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Update From the 2011 International Schwannomatosis Workshop: From Genetics to Diagnostic Criteria

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Abstract

Schwannomatosis is the third major form of neurofibromatosis and is characterized by the development of multiple schwannomas in the absence of bilateral vestibular schwannomas. The 2011 Schwannomatosis Update was organized by the Children's Tumor Foundation (www.ctf.org) and held in Los Angeles, CA, from June 5–8, 2011. This article summarizes the highlights presented at the Conference and represents the "state-of-the-field" in 2011. Genetic studies indicate that constitutional mutations in the *SMARCB1* tumor suppressor gene occur in 40–50% of familial cases and in 8–10% of sporadic cases of schwannomatosis. Tumorigenesis is thought to occur through a four-hit, three-step model, beginning with a germline mutation in *SMARCB1* (hit 1), followed by loss of a portion of chromosome 22 that contains the second *SMARCB1* allele and

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one *NF2* allele (hits 2 and 3), followed by mutation of the remaining wild-type *NF2* allele (hit 4). Insights from research on HIV and pediatric rhabdoid tumors have shed light on potential molecular pathways that are dysregulated in schwannomatosis-related schwannomas. Mouse models of schwannomatosis have been developed and promise to further expand our understanding of tumorigenesis and the tumor microenvironment. Clinical reports have described the occurrence of intracranial meningiomas in schwannomatosis patients and in families with germline *SMARCB1* mutations. The authors propose updated diagnostic criteria to incorporate new clinical and genetic findings since 2005. In the next 5 years, the authors expect that advances in basic research in the pathogenesis of schwannomatosis will lead toward clinical investigations of potential drug therapies.

Keywords

neurofibromatoses; schwannoma; rhabdoid tumor

INTRODUCTION

Schwannomatosis is a form of neurofibromatosis that is clinically and genetically distinct from neurofibromatosis type 1 (NF1) and neurofibromatosis type 2 (NF2). Schwannomatosis is characterized by the predisposition to develop multiple schwannomas, but the clinical spectrum continues to expand as more cases are reported. Because the phenotype of schwannomatosis overlaps with NF2, the first published reports did not appear in the literature until the 1990s. By 2003, genetic studies showed that the *NF2* locus was excluded as the cause for familial schwannomatosis. The candidate region for the causative gene was narrowed during the 2000s, and in 2007, Hulsebos et al. reported a constitutional *SMARCB1* mutation in a family affected by schwannomatosis. Since 2007, research on the genetic and molecular basis of schwannomatosis has expanded. The Children's Tumor Foundation sponsored a conference held in Los Angeles, CA, on June 5–8, 2011 in which about 30 schwannomatosis researchers and clinicians discussed recent advances in the genetics, biology, and clinical description of schwannomatosis. This article provides a synopsis of the highlights presented at the Conference and as such, is a "state-of-the-field" for schwannomatosis research in 2011.

MEETING UPDATE

Genetics

Genetic identification of SMARCB1—Schwannomatosis is characterized by the development of multiple schwannomas. Only about 15 years ago, schwannomatosis was recognized as a clinical entity that is distinct from neurofibromatosis type 2 (NF2) [MacCollin et al., 1996; Evans et al., 1997]. Genetic evidence that schwannomatosis is different from NF2 was initially provided by the molecular analysis of schwannomas of schwannomatosis patients. These studies revealed that the multiple tumors of individual patients harbored independent mutations in the *NF2* gene, which were not present in their respective constitutional DNAs [Jacoby et al., 1997; Kaufman et al., 2003]. Subsequent linkage analyses in schwannomatosis families excluded the *NF2* gene as the

schwannomatosis-predisposing gene and suggested a location of this gene near marker D22S1174, which is about 6 cM centromeric to the *NF2* gene on chromosome 22. Hulsebos et al. [2007] considered the *SMARCB1* gene to be an attractive candidate gene for schwannomatosis, because it was a known tumor suppressor gene and located at a very short distance from marker D22S1174. Indeed, they identified an inactivating germline mutation in exon 1 of the *SMARCB1* gene in a father and daughter who both had schwannomatosis. In addition, in accordance with the tumor suppressor gene model, they found inactivation of the wild-type copy of the *SMARCB1* gene, by a second inactivating mutation or by deletion, in schwannomas of the patients. These findings identified the *SMARCB1* gene as a predisposing gene in schwannomatosis.

