die too early to manifest recurrent tumors.

SMARCAL1 does nonetheless play a role in genome maintenance and the DNA stress response [Bansbach et al., 2009; Ciccina et al., 2009; Postow et al., 2009; Yuan et al., 2009; Yusufzai et al., 2009]. As an annealing helicase, SMARCAL1 resolves single to double-stranded DNA transitions [Yusufzai and Kadonaga 2008] and contributes to the maintenance of genomic integrity [Bansbach et al., 2009; Ciccina et al., 2009; Postow et al., 2009; Yuan et al., 2009; Yusufzai et al., 2009]. It also participates in the reactivation of stalled replication forks, associates with double-strand DNA breaks, and facilitates cell cycle progression from the G2 to the M phase [Bansbach et al., 2009; Ciccina et al., 2009; Postow et al., 2009; Yuan et al., 2009; Yusufzai et al., 2009]. Four domains within the SMARCAL1 enzyme are critical to these functions: a nuclear localization signal (NLS), a replication protein A (RPA) binding domain, two tandem HARP domains with annealing helicase activity, and a SWI/SNF ATPase and DNA binding domain [Bansbach et al., 2009; Ciccina et al., 2009; Coleman et al., 2000; Elizondo et al., 2009; Ghosal et al., 2011; Postow et al., 2009; Yuan et al., 2009; Yusufzai et al., 2009]. The deletion of the NLS, RPA and HARP domains is associated with SIOD in humans [Boerkoel et al., 2002; Clewing et al., 2007b], and herein we show that mice with deletion of the homologous domains are hypersensitive to genotoxic agents. Extension of these observations to humans shows that one SIOD patient was hypersensitive to anticancer agents [Basiratnia et al., 2011], another with NHL tolerated standard doses of R-CHOP chemotherapy (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone) but then developed severe infection and delayed neutrophil recovery when treated with one cycle of ifosfamide (76.5ml/hr × 24 hours) and etoposide (61mg) for relapse and another with osteosarcoma tolerated and survived chemotherapeutic regimens adjusted for immunodeficiency. Definitive evidence of whether SIOD patients are hypersensitive to genotoxic agents will therefore only come as more patients are treated for cancer; however, in the interim, caution in the use of such agents in SIOD patients seems advisable.

Since our earlier studies have shown that the *Smarca11^{del/del}* mice did not express features of SIOD unless gene expression was compromised [Baradaran-Heravi et al., 2012], we hypothesized that exposure to genotoxic agents also induced features of SIOD. Indeed, treatment with genotoxic agents did cause growth retardation and skeletal features similar to those observed in SIOD. Additionally, exposure to CPT-11 did not induced a continued loss of growth potential after its removal suggesting that the growth of SIOD patients will be retarded by exposure to genotoxic agents during bone marrow ablation or treatment for cancer but that their growth rate will likely return to baseline when the genotoxic therapy is removed.

In the management of SIOD, bone marrow transplantation is often considered for the immunodeficiency. Of concern, however, is the one SIOD patient who developed renal

failure shortly after bone marrow transplantation [Petty et al., 2000]. The question arises therefore as to whether this was the natural progression of SIOD or prematurely induced by the exposure to the genotoxic agents. The failure of genotoxic agents to induce albuminuria and renal sclerosis in *Smarcal1^{del/del}* mice suggests that despite the potential hypersensitivity of SIOD patients to chemotherapy or bone marrow ablation, short-term treatment with genotoxic agents is unlikely to hasten renal failure. Nonetheless, since the four other SIOD patients treated with bone marrow transplantation died from EBV infection, cerebral haemorrhage, pulmonary embolism or pancreatitis shortly after transplant (C. F. B., unpublished data), definitive evidence of whether genotoxic agents cause long term sequelae in SIOD will only come as more patients survive cancer therapy and bone marrow transplantation. In the interim, the hypersensitivity of the *Smarcal1^{del/del}* mice suggests caution in the use of such agents in SIOD patients.

