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Schimke Immuno-Osseous Dysplasia: A Cell Autonomous Disorder?

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SMARCAL1 (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a-like protein 1) encodes a SWI/SNF ATP-dependent chromatin remodeling protein. Mutations in *SMARCAL1* cause the autosomal-recessive multisystem disorder Schimke immuno-osseous dysplasia (SIOD); this suggests that the SMARCAL1 protein is involved in the development or maintenance of multiple organs. Disease within these many tissues could arise by a cell autonomous or a cell non-autonomous mechanism. Consistent with a cell autonomous mechanism, we did not find any disease recurrence in transplanted organs or protection of other tissues by the organ grafts. In order to better understand the role of SMARCAL1 during normal development and in the pathogenesis of SIOD, we characterized the spatial and temporal expression of the murine homolog (Smarcal1). The Smarcal1 mRNA and protein were expressed throughout development and in all tissues affected in patients with SIOD including the bone, kidney, thymus, thyroid, tooth, bone marrow, hair, eye, and blood vessels. Significantly, the expression profile of Smarcal1 in the mouse has led us to reexamine and identify novel pathology in our patient population resulting in changes in the clinical management of SIOD. The expression of Smarcal1 in affected tissues and the non-recurrence of disease in grafted organs lead us to hypothesize a cell autonomous function for SMARCAL1 and to propose tissue-specific mechanisms for the pathophysiology of SIOD.
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INTRODUCTION

Schimke immuno-osseous dysplasia (SIOD, OMIM 242900) is an autosomal recessive multisystem disorder. The relatively invariant features of this disease are spondyloepiphyseal dysplasia, renal insufficiency, and T-cell immunodeficiency [Schimke et al., 1971; Ehrich et al., 1990; Spranger et al., 1991; Saraiva et al., 1999]. Additional features that are variably expressed among SIOD patients include hypothyroidism, abnormal dentition, bone marrow failure, thin hair, corneal opacities, arteriosclerosis, and headaches [Spranger et al., 1991; Saraiva et al., 1999; Boerkoel et al., 2000; da Fonseca, 2000; Dhillon et al., 2001; Kilic et al., 2005].

The severity of SIOD is a continuum. At one end of the spectrum, severely affected patients develop symptoms early and usually die within the first 5 years of life [Boerkoel et al., 2000]. In contrast, mildly affected patients develop symptoms late in the first decade or early in the second decade of life and can live into middle age [Hashimoto et al., 1994; Lama et al., 1995; Boerkoel et al., 2000; Ieshima, 2000]. Patients with severe disease frequently develop hypothyroidism, episodic cerebral ischemia, migraine-like headaches, and bone marrow failure [Schimke et al., 1971; Spranger et al., 1991; Ehrich et al., 1995; Schmidt et al., 1997; Saraiva et al., 1999; Boerkoel et al., 2000], whereas patients with milder disease rarely manifest these additional problems [Boerkoel et al., 2000].

Putative loss-of-function mutations in *SMARCAL1* (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a-like 1) result in

Leah I. Elizondo and Cheng Huang contributed equally to this work.

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SIOD [Boerkoel et al., 2002]. *SMARCAL1* encodes a protein homologous to the superfamily 2 (SF2) helicases and the sucrose non-fermenting type 2 (SNF2) family of chromatin remodeling proteins [Coleman et al., 2000]. SF2 helicases include DNA and RNA helicases that unwind duplex DNA and RNA, respectively [Marians, 1997; Korolev et al., 1998]. The SNF2-related proteins restructure DNA-histone interactions and thus mediate chromatin remodeling [Pazin and Kadonaga, 1997; Havas et al., 2001].

Mutations of other SF2 helicases and SNF2 chromatin remodeling proteins have been associated with several diseases. Diseases associated with SF2 helicases include Werner syndrome (OMIM # 277700), Bloom syndrome (OMIM # 210900), and Rothmund-Thompson syndrome (OMIM # 268400); these diseases are characterized by defective DNA recombination [Duker, 2002]. Diseases associated with SNF2 chromatin remodeling proteins include ATR-X syndrome (OMIM # 301040), Cockayne syndrome type II (OMIM # 133540), Cerebro-oculo-facio-skeletal syndrome (OMIM # 214150), and neoplasia. The SNF2 chromatin remodeling proteins associated with neoplasia include SMARCA2 (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 2, OMIM # 600014), SMARCA4 (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 4, OMIM # 603254), and SMARCB1 (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily b, member 1, OMIM # 601607).