Studies by others have confirmed the involvement of the *SMARCB1* gene in 40–50% of familial and less than 10% of sporadic cases of schwannomatosis [Boyd et al., 2008; Hadfield et al., 2008; Sestini et al., 2008; Rousseau et al., 2011; Smith et al., 2012]. In these studies, additional somatic inactivation of the *NF2* gene was reported for the schwannomas. In *SMARCB1* mutation-positive schwannomas, the deletions always involved loss of one copy of chromosome 22 [Hadfield et al., 2010b]. These observations suggest a four-hit, three-step model of tumorigenesis, in which the mutated germline *SMARCB1* gene copy is retained in the tumor (hit 1), whereas chromosome 22, or at least a segment containing the wild type *SMARCB1* gene copy and a wild-type copy of the *NF2* gene, is lost (hits 2 and 3), followed by mutation of the remaining wild-type *NF2* gene copy (hit 4) (Fig. 1).

Conflicting evidence exists with regard to the involvement of the *SMARCB1* gene in the development of multiple meningiomas. Somatic mutations in the *SMARCB1* gene have been shown to occur in sporadic meningiomas, although at low frequency (<3% of cases) [Bruder et al., 1999; Schmitz et al., 2001; Rieske et al., 2003]. However, germline mutations in the *SMARCB1* gene could not be detected in 47 patients with multiple meningiomas, including eight familial cases [Hadfield et al., 2010a]. On the other hand, the inheritance of a germline mutation in the *SMARCB1* gene was found in two families with schwannomatosis and multiple meningiomas [Bacci et al., 2010; Christiaans et al., 2011]. For the meningiomas in one of these families, it could be demonstrated that the four-hit, three step model also applies to these tumors, and that the cranial meningiomas were preferentially located at the falx cerebri [van den Munckhof et al., 2012].

Despite the frequent combination of germline *SMARCB1* and somatic *NF2* mutations, a large proportion of familial schwannomatosis patients and the majority of sporadic schwannomatosis patients have no known causative mutation [Boyd et al., 2008; Hadfield et al., 2008; Sestini et al., 2008; Rousseau et al., 2011; Smith et al., 2012]. In addition, there are currently no known causative genes for multiple meningiomas unrelated to NF2 or schwannomatosis. This leaves open the possibility of discovering further predisposing, or modifier, genes. Recent developments in technologies such as whole genome and whole exome sequencing analysis have now made them accessible for use in identification of these novel genes, which will help to elucidate the overall affected cellular pathways involved. This will then indicate targets for the development of novel therapies.

GENOTYPE–PHENOTYPE CORRELATIONS

In addition to schwannomatosis, germline mutations in the *SMARCB1* gene cause the inherited prediposition to rhabdoid tumors characterized by the presence of malignant rhabdoid tumors of the kidney, atypical teratoid/rhabdoid tumors (AT/RT) of CNS, and extra-renal rhabdoid tumors that develop in childhood [Versteege et al., 1998; Hulsebos et al., 2007]. The factors determining which patients will develop schwannomatosis and which will develop rhabdoid tumors are unclear. Non-penetrance of rhabdoid tumors in patients with germline mutations of *SMARCB1* leaves open the possibility that surviving mutation carriers will develop schwannomas later in life. However, it is extremely rare to find both phenotypes in the same family. Currently, there are only three reported families in which either rhabdoid tumors or schwannomas are seen in different family members with the same inherited mutation. The schwannomas seen in these patients seem to differ histologically from those typically found in schwannomatosis [Swensen et al., 2009; Eaton et al., 2011a; Carter et al., 2012]. The causative mutations in these families are predicted to cause truncation of the protein.

There is a direct correlation between rhabdoid tumors and *SMARCB1* inactivation, as >95% of tumors show biallelic loss of this gene due to deletions, nonsense and frameshift mutations, or lack of mRNA and protein. Germline mutations in *SMARCB1* are found in one third of patients with rhabdoid tumors and tend to be truncating (frameshift/nonsense) mutations, or deletions of one or more exons, leading to a complete knockout of the *SMARCB1* gene [Bourdeaut et al., 2011; Eaton et al., 2011b]. This is coupled with a somatic mutation of the second allele, leading to biallelic inactivation of *SMARCB1*.