Regarding screening of SIOD patients for cancer, extra hematologic surveillance or imaging appear unnecessary. Patients should maintain close contact with their physicians, and symptoms that cannot be accounted for otherwise should be evaluated promptly as potential early indicators of cancer.

In summary, SIOD patients have an increased risk for NHL that is likely attributable to their T-cell immunodeficiency and possibly for osteosarcoma; therefore patients should be evaluated promptly for cancer when they have unexplained symptoms. SIOD patients do not have a detectable defect of NER or NHEJ to explain their ectodermal or immunological features, but as shown by the hypersensitivity of *Smarcal1^{del/del}* mice to some DNA damaging agents, some SIOD patients are likely to be hypersensitive to genotoxic drugs. We conclude therefore that extra caution should be observed during bone marrow ablation and administration of genotoxic agents to SIOD patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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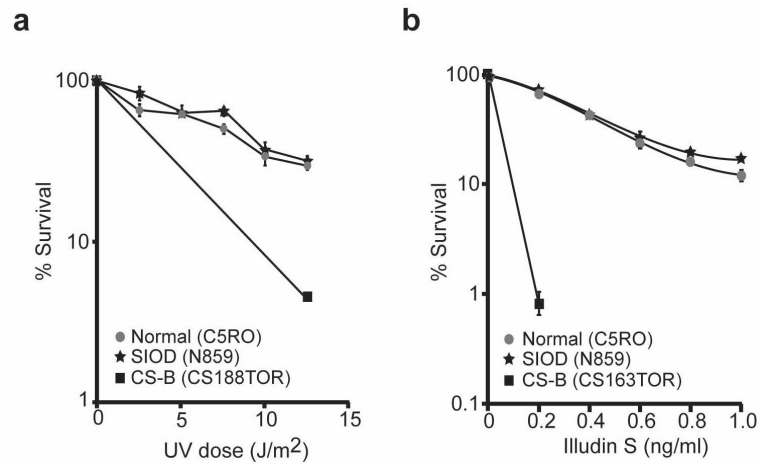
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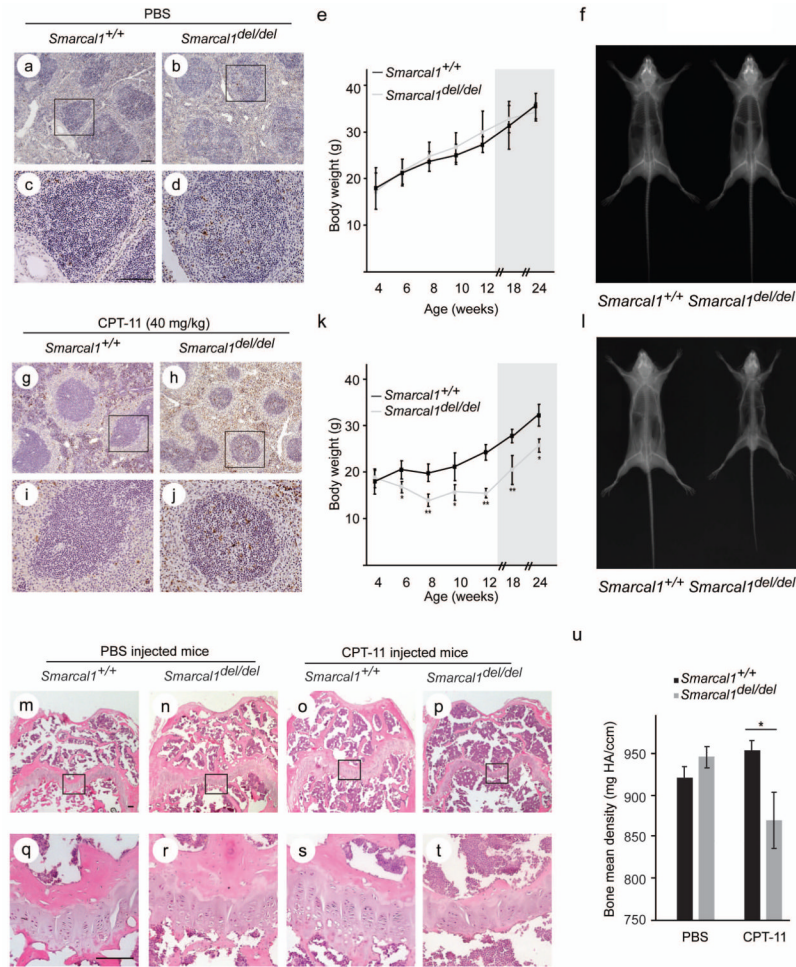
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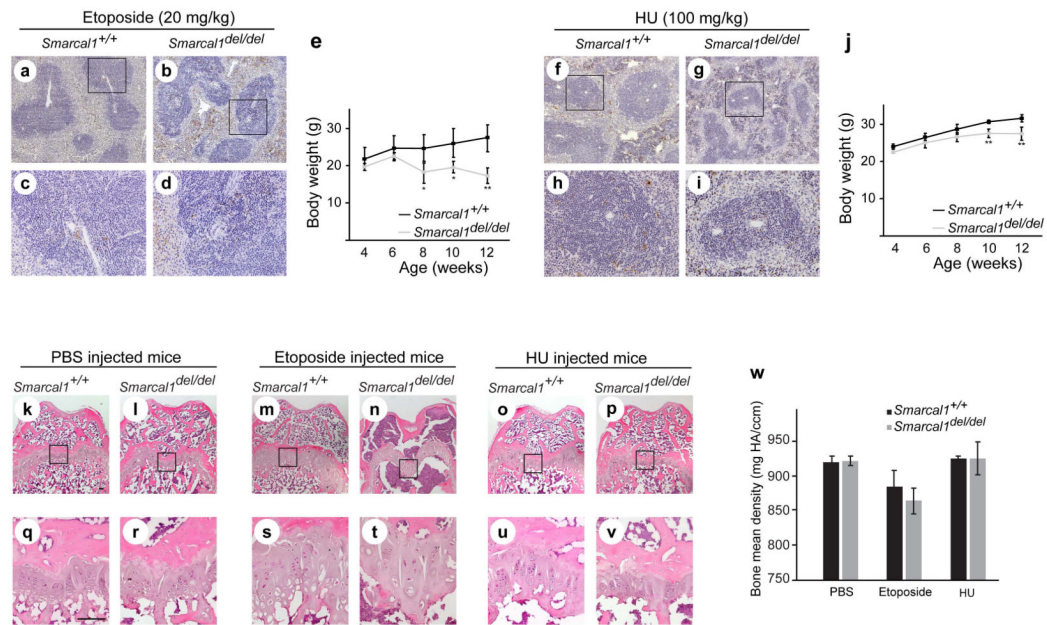
**FIG. 1.**