Prior studies have postulated that SIOD might be an autoimmune disorder [Spranger et al., 1991; Kaitila et al., 1998], a connective tissue disorder [Ludman et al., 1993; Lama et al., 1995; Boerkoel et al., 1998], a vascular endothelial disorder [Ehrich et al., 1995; Ehrich and Filler, 1996; Lücke et al., 2005], a metabolic disorder affecting chondrocyte and T-cell differentiation [Spranger et al., 1991; Lama et al., 1995], or a cellular proliferation disorder [Boerkoel et al., 2000; Boerkoel et al., 2002]. As a first step toward clarifying the pathophysiology of SIOD, we herein summarize the clinical features of SIOD and correlate those with the expression profile of the murine homolog of SMARCAL1 (Smarcal1). Clinically, we documented that tissue and organ grafts do not rescue other organs or tissues, and that disease did not reoccur in these organ grafts. Experimentally, we found that Smarcal1 was expressed in proliferating and non-proliferating cells and in each tissue affected in SIOD as well as in additional tissues. Directed by this expression pattern, we have reexamined our patient population and have identified pathology in some of these additional tissues. Our results highlight the importance of translational research in vertebrate models where experimental results can directly provide additional information to enhance patient care.

MATERIALS AND METHODS

Human Subjects

Patients referred to this study gave informed consent approved by the Institutional Review Board of Baylor College of Medicine (Houston, TX, IRB protocol: H-9669) or the Hospital for Sick Children (Toronto, ON, Canada). The clinical data for patients were obtained from questionnaires completed by the referring physician as well as from medical records and summaries provided by that physician.

Vertebrate Animals

Mice used in this study were housed, bred, and sacrificed in accordance with accepted ethical guidelines. These procedures were approved by the Institutional Review Board of Baylor College of Medicine (IRB protocol: AN-2983).

Northern Blot Analysis

The mouse developmental Northern blot (20 μg of total RNA/lane, SeeGene, Seoul, Korea) and the adult tissue Northern blot (2 μg of mRNA, Ambion, Inc., Austin, TX, USA) were probed with ^{32}P DNA probes synthesized from the 5′UTR of the Smarcal1 cDNA using a random-primed DNA labeling kit (Amersham Biosciences, Piscataway, NJ, USA, RPN1633). To standardize for RNA loading in each lane, each blot was stripped of the Smarcal1 probe with Tris-HCl buffer at 100°C and rehybridized with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe.

Western Blotting

The mouse tissues were dissected, snap frozen in liquid nitrogen, and stored at −80°C. Tissues were homogenized in 2× SDS sample buffer (Invitrogen Corporation, Carlsbad, CA, USA) and boiled for 5 min. The samples were fractionated on a 10% SDS-PAGE gel and transferred to a PVDF membrane. After blocking with phosphate buffered saline containing 0.2% I-Block (Applied Biosystems, Foster City, CA, USA) overnight at 4°C, a 1:2,500 dilution of the anti-SMARCAL1 rabbit polyclonal antibody and a 1:5,000 dilution of anti-GAPDH mouse monoclonal antibody (MAB374, Chemicon International, Inc., Temecula, CA, USA) were incubated with the membrane for 1 hr at room temperature. Following incubation with the primary antibodies, the Western blots were washed with blocking buffer four times for 15 min each at room temperature and then incubated with alkaline phosphatase conjugated secondary antibodies (anti-rabbit IgG and anti-mouse IgG: A2556 and A3562, respectively, Sigma-Aldrich, St. Louis, MO, USA) for 1 hr at room temperature. The blots were then washed four times for 15 min each at room temperature.

The bound antibody was detected by chemiluminescence using CDP-Star (Applied Biosystems) according to the manufacturer's specifications.

Immunohistochemistry

The anti-SMARCAL1 serum and the immunohistochemistry procedures were as described by Kilic et al. [2005] with the following modifications [Kilic et al., 2005]. We dissected mouse embryos and tissues directly in a 4% paraformaldehyde (PFA) bath and then fixed the tissues in 4% PFA overnight at 4°C. Kidney fixation required that the tissue be hemisected to allow for the penetration of the 4% PFA through the capsule. Following incubation with the polyclonal rabbit anti-human SMARCAL1, the sections were incubated with biotinylated goat anti-rabbit secondary antibodies (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA). Images were acquired using a Zeiss Axiovert 200 microscope, a Zeiss AxiocamHR camera, and the Zeiss Axiovision imaging system.

RESULTS

SIOD Patient Treatment Summary

To ascertain the progression of symptoms and results of organ transplantation in SIOD patients, we reviewed the clinical data of 41 SIOD patients with identified *SMARCAL1* mutations. There were no consistent markers suggestive of an autoimmune disease such as an elevated C-reactive protein, erythrocyte sedimentation rate, anti-neutrophil antibodies, or inflammatory infiltrates on tissue biopsies. Except for the elevated thyroid stimulating hormone (TSH) and low T3 and T4 levels found in 11 patients, there were no other consistent endocrine deficiencies. Treatment with levothyroxine did not prevent progression of renal, vascular, or immunological disease.

Fourteen of the 41 patients received a renal transplant and none of the transplanted kidneys had recurrence of focal segmental glomerulosclerosis, the renal pathology observed in SIOD. Seven of these patients subsequently developed symptoms of cerebral ischemia. Thus, the renal transplantation did not protect them from the cerebrovascular complications.

As previously reported, bone marrow transplantation successfully rescued the immunodeficiency in one patient; however, this did not prevent development of renal failure [Petty et al., 2000], and combined bone marrow and renal transplantation in the same patient did not prevent the development of central nervous system symptoms. Consistent with the lack of elevation of autoimmune markers, this distinction between transplanted and non-transplanted tissue further supports the hypothesis that SIOD is a cell autonomous defect rather than having an autoimmune or hormonal origin.