In contrast, inherited mutations found in familial schwannomatosis are more likely to be non-truncating (missense or splice-site) mutations [Boyd et al., 2008; Hadfield et al., 2008], which might account for the milder phenotype as these are potentially hypomorphic. However, sporadic schwannomatosis patients may carry truncating (frameshift or nonsense) or non-truncating (missense/splice-site) mutations [Rousseau et al., 2011; Smith et al., 2012], which are predicted to knock out the protein product. Schwannomatosis-associated *SMARCB1* mutations do seem to be more frequent at either end of the gene whereas mutations associated with the inherited prediposition to rhabdoid tumors are more centrally located. It is possible that the specific combination of resulting somatic mutations, including the frequent co-mutation of the *NF2* gene in tumors from schwannomatosis patients [Boyd et al., 2008; Hadfield et al., 2008], may regulate the severity of the resulting phenotype. However, given the relatively small number of families published with each phenotype more work is required to understand why the mutation patterns in schwannomatosis families are distinct and usually separate from patients with the inherited prediposition to rhabdoid tumors.

INHERITANCE

The majority of cases of schwannomatosis are sporadic [Evans et al., 1997; Antinheimo et al., 2000; Gonzalvo et al., 2011]. Case series suggest that only around 15–25% of cases are inherited from an affected parent. Confirmation of a germline *SMARCB1* mutation will

prove the inherited nature of disease and confirm a 50% risk of transmission to the next generation. However, only about 10% of sporadic cases and 40–60% of inherited cases have an identifiable mutation [Boyd et al., 2008; Hadfield et al., 2008; Rousseau et al., 2011]. The transmission risk can be assumed to be 50% in an individual with schwannomas who has a proven family history regardless of *SMARCB1* status, but the risks for a sporadic case are less clear. Even with exclusion of *SMARCB1*, it must be assumed that there is still a small risk of transmission to offspring. Quantifying transmission risk is also complicated by phenotypic overlap between patients with sporadic schwannomatosis and mosaic NF2, since the latter patients may fulfill current schwannomatosis criteria [Baser et al., 2006; Murray et al., 2006]. Mosaicism also exists for *SMARCB1* [Hulsebos et al., 2010], although the frequency of *SMARCB1* mosaicism is still not clear. Nonetheless, mosaicism may account for a further proportion of sporadic schwannomatosis cases. Non-penetrance has been described in schwannomatosis although this appears at present to be less likely in *SMARCB1* families. Ideally, molecular analysis of the *NF2* and *SMARCB1* genes from two separate tumors from an individual are required to confirm an underlying diagnosis.

CELL BIOLOGY

Function of SMARCB1

SMARCB1 and the SWI/SNF complex—Eukaryotic epigenetic regulation is mediated by chromatin remodeling factors that are high molecular weight multi-subunit protein complexes [Martens and Winston, 2003; Cairns, 2007; Osley et al., 2007; Wang et al., 2007; van Vugt et al., 2007; Racki and Narlikar, 2008]. These complexes can be divided into two general groups: Those that covalently modify histones and DNA by acetylation/deacetylation or methylation/demethylation, and those that use the energy of ATP to reorganize/reposition the nucleosomes. The latter complexes do not modify the chromatin in a covalent manner although they may indirectly influence such modifications [Banine et al., 2005; Datta et al., 2005].

SMARCB1 (also called *hSNF5*, *INI1*, and *BAF47*) is a component of the mammalian SWI/SNF complex, a prototypical ATP-dependent chromatin remodeling complex consisting of at least nine subunits that are conserved among eukaryotes (Fig. 2) [Wang et al., 1996a; Wang et al., 1996b; Narlikar et al., 2002; Martens and Winston, 2003]. Among these are four core subunits required for chromatin remodeling, including an ATPase (SWI2/SNF2/BRG1 or BRM1), SMARCB1, BAF155, and BAF170 [Martens and Winston, 2003]. Among these, BRG1 and BAF155 are known to play critical roles in mammalian nervous system development [Kim et al., 2001; Seo et al., 2005; Matsumoto et al., 2006; Lessard et al., 2007].

Several functionally distinct classes of SWI/SNF complexes have been recognized in eukaryotes. Two major classes include hSWI/SNF-A or BAF (BRG1-associated Factor) complex and hSWI/SNF-B or PBAF complex. The two classes of complexes are distinguished by the presence of specific subunits [Martens and Winston, 2003; van Vugt et al., 2007]. Additional complexes containing mixtures of components of SWI/SNF and HDAC1 complexes have also been identified (See Fig. 2) [Martens and Winston, 2003]. Since SMARCB1 is present in all of these complexes, understanding the distinct roles

played by these various complexes will be essential for deciphering the role of SMARCB1 in schwannomatosis.