Analysis of SMARCAL1 participation in nucleotide excision repair. **a:** Survival curve comparing the sensitivity of SIOD (N859), unaffected (C5RO), and Cockayne syndrome B (CS-B, CS188TOR) skin fibroblasts to killing by UV radiation (254 nm). This is a measure of both TC-NER and global NER. **b:** Survival curve comparing the sensitivity of SIOD (N859), unaffected (C5RO), and CS-B (CS163TOR) skin fibroblasts to killing by illudin-S. This is a measure of TC-NER.

**FIG. 2.**

Hypersensitivity of *Smarcal1*^{del/del} mice to CPT-11. **a-d**: Photomicrographs of TUNEL-positive nuclei in splenic tissue from postnatal day (P) 36 *Smarcal1*^{del/del} and *Smarcal1*^{+/+} mice following 5 daily ip-injections with PBS. Panels c and d are higher magnification photomicrographs of the boxed areas in panels a and b, respectively. **e**: Graph showing the average weight gain of *Smarcal1*^{del/del} (n = 4) and *Smarcal1*^{+/+} (n = 4) mice receiving daily ip-injections of PBS from P28 through P84 and during 90 days following treatment (shaded area). **f**: Representative skeletal radiographs of *Smarcal1*^{del/del} and *Smarcal1*^{+/+} mice at P174 following 8 weeks of daily injections with PBS. **g-j**: Photomicrographs of TUNEL-positive nuclei in splenic tissue from P36 *Smarcal1*^{del/del} and *Smarcal1*^{+/+} mice following 5 daily ip-injections with CPT-11. Panels i and j are higher magnification photomicrographs of the boxed areas in panels g and h, respectively. **k**: Graph showing the average weight gain of *Smarcal1*^{del/del} (n = 3) and *Smarcal1*^{+/+} (n = 4) mice receiving daily ip-injections of CPT-11 from P28 through P84 and during 90 days following treatment (shaded area). **l**: Representative skeletal radiographs of *Smarcal1*^{del/del} and *Smarcal1*^{+/+} mice at P173 following 8 weeks of daily injections with CPT-11. **m-t**: Photomicrographs of representative sections of the distal femoral growth plate of *Smarcal1*^{+/+} and *Smarcal1*^{del/del} male mice treated with PBS or CPT-11 for 8 weeks. The tissue was stained with H&E. Panels q-t are higher magnification photomicrographs of the boxed areas in panels m-p, respectively. Note the poorly organized columns of chondrocytes in the smaller hypocellular growth plates of the CPT-11 treated *Smarcal1*^{del/del} mice. **u**: Graph showing the mean bone density of the

distal femur of *Smarcal1*^{+/+} and *Smarcal1*^{del/del} mice treated either with PBS or CPT-11. Scale bars = 100 μ m. Error bars represent one standard deviation. For statistically significant differences between *Smarcal1*^{del/del} and *Smarcal1*^{+/+} mice, a * indicates $P < 0.05$ and a ** indicates $P < 0.01$. Abbreviations: CPT-11, irinotecan; ip, intraperitoneal; mg HA/ccm, milligrams hydroxyapatite per cubic centimetre.

**FIG. 3.**

Hypersensitivity of *Smarcal1^{del/del}* mice to etoposide and HU. **a-d**: Photomicrographs of TUNEL-positive nuclei in splenic tissue from P36 *Smarcal1^{del/del}* and *Smarcal1^{+/+}* mice following 5 daily ip-injections with etoposide. Panels c and d are higher magnification photomicrographs of the boxed areas in panels a and b, respectively. **e**: Graph showing the average weight gain of *Smarcal1^{del/del}* (n = 4) and *Smarcal1^{+/+}* (n=3) mice receiving daily ip-injections of etoposide from P28 through P84. **f-i**: Photomicrographs of TUNEL-positive nuclei in splenic tissue from P36 *Smarcal1^{del/del}* and *Smarcal1^{+/+}* mice following 5 daily ip-injections with hydroxyurea (HU). Panels h and i are higher magnification photomicrographs of the boxed areas in panels f and g, respectively. **j**: Graph showing the average weight gain of *Smarcal1^{del/del}* (n = 5) and *Smarcal1^{+/+}* (n = 4) mice receiving daily ip-injections of HU from P28 through P84. **k-v**: Photomicrographs of representative sections of the distal femoral growth plate of *Smarcal1^{+/+}* and *Smarcal1^{del/del}* male mice treated with PBS, etoposide, or HU for 8 weeks. The tissue was stained with H&E. Panels q-v are higher magnification photomicrographs of the boxed areas in panels k-p, respectively. Note the poorly organized columns of chondrocytes in the hypocellular growth plates of the etoposide and HU treated *Smarcal1^{del/del}* mice. Also note the near absence of trabeculae in the etoposide treated *Smarcal1^{del/del}* mice. **w**: Graph showing the bone mean density of the distal femur of *Smarcal1^{+/+}* and *Smarcal1^{del/del}* mice treated with PBS, etoposide or HU. Scale bars = 100 μ m. Error bars represent one standard deviation. For statistically significant differences between *Smarcal1^{del/del}* and *Smarcal1^{+/+}* mice, a * indicates $P < 0.05$ and a ** indicates $P < 0.01$. Abbreviations: HU, hydroxyurea; ip, intraperitoneal; mg HA/ccm, milligrams hydroxyapatite per cubic centimetre.