Smarcal1 Expression During Mouse Development

The Smarcal1 protein is 76% similar and 70% identical to human SMARCAL1 and shares the same functional domains (Fig. 1A). By Northern blot analysis, Smarcal1 mRNA was expressed in embryos from embryonic day (E) 4.5 to E18.5 (Fig. 1B) and in adult tissues (Fig. 1C). The 3-kb transcript in the mouse is comparable in size to that observed in the human [Boerkoel et al., 2000; Coleman et al., 2000] and was the only transcript identifiable in the National Center for Biotechnology Information (NCBI) mouse expressed-sequence-tag database or by 5′ and 3′ RACE. Using an antiserum against human SMARCAL1 that cross-reacts with Smarcal1 [Kilic et al., 2005], our Western analyses detected a single protein band of 100 kDa, the size predicted from the sequence of the 3-kb transcript (Fig. 1D).

Consistent with our Northern analyses, Smarcal1 end sequence tags (ESTs) were detected in most mouse tissues (http://www.ncbi.nlm.nih.gov/UniGene/ESTProfileViewer. cgi?uglist=Mm.274232). The Smarcal1 ESTs were first detected in the preimplantation embryo and then reported for each stage through adulthood. Within the adult mouse, ESTs were reported for bone, bone marrow, brain, eye, heart, kidney, liver, lung, lymph node, mammary gland, ovary, pancreas, pituitary, skin, spleen, stomach, testis, thymus, and uterus.

Distribution of Smarcal1 Expression in Tissues Usually Affected by SIOD

To define further the temporal and tissue-specific expression of Smarcal1 during development, we performed in situ hybridization and immunohistochemistry using mice staged from E7.5 to postnatal day (P) 7.

Bone. Generally individuals with SIOD have spondyloepiphyseal dysplasia in which their vertebrae are ovoid and mildly flattened and their femoral epiphyses are small and laterally displaced [Schimke et al., 1971; Ehrich et al., 1990; Spranger et al., 1991; Boerkoel et al., 2000]. In the mouse, the Smarcal1 mRNA (summarized in Fig. 4) and protein were expressed in the anlagen of all bones of the axial and appendicular skeleton; expression was first detected at E15.5 but was not prominent until E18.5. Interestingly, Smarcal1 was expressed throughout the growth plate of developing long bones (Fig. 2A,C) including the resting, proliferative, and hypertrophic zones; this contradicts our initial hypothesis that SIOD was solely due to a defect in proliferation. Thus, loss of SMARCAL1 expression in SIOD patients may affect not only the proliferation but also the differentiation of chondrocytes.

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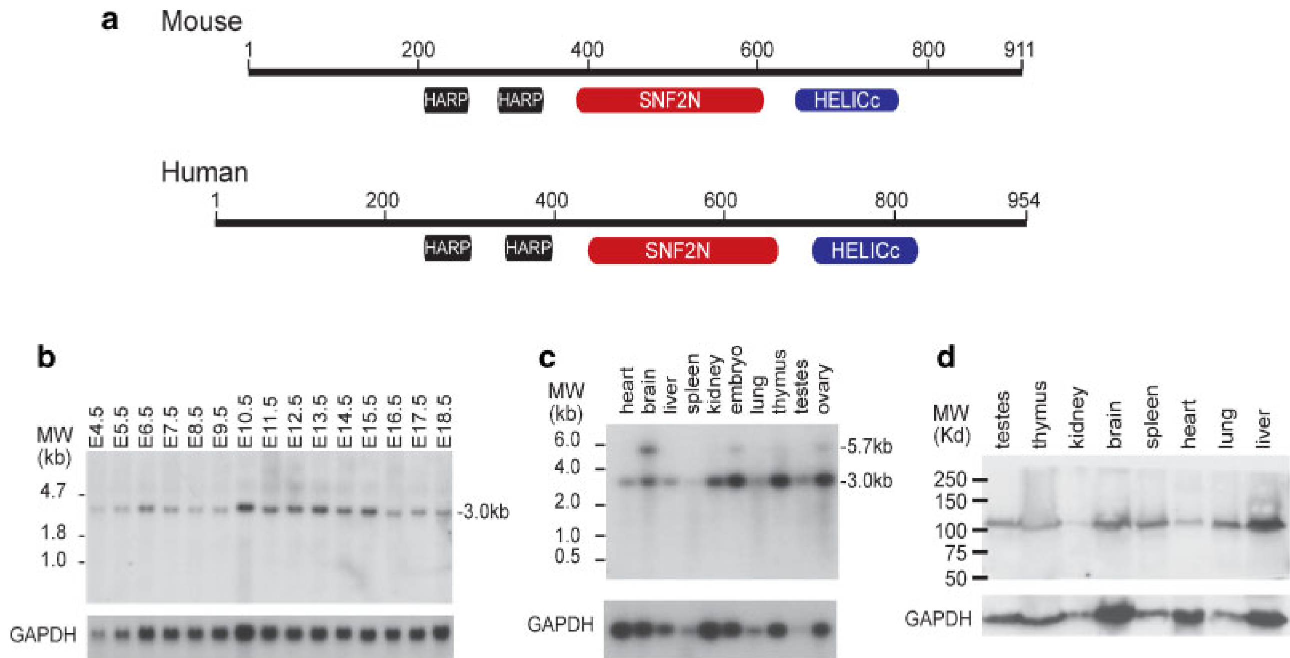