The functions of non-catalytic subunits of SWI/SNF complexes, including SMARCB1, are not completely understood. The components of the SWI/SNF complex do not have sequence-specific DNA binding activity and hence they are targeted to specific promoter regions via protein–protein interactions with sequence-specific transcription factors, either activators or repressors [Cairns, 1998; Cheng et al., 1999; Kingston and Narlikar, 1999; Martens and Winston, 2003; Cairns, 2007; Racki and Narlikar, 2008].

Structure/function analysis of SMARCB1—SMARCB1 was originally called INI1 and was originally identified as an interacting protein for HIV-1 integrase (IN) [Kalpana et al., 1994]. Structure/function analysis has revealed that SMARCB1 has three conserved domains. Two of these domains (Rpt I and Rpt II) are direct and imperfect repeats of each other, and the Rpt I domain is necessary and sufficient to bind to HIV-1 IN in vitro and in vivo [Morozov et al., 1998; Yung et al., 2001]. SMARCB1 is also engaged in protein–protein interactions with other viral and cellular proteins (Fig. 3) [Wu et al., 1996; Rozenblatt-Rosen et al., 1998; Cheng et al., 1999; Lee et al., 1999]. Viral proteins include the E1 protein of human papilloma virus 18 and the EBNA-2 protein of Epstein–Barr virus [Wu et al., 1996; Lee et al., 1999; Wu et al., 2000]. The cellular proteins include MYC, MLL (HXR), GADD34, and AKT [Rozenblatt-Rosen et al., 1998; Cheng et al., 1999; Wu et al., 2002; Foster et al., 2006]. Furthermore, SMARCB1 harbors additional functional domains including a nuclear export signal that is constitutively activated by the deletion of the C-terminal regions, a homology region III, a non-specific DNA binding domain, and a region involved in nuclear import (Fig. 3) [Morozov et al., 1998; Craig et al., 2002]. Mutations of *SMARCB1* in schwannomatosis span all these domains and hence understanding of specific roles played by these domains will provide insight into its function.

Role of SMARCB1 in HIV-1 replication—Understanding its interactions with HIV-1 IN may reveal clues about the functions of SMARCB1. It has been reported that SMARCB1 has a multifaceted role in HIV-1 replication. Binding of IN to SMARCB1 may facilitate targeting of incoming HIV-1 viral DNA into open chromatin regions in infected cells [Kalpana et al., 1994; Lesbats et al., 2011]. SMARCB1 is also required for post-integrational events of HIV-1 viral assembly and particle production in the cytoplasm [Yung et al., 2001; Yung et al., 2004; Sorin and Kalpana, 2006]. These studies indicate an undefined function for SMARCB1 outside the nucleus in the cell, and understanding these roles may provide novel insight into its role in schwannomatosis and other cancers. Furthermore, SMARCB1 also has been demonstrated to bind to the HIV-1 trans-activator protein, Tat, and to activate transcription of the HIV-1 promoter within its long terminal repeats [Ariumi et al., 2006; Mahmoudi et al., 2006].

Crosstalk between merlin and the HIV-1 host proteins—Studies indicate a four-hit mechanism for genesis of schwannomatosis where mutations in both *NF2* and *SMARCB1* genes lead to pathogenesis. Currently, the functional interaction and relationship between

SMARCB1 and *NF2* are unknown. However, it is intriguing to note that there is interplay between *NF2* and the cellular factors that influence HIV-1 replication.

$CLR4^{DCAF1}$ or VprBP is an E3 ubiquitin ligase that binds to an HIV-1 encoded accessory protein called Vpr. This association of Vpr with $CLR4^{DCAF1}$ appears to be required for inducing G2/M arrest [DeHart et al., 2007; Wen et al., 2012]. Recent studies indicate that merlin also associates with $CLR4^{DCAF1}$ in the nucleus and suppresses its activity and tumorigenesis [Li et al., 2010].

TRBP (transactivator-responsive RNA binding protein) is another HIV-1 host protein that binds to HIV-1 encoded Tat [Bannwarth and Gatignol, 2005; Corbeau, 2008; Clerzius et al., 2011]. TRBP is a Dicer co-factor required for cellular RNA interference (RNAi). HIV-1 Tat suppresses the RNAi by sequestration of TRBP away from the Dicer complex. Merlin also seems to inhibit TRBP, which has growth-promoting potential, by ubiquitination and degradation of TRBP protein [Lee et al., 2004]. Whether or not these interactions of merlin with HIV-1 host proteins have anything to do with SMARCB1 is unclear at this point.