TABLE I

Comparison of the prominent clinical features of SIOD to those of some DNA repair disorders [Ciccia and Elledge 2010; Friedberg et al., 2006; Kubota et al., 1993; Sriver et al., 2001].

Clinical Feature	BS	FA	CS	AT	NBS	XP	WS	RTS	RS	SIOD
Growth deficiency	+	+	+	+/-	+	+	+	+	+	+
Radial ray defects	-	+	-	-	-	-	-	+	+	-
Cognitive deficits	-	+	+	-	+	+	-	-	-	-
Ataxia	-	-	+	+	-	+/-	-	-	-	-
Corneal or lens defects	+	+	+	-	-	+	+	+	-	+
Immunodeficiency	+	-	-	+	+	-	-	+/-	-	+
Single cytopenias	+	+	-	+	+	-	-	+/-	-	+
Pancytopenia	-	+	-	-	-	-	-	+/-	-	+
Malignancy predisposition	+	+	-	+	+	+	+	+	+	-
Photosensitivity	+	-	+	-	-	+	-	+	+	-
Radiosensitivity	-	+/-	-	+	+	-	-	+	NR	-
Abnormal pigmentation	+	+	+	+/-	+	+	+	+	+	+
Telangiectasias	+	-	-	+	-	+/-	-	+	-	-
Thin hair	-	+	+	-	-	-	+	+	-	+
Premature atherosclerosis	-	-	+	+	-	-	+	-	NR	+
Hypogonadism	+	+	+	+	+	+	+	+	NR	+
Chromosomal instability	+	+	-	+	+	-	+	+	NR	-
Poor lymphocyte mitogenic response	+	-	-	+	+	-	-	+/-	-	+
DNA repair defect	HR	ICL repair, HR	TC-NER	DSB repair	DSB repair, replication fork repair	NER	HR, BER, telomere maintenance	BER, HR?	Resolution of stalled replication/transcription intermediates	Replication fork repair

Abbreviations: BS, Bloom syndrome; FA, Fanconi anemia; CS, Cockayne syndrome; AT, Ataxia telangiectasia; NBS, Nijmegen breakage syndrome; XP, Xeroderma pigmentosum; WS, Werner syndrome; RTS, Rothmund-Thomson syndrome; RS, RAPADILINO syndrome; +, common feature; -, uncommon feature; +/-, presented as case reports; NR, not reported; HR, Homologous recombination; ICL, interstrand crosslink; TC-NER, Transcription-coupled nucleotide excision repair; DSB, Double-strand break; BER, Base excision repair.

TABLE II

Response of *Smarcal1^{+/+}* and *Smarcal1^{del/del}* mice to exposure to CPT-11, etoposide and hydroxyurea.

Compound	Dose (mg/kg)	Frequency	Phenotype	
			<i>Smarcal1^{+/+}</i>	<i>Smarcal1^{del/del}</i>
CPT-11	40	daily × 56 days	Intermittent diarrhea	Chronic diarrhea, weakness, severe growth arrest
	80	daily × 5 days (1 week rest) daily × 10 days	Weight loss (2:3), mild diarrhea (2:3)	Weight loss (3:3), diarrhea (3:3), severe weakness and death (2:3)
Etoposide	20	daily × 56 days	Normal	Severe growth arrest
	40	daily × 5 days	Weight loss (3:3), severe weakness (2:3), death (1:3)	Weight loss and severe weakness (4:4), death (2:4)
Hydroxyurea	100	daily × 56 days	Normal	Mild growth arrest
	500	daily × 10 days	Weight loss (4:4), death (1:4)	Weight loss (4:4), severe weakness and death (3:4)