Fig. 1. Spatial and temporal expression profiles of Smarcal1. **a**: Diagram comparing the structure of the Smarcal1 and SMARCAL1 proteins. Briefly the represented domains are HARP: HepA related protein domain (This is the defining domain of the SMARCAL1 class of enzymes; its function is unknown), SNF2: sucrose non-fermenting 2 domain (DNA-coupled ATPase), and HELICc: helicase c domain (function undefined in SMARCAL1). **b**: Northern blot analysis of Smarcal1 at different developmental stages of mouse embryos and adult tissues. E4.5-E18.5 mouse embryos were used for Northern blot analysis. Twenty micrograms of total RNA was loaded into each lane. GAPDH was blotted as an internal control. **c**: Northern blot analysis using adult mouse tissues. **d**: Western blot analysis of Smarcal1 protein expression in adult mouse tissues. One protein band was detected in all tissue analyzed. MW, molecular weight; kb, kilobases; kDa, kilodaltons.

Thymus. T-cell deficiency occurs in nearly all patients with SIOD and results in opportunistic infections in approximately half of the patients. We examined the expression of Smarcal1 mRNA (summarized in Fig. 4) and protein within the thymus since it is within this tissue that T-cell precursors mature. Within the thymus, Smarcal1 was expressed from E13.5 to P7 in a small subset of cells. Based on morphology, most of these cells appear to be a subset of the lymphocytes and not epithelial reticular cells (Fig. 2E,G). This expression pattern would support the hypothesis that the loss of SMARCAL1 expression results in a cell autonomous affect on T cells.

Kidney. All patients with SIOD develop renal dysfunction. This begins as proteinuria and frequently progresses to end-stage renal failure [Schimke et al., 1971; Ehrich et al., 1990; Ludman et al., 1993; Boerkoel et al., 2000]. The renal pathology most commonly reported is focal segmental glomerulosclerosis (FSGS) [Ehrich et al., 1990]. The Smarcal1 protein was first detected in renal tubules and glomeruli at E18.5 and continued to be expressed in postnatal renal tubules and in podocytes within glomeruli (Fig. 2I,K,M). Since glomerular filtration and tubular absorption regulate the composition of the urine, mutation of SMARCAL1 may lead to proteinuria by affecting both glomerular and tubular function.

Distribution of Smarcal1 Expression in Tissues Variably Affected by SIOD

In addition to spondyloepiphyseal dysplasia, renal failure, and T-cell deficiency, SIOD patients have variable involvement of other organ systems. These include defects in the thyroid, other blood cell lineages in addition to T cells, hair, eyes, and blood vessels. This variability cannot be readily ascribed to differences in the SMARCAL1 mutations because it manifests both among and within families [Lücke et al., 2005].

Thyroid. As described above, a quarter of SIOD patients with SMARCAL1 mutations had elevated TSH levels. The Smarcal1 mRNA (summarized in Fig. 4) and protein were expressed in the developing thyroid by E15.5 (Fig. 3A), and the Smarcal1 protein showed continued expression within the nucleus of P0 thyroid follicular cells (Fig. 3C). Follicular cells synthesize and secrete tetraiodothyronine (T4) and triiodothyronine (T3). This expression pattern and the responsiveness of the elevated TSH levels in patients to levothyroxine therapy suggest that mutation of SMARCAL1 disrupts pathways leading to the synthesis and/or the secretion of T4 and T3 [Van Vliet, 2003]; therefore, primary thyroid dysfunction, not nephrotic syndrome, likely accounts for the clinical observation of reduced T3 and T4 levels and elevated TSH levels.