Tumor suppressor function ofSMARCB1—Various studies have indicated that SMARCB1 exerts its tumor suppressor function by: (i) inducing G1 arrest; (ii) inducing mitotic arrest; (iii) inhibiting aneuploidy and causing diploidization; and (iv) inducing senescence [Kalpana and Smith, 2010; Wilson and Roberts, 2011]. Induction of G0/G1 arrest by SMARCB1 is correlated with down-regulation of cyclin D1 [Zhang et al., 2002] and up-regulation of $p16^{INK4a}$ [Betz et al., 2002]. The cyclin D1 gene appears to be essential for genesis of tumors due to loss of *Smarcb1* in mouse models as *Smarcb1* +/− heterozygous mice in combination with *cyclin D1*−/− null genotype resulted in no tumor formation [Tsikitis et al., 2005]. Furthermore, cyclin D1 appears to be essential for survival of established tumors. Knocking-down cyclin D1 by siRNA in rhabdoid tumor cells induced cell cycle arrest and apoptosis in vitro [Alarcon-Vargas et al., 2006]. Interestingly enough it has been postulated that merlin exerts its tumor suppressor function via repression of PAK-induced cyclin D1 expression [Xiao et al., 2005]. It is possible then that SMARCB1 and merlin loss could co-operate in cyclin D1 induction. Small molecular weight pan-cyclin-dependent kinase inhibitors such as Flavopyridol were effective at nanomolar concentrations to inhibit rhabdoid tumor cells in vitro and primary tumor models in vivo [Smith et al., 2008; Smith et al., 2011]. These studies suggest that targeting cyclin D1 is an effective strategy to inhibit at least a subset of tumors arising due to loss of SMARCB1. Whether this strategy would also be effective for inhibiting schwannoma growth in schwannomatosis animal models and patients should be investigated.

In addition to regulating the cell cycle, SMARCB1 and the SWI/SNF components regulate lineage specific gene expression and embryonic stem cell programming. SMARCB1 and SWI/SNF complexes have been shown to be involved in the control of neurogenesis, myogenesis, adipogenesis, osteogenesis, and hematopoiesis [Young et al., 2005; Caramel et al., 2008; Yoo and Crabtree, 2009; Krosl et al., 2010; Albini and Puri, 2010; Nowak et al., 2012]. SMARCB1 and SWI/SNF complexes also regulate embryonic stem cell programming by antagonizing the activity of polycomb group proteins [Wilson et al., 2010].

Downstream pathways affected by SMARCB1—SWI/SNF complexes are involved in both activation and repression of a selective subset of genes [Martens and Winston, 2003]. The components of SWI/SNF complex do not posses sequence-specific DNA binding activity and hence are recruited to the promoter region by interaction of one of the subunits with gene-specific transcription factors [Cairns, 1998; Kingston and Narlikar, 1999; Cairns, 2007; Racki and Narlikar, 2008]. SMARCB1 appears to be a tethering protein that recruits SWI/SNF complexes to promoter sites by its interaction with an activator (for example, MYC) or repressor proteins bound to the promoter [Cheng et al., 1999; Martens and Winston, 2003].

Several studies that compared the gene expression profile in rhabdoid cells when SMARCB1 is re-expressed have lead to the identification of a number of downstream pathways that are directly or indirectly affected by SMARCB1. As described above, SMARCB1 affects cyclin D1 and p16 and hence regulates p16-cyclin D1/CDK4-pRb-E2F. SMARCB1 also appears to affect (i) mitotic spindle checkpoint; (ii) the interferon signaling pathway; and (iii) the Gli-Hedgehog pathway [Medjkane et al., 2004; Vries et al., 2005; Morozov et al., 2007; Jagani et al., 2010; Lee et al., 2011]. For example, SMARCB1 and the SWI/SNF complexes are involved in induction of interferon signaling [Agalioti et al., 2002; Liu et al., 2002; Pattenden et al., 2002; Cui et al., 2004; Morozov et al., 2007]. Reintroduction of *SMARCB1* into *SMARCB1*−/− MON cells results in the activation of a statistically significant high abundance of IFN signal induced genes (ISGs) [Morozov et al., 2007].