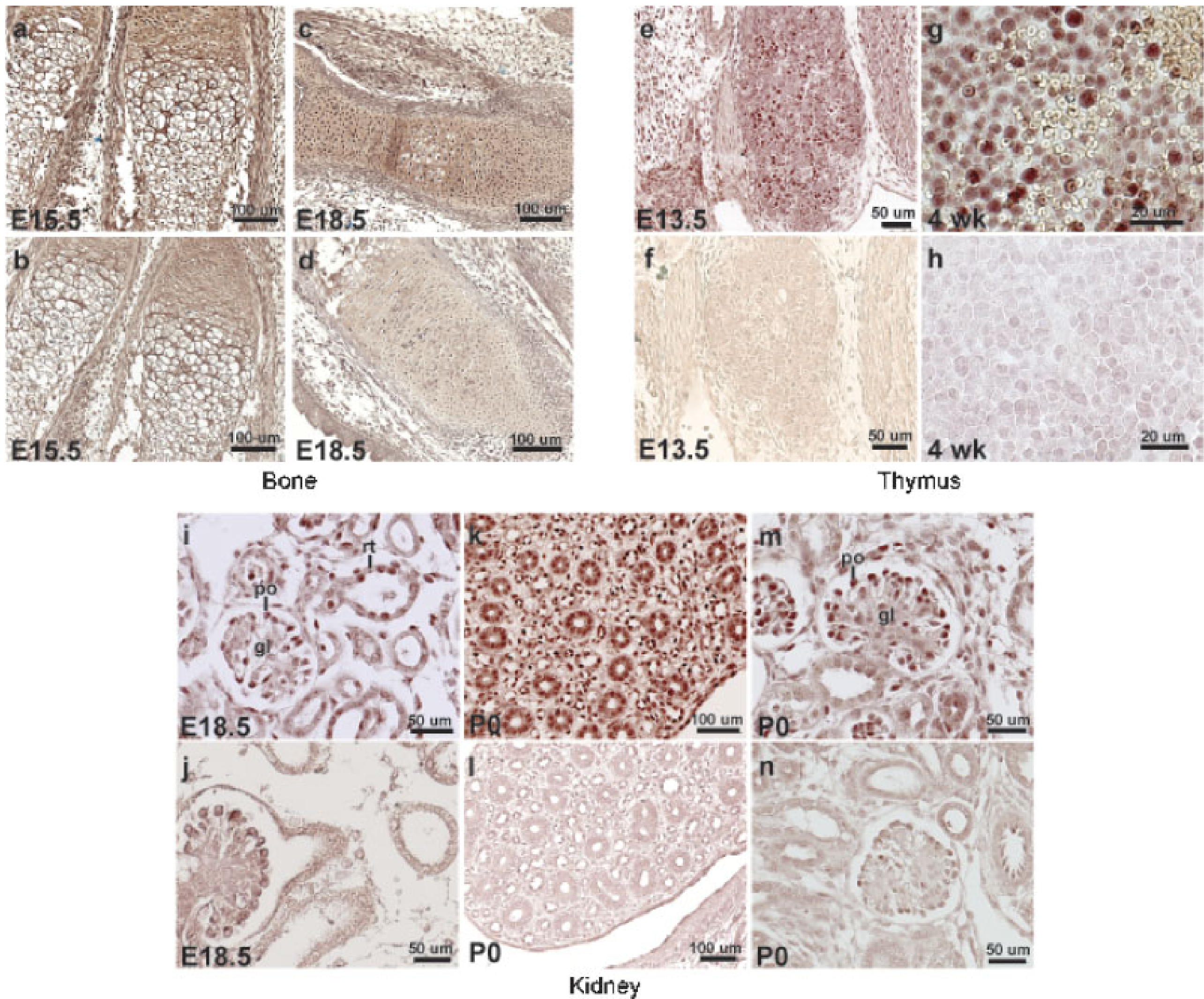

Fig. 2. Smarcal1 protein expression in mouse tissues commonly affected in SIOD. The immunohistochemistry was performed using immune (upper panel) and preimmune (lower panel) serum to stain serial sections. **a**, **b**: E15.5 forelimb showing expression in chondrocytes throughout the growth plate. **c**, **d**: E18.5 digit showing expression throughout the growth plate. **e**, **f**: E13.5 thymus. **g**, **h**: Higher magnification of a 4-week-old thymus. **i**, **j**: E18.5 kidney with staining in renal tubules and glomeruli cells. **k**, **l**: P0 renal tubules. **m**, **n**: P0 kidney showing staining in podocytes within the glomerulus. Ch: chondrocytes; gl: glomerulus; rt: renal tubules; po: podocytes.

Teeth. Another pathology of SIOD is small or absent secondary teeth [da Fonseca, 2000; Ludman et al., 1993]. To explore a possible role for Smarcal1 in teeth development, we analyzed the expression pattern of Smarcal1 in developing murine teeth. At E18.5, Smarcal1 mRNA was weakly expressed in the dental epithelium and the dental lamina (summarized in Fig. 4). By P0, Smarcal1 mRNA and protein were expressed in the developing ameloblasts, odontoblasts, enamel (stellate) reticulum, and dental papilla (Fig. 3E). Since mice do not develop secondary teeth, we hypothesize that SMARCAL1 may be similarly expressed in human secondary teeth and regulate the development, migration, or proliferation of the secondary tooth anlagen. This would be consistent with the hypodontia observed in SIOD patients.

Hematopoietic system. In addition to T-cell deficiency, several SIOD patients suffer from deficiencies of other blood cell lineages [Boerkoel et al., 2000]. Expression of the Smarcal1 protein was detected in a subset bone marrow cells beginning at E18.5 (Fig. 3G). The cellular morphology was not indicative of a particular lineage or of stromal cells. Despite the small number of cells expressing Smarcal1, loss of SMARCAL1 expression in patients could reduce the production of several blood cell lineages either by affecting progenitors or by impairing an inductive interaction.

Hair. Abnormally fine hair is observed in approximately 75% of SIOD patients [Boerkoel et al., 2000]. Beginning at E18.5, we observed expression of Smarcal1 mRNA (summarized in Fig. 4) and protein in the hair bulbs and proximal

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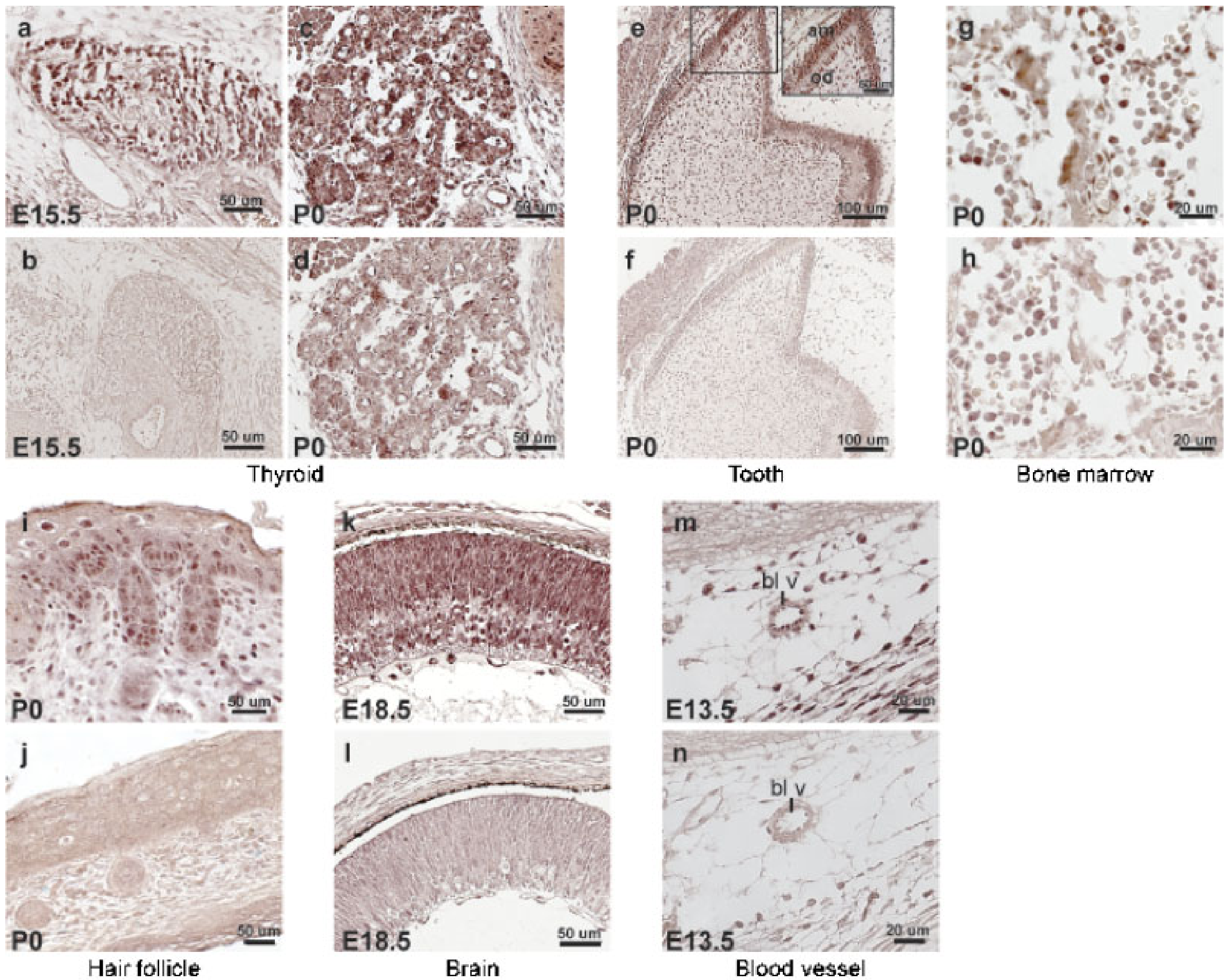

Fig. 3. Smarcal1 protein expression in mouse tissues variably affected in SIOD. The immunohistochemistry was performed using immune (upper panel) and preimmune (lower panel) serum to stain serial sections. **a**, **b**: E15.5 thyroid. **c**, **d**: P0 thyroid epithelium; note that the follicular cells stain positive for Smarcal1 protein. **e**, **f**: P0 tooth with Smarcal1 expression in the developing ameloblasts and odontoblasts. **g**, **h**: Smarcal1-positive cells within P0 bone marrow. **i**, **j**: P0 hair follicles. **k**, **l**: Retina of an E18.5 eye. **m**, **n**: Cerebellar blood vessel at E13.5. am: ameloblasts; od: odontoblasts; bl v: blood vessel.

cortex of the hair shaft (Fig. 3I). Since hair growth occurs from cells at the base of the bulb, this expression pattern suggests that mutation of *SMARCAL1* may cause impairment in hair growth.