In addition to inducing the ISGs, SMARCB1 repressed a set of mitotic genes including PLK1 and Aurora A [Morozov et al., 2007; Lee et al., 2011]. Aurora A and PLK1 are serine/threonine kinases that play multiple roles in mitosis. Aurora A is frequently over-expressed in many cancers and is indicative of poor prognosis [Nadler et al., 2008; Wang et al., 2009; Zhang et al., 2009]. Aurora A is an attractive target for developing anti-cancer therapy and, many Aurora A kinase inhibitors are currently under investigation in phase I clinical trials [Pollard and Mortimore, 2009; Dar et al., 2010; Kitzen et al., 2010; Wurzenberger and Gerlich, 2011; Yan et al., 2011]. Aurora A is a repressed downstream target of SMARCB1 and SMARCB1 associates with Aurora A promoter in RT and normal cells but not in non-RT cells [Lee et al., 2011]. Loss of SMARCB1 leads to aberrant over-expression of Aurora A in mouse and human tumors and knocking down Aurora A leads to inhibition of rhabdoid cell growth [Lee et al., 2011]. Targeting expression or activity of this gene is a novel therapeutic strategy for these tumors. At this point it is unclear if Aurora A is de-repressed in schwannomatosis due to *SMARCB1* mutations.

MOUSE MODELS

A number of mice have been generated in which particular proteins within the SWI/SNF complex have been disrupted. The functional complexity of the SWI/SNF unit is reflected by the distinct phenotypes observed in mice lacking different subunits. For example, mice lacking *Brm*, an ATPase subunit, develop normally but demonstrate hyperproliferative phenotypes [Reyes et al., 1998]. In contrast, mice lacking *Brg1* (the other SWI/SNF ATPase), *Smarcb1* or *BAF155* die at the peri-implantation stage [Klochendler-Yeivin et al.,

2000; Roberts et al., 2000; Guidi et al., 2001; Tsikitis et al., 2005; Bultman et al., 2008]. In all three of these knockout mice, inner cell mass development is altered, and *Brg1* and *Smarcb1* inactivation also affects the trophectoderm lineage.

Mice haploinsufficient for SWI/SNF subunits demonstrate a number of distinct phenotypes. For example, *Brg1* heterozygotes are susceptible to mammary carcinomas without evidence of loss of heterozygosity [Bultman et al., 2008]. In contrast, *Smarcb1* heterozygotes undergo normal development and 5–35% later present with rhabdoid tumors and other tumor types, many of which are highly aggressive and metastatic [Klochendler-Yeivin et al., 2000; Roberts et al., 2000; Guidi et al., 2001; Tsikitis et al., 2005]. Where examined, tumorigenesis in these mice correlates with loss or inactivation of the remaining wild-type *Smarcb1* allele. Roberts et al. [2002] generated both an inactivating-conditional *Smarcb1* allele and a reversibly inactivating-conditional *Smarcb1* allele in mice. Inhibition of Smarcb1 expression in a variety of adult tissues resulted in bone marrow failure and rapid death, whereas sporadic inactivation of *Smarcb1* in hematopoietic tissues and in other organs resulted in rapid onset of lymphomas and rhabdoid tumors. Neither *Brg1* nor *Smarcb1* heterozygotes have been reported to develop schwannomas.

The phenotypes of animals with other conditional mutations of *SWI/SNF* subunits demonstrate distinct roles for these proteins that were not predicted from null or haploinsufficient mice. Matsumoto et al. [2006], for example, demonstrated that loss of *Brg1* in neural progenitor cell populations results in precocious neuronal differentiation at the expense of glial cell differentiation in the brain and spinal cord. These effects are linked to a switch in neuron-associated SWI/SNF subunits [Lessard et al., 2007]. Gresh et al. [2005] specifically inactivated *Smarcb1* in the developing liver, resulting in failed hepatic cell differentiation. Collectively, these mouse studies demonstrate that SWI/SNF factors, including *Smarcb1*, suppress tumor formation and are absolutely required for the survival and differentiation of non-malignant cells. These studies also highlight the diverse functions of SWI/SNF subunits and the need to examine these proteins using conditional knock-out approaches.

Phenotypic analysis of *Smarcb1*-deficient Schwann cells is hindered by the fact that homozygous *Smarcb1* mutant embryos lack normal extra-embryonic development and fail at the peri-implantation stage. To circumvent this early embryonic-lethal phenotype, and to examine whether loss of *Smarcb1* in Schwann cells confers a growth advantage that is sufficient to induce schwannoma formation or other schwannomatosis-related phenotypes (e.g., pain) in vivo, a number of groups are developing *Smarcb1* conditional knockout mice to target Schwann cells.

One of the promoters used to generate Schwann cell-targeted disruption of *Smarcb1* is the protein zero (P0) gene, one of the major proteins of peripheral myelin. A 1.1 kb stretch of the 5′ flanking sequence of the rat *P0* gene is sufficient to direct expression of heterologous genes to Schwann cells in vivo [Messing et al., 1992], although expression in neural crest cells has also been reported [Yamauchi et al., 1999]. This expression follows the developmental schedule of the endogenous *P0* gene showing a dramatic increase during the first week after birth. Moreover, transgenic mice expressing either SV40 large T-antigen or a

naturally occurring mutant *Nf2* allele under the rat *P0* promoter develop schwannomas [Messing et al., 1994; Giovannini et al., 1999]. Therefore, this *P0* promoter efficiently targets schwannoma precursor cells. Based on these data, *P0-Cre;Smarcb1*$^{flox/flox}$ mice are being generated to direct *Cre* expression to the schwannoma precursor cells.

Another strategy to assess the function of *Smarcb1* in Schwann cells is to utilize mouse lines in which gene loss can be induced in Schwann cells. Mice are therefore being generated in which *Smarcb1* disruption is induced using the proteolipid protein gene following injection of post-natal or adult animals with tamoxifen. This strategy has been used successfully to disrupt genes in Schwann cells in embryonic, post-natal and adult mice [Doerflinger et al., 2003]. Utilizing both of these strategies will likely result in complementary mouse models that define how loss of *Smarcb1* in Schwann cells influences both developmental and adult phenotypes.

Additional somatic inactivation of *NF2* or other genes may play a role in the development of schwannomas in schwannomatosis patients. Although Hulsebos et al. [2007] were unable to detect *NF2* gene aberrations in their patients, others found *NF2* gene mutations and loss in a considerable fraction of the investigated tumors from schwannomatosis patients [Jacoby et al., 1997; Kaufman et al., 2003; MacCollin et al., 2003; Boyd et al., 2008; Hadfield et al., 2008; Sestini et al., 2008]. Analysis of mice carrying both conditional mutant *Nf2* and *Smarcb1* alleles will be critical to assess how inactivation of Snf5/*Nf2* influences the development of schwannomatosis-associated phenotypes.

CLINICAL FEATURES

Diagnostic Criteria

The diagnostic criteria set forth in 2005 [MacCollin et al., 2005] predated the ability to perform molecular testing for schwannomatosis, and also did not take account of the possibility of multiple meningiomas as a presenting feature. Given current knowledge of the disorder, we propose the following new criteria for diagnosis:

Molecular Diagnosis:

- Two or more pathologically proved schwannomas or meningiomas AND genetic studies of at least two tumors with loss of heterozygosity (LOH) for chromosome 22 and two different *NF2* mutations; if there is a common *SMARCB1* mutation, this defines *SMARCB1*-associated schwannomatosis
- One pathologically proved schwannoma or meningioma AND germline *SMARCB1* pathogenic mutation

Clinical Diagnosis:

- Two or more non-intradermal schwannomas, one with pathological confirmation, including no bilateral vestibular schwannoma by high-quality MRI (detailed study of internal auditory canal with slices no more than 3 mm thick). Recognize that some mosaic NF2 patients will be included in this diagnosis at a young age and that

some schwannomatosis patients have been reported to have unilateral vestibular schwannomas or multiple meningiomas.

- One pathologically confirmed schwannoma or intracranial meningioma AND affected first-degree relative
- Consider as possible diagnosis if there are two or more non-intradermal tumors but none has been pathologically proven to be a schwannoma; the occurrence of chronic pain in association with the tumor(s) increase the likelihood of schwannomatosis

Patients with the following characteristics do not fulfill diagnosis for schwannomatosis:

- Germline pathogenic *NF2* mutation
- Fulfill diagnostic criteria for NF2
- First-degree relative with NF2
- Schwannomas in previous field of radiation therapy only