Eye. Some SIOD patients also suffer from ocular abnormalities such as corneal opacities, myopia, astigmatism, and optic nerve atrophy [Boerkoel et al., 2000]. Beginning at E18.5, Smarcal1 is expressed in the retina (Fig. 3K) and lens (summarized in Fig. 4). Thus, loss of functional SMARCAL1 could directly affect the cells involved in the ocular abnormalities observed among SIOD patients.

Blood vessels. Approximately half of patients suffer from central nervous system ischemia and many of those have indications of arteriosclerosis [Spranger et al., 1991; Ehrich et al., 1995]. In the mouse, we detected Smarcal1 protein expression in the endothelium of cranial and systemic blood vessels as early as E11.5 and this expression continued into adulthood (Fig. 3M). This expression profile is consistent with the proposal that *SMARCAL1* mutations cause endothelial dysfunction and secondary arteriosclerosis [Lücke et al., 2004].

Distribution of Smarcal1 Expression in Tissues Reportedly Unaffected by SIOD

Besides the tissues reported affected by SIOD, we observed that the Smarcal1 mRNA and protein were expressed in several additional tissues (summarized in Fig. 4). Smarcal1 is highly expressed in neural tissue, such as the brain, sympathetic trunk, spinal cord, and dorsal root ganglia. It is also expressed in the olfactory epithelium from E14.5 into adulthood. Outside of the nervous system, Smarcal1 mRNA and protein can be detected within the developing heart, skeletal muscle, pancreas, germ cells, testes, and ovaries. Expression within these tissues suggests that

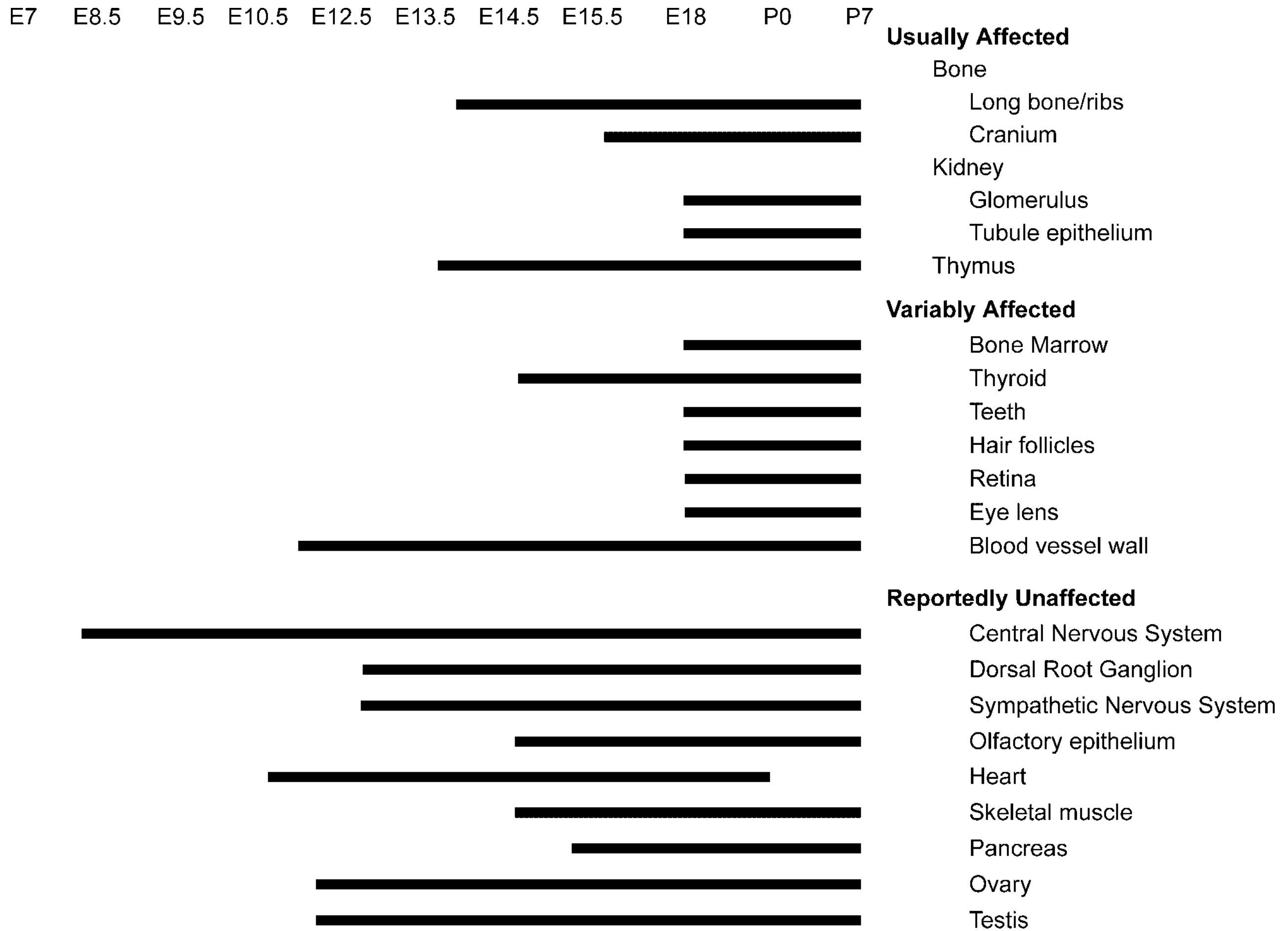

Fig. 4. Summary of the developmental expression of the Smarcal1 mRNA and protein from embryonic day 7 to postnatal day 7. The bars represent the stages when Smarcal1 was observed in the corresponding tissue as determined by mRNA in situ hybridization and immunohistochemistry.

mutations in *SMARCAL1* may have additional effects beyond those that have been clinically reported.

DISCUSSION

Mutations of *SMARCAL1* cause SIOD, a multisystem disorder [Coleman et al., 2000; Boerkoel et al., 2002]. The pathophysiology of SIOD has been variously postulated to be an autoimmune disorder [Spranger et al., 1991; Kaitila et al., 1998], a connective tissue disorder [Ludman et al., 1993; Lama et al., 1995; Boerkoel et al., 1998], a vascular endothelial disorder [Ehrich et al., 1995; Ehrich and Filler, 1996; Lücke et al., 2005], a metabolic disorder affecting chondrocyte and T-cell differentiation [Spranger et al., 1991; Lama et al., 1995], or a cellular proliferation disorder [Boerkoel et al., 2000; Boerkoel et al., 2002]. This report documents that SIOD patients with identified *SMARCAL1* mutations do not express markers of autoimmune disease or consistent endocrine disturbances. We also found that disease did not recur in transplanted renal and bone marrow tissue and, that neither organ transplant protected other tissues from disease progression. In addition, Lücke et al. [2004], recently showed that atherosclerosis did not develop in the renal graft of an SIOD patient [Lücke et al., 2004]; therefore, even the blood vessels of the transplanted organ are protected from systemic disease. These results strongly suggest that the disease is intrinsic to the affected organs and is not imposed from outside as would be observed with an autoimmune or endocrine disorder. Consistent with this, we found that Smarcal1 was expressed in each murine tissue equivalent to those affected in SIOD patients but was not expressed in unaffected tissues such as the liver and lungs. These results strongly support the hypothesis that loss of SMARCAL1 causes disease through a cell autonomous mechanism.

SMARCAL1 encodes a putative SF2 helicase or SNF2 chromatin remodeling factor. The SF2 helicases associated with human disease prevent hyperrecombination and the resultant chromosome breakage [Duker, 2002]. Unlike patients with those diseases,

SIOD patients do not have an increased cancer incidence and their cells do not show increased rates of chromosome breakage and sister chromatid exchange [Boerkoel et al., 2000]. The SNF2 chromatin remodeling factor ERCC6 facilitates transcription-coupled DNA repair; however, in contrast to Cockayne syndrome patients, SIOD patients do not have hypersensitivity to ultraviolet radiation and their cells do not show decreased RNA transcription following exposure to ultraviolet radiation [Boerkoel et al., 2000]. Additionally, cells from SIOD patients exhibit normal DNA repair following exposure to gamma radiation [Boerkoel et al., 2000]. These results suggest that SMARCAL1 is not a cell autonomous regulator of DNA repair.

Based on the poor growth of SIOD patients and the unresponsiveness of their T-cells to mitogens, we had previously proposed that SMARCAL1 might regulate cellular proliferation [Boerkoel et al., 2002]. However, Smarcal1 was not expressed in the proliferating cells of the developing liver, lung, and skin and was highly expressed in postmitotic neurons and resting chondrocytes. Thus, although SMARCAL1 might regulate cellular proliferation in some tissues, it does not appear to regulate the proliferation of all tissues or to be limited to regulation of cellular proliferation.

Interestingly, we also observed expression of Smarcal1 in several tissues that have not been reported as affected in patients with SIOD. These tissues included the central nervous system (brain and spinal cord), the peripheral nervous system (sympathetic trunk, dorsal root ganglia, retina, and neurosensory tissues such as the olfactory epithelium and vibrissae), skeletal muscle, pancreas, germ cells, and reproductive organs. To ascertain the utility of this expression pattern in identifying additional pathologies in SIOD, we focused on the function of the central nervous system. Firstly, we observed that nearly half of SIOD patients have severe migraine-like headaches [Kilic et al., 2005]. Secondly, we found neural migration defects in the postmortem brain tissue of SIOD patients [Boerkoel, paper in progress]. Given the success of this strategy for identifying more subtle features of SIOD, we are reevaluating patients for disease in the other Smarcal1-expressing tissues.

In summary, the clinical evidence suggests that SMARCAL1 mutations cause disease by a cell autonomous mechanism. Additionally, consistent with a cell autonomous disease, we observed Smarcal1 expression in all murine tissues equivalent to those affected in SIOD patients. Further confirming the hypothesis of cell autonomy, we also found disease in tissues expressing Smarcal1 but not previously reported as affected in patients with SIOD. On this basis, we recommend that physicians be aware of the possibility of disease in other expressing tissues that have not been previously reported.

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