PATHOLOGIC DIAGNOSIS OF SCHWANNOMATOSIS-ASSOCIATED SCHWANNOMAS

Several studies have documented the presence of hybrid tumors (benign nerve sheath tumors with characteristic features of both neurofibromas and schwannomas) in the setting of NF1, NF2, and schwannomatosis [MacCollin et al., 2005; Harder et al., 2012]. The presence of abundant myxoid stroma in a schwannoma should alert the pathologist to the possibility of an association with any of the neurofibromatoses (as opposed to sporadic schwannoma in a non-syndromic person). In addition to histological features (hybrid tumors, myxoid schwannomas), a mosaic pattern of staining for SMARCB1/INI1 is suggestive of a tumor related to NF2 or schwannomatosis. In an immunohistochemical study of a large cohort of schwannomas, a mosaic pattern of SMARCB1 expression was noted in tumors resected from patients with NF2 and familial schwannomatosis (>90% of tumors). In contrast, a mosaic pattern of expression is unusual (5%) in sporadic, non-syndromic tumors [Patil et al., 2008]. Thus, the finding of mosaic SMARCB1 expression on immunohistochemistry and/or hybrid histology suggests that a schwannoma may be associated with a form of neurofibromatosis. In these cases, the pathologic findings may direct the clinican to refer a patient with a solitary schwannoma for evaluation of neurofibromatosis.

TUMOR BURDEN IN SCHWANNOMATOSIS

Time and financial constraints limit the usefulness of regional MRI to comprehensively phenotype tumor burden in patients with schwannomatosis. For this reason, important features of schwannomatosis including whole-body tumor burden and distribution of schwannomas across body parts are still not well understood. To better understand the clinical phenotype of schwannomatosis, teams at Massachusetts General Hospital and University Hospital, Hamburg, have used whole-body MRI (WBRMI) to assess patients with schwannomatosis [Plotkin et al., 2012]. This technique involves the use of a standard MRI scanner with commercially available software to create a single DICOM image that

extends from head to ankle in most patients. No contrast is required and a patient can be scanned in about 45 min without the need for ionizing radiation.

To date, 51 patients with schwannomatosis have been scanned. The mean age at diagnosis was 42 years; the mean age at the time of MRI scan was 48.5 years (range: 25–97 years). Fifty-one percent were male, and 16% had familial disease. WBMRI identified internal nerve sheath tumors in 71% of patients, including seven of eight patients that did not have a history of internal tumors. Internal tumors were classified radiologically as either discrete (locally circumscribed) or plexiform (invasive tumors or those that involved multiple nerve roots). In patients with tumors on WBMRI, 29/36 (81%) had only discrete appearing tumors, 3/36 (8%) had only plexiform appearing tumors, and 4/36 (11%) had both types. For patients with tumors, the median tumor count was 4 (range: 1–27 tumors) and the median whole-body tumor volume was 39.4 ml (range: 7.0–1371.5 ml). Median volume of plexiform appearing tumors was smaller in schwannomatosis patients (32 ml) than in a group of similarly imaged NF1 and NF2 patients, who had median tumor volumes of 206 and 104 ml, respectively. The distribution of tumors across body parts was measured using the distribution coefficient which ranges from 0 (representing tumor volume that is equally distributed throughout the body) to 1 (representing tumor volume located in only one body area) [Plotkin et al., 2012]. In the cohort of schwannomatosis patients, the median distribution coefficient was 0.9 (range: 0.26–1), indicating that total tumor volume is not distributed evenly across the body. This finding may reflect the relatively high frequency of anatomically limited schwannomatosis or the relatively low number of tumors in most patients.

In order to explore the relationship between tumor burden and pain, schwannomatosis patients were asked to rate their pain level at the time of imaging using a visual analog pain scale [scores ranges from 0 (no pain) to 10 (most pain)]. The median pain score for all schwannomatosis patients was 1.7. Surprisingly, the median pain score for patients with familial schwannomatosis was 0.5 whereas the median score for patients with sporadic schwannomatosis was 2.5.

CURRENT TREATMENT OPTIONS

Current Treatment Options

Pain medications—Chronic pain is common in patients with schwannomatosis [Merker et al., 2012]. The etiology of pain in schwannomatosis is unclear: there is no clear relationship between tumor number, size, location, and the intensity of pain, and the pain has both neuropathic and nociceptive features. Perhaps for this reason, there is no widely accepted approach for treating pain in these patients. Conference attendees supported the use of neuropathic pain medication without favoring a single approach for schwannomatosis. Reasonable options include calcium channel alpha 2-delta ligands (e.g., gabapentin, pregabalin). In addition, several medications designated as antidepressants (e.g., amitriptyline, nortriptyline, duloxetine) can have potent anti-neuropathic pain effects. Medications used for mood stabilization (e.g., lamotrigine, valproate) can also be effective in chronic pain scenarios. It is not clear whether these drugs directly modulate the pain mechanism or treat the depression and anxiety associated with chronic pain.

Surgical considerations—Surgery is the treatment of choice for symptomatic schwannomas and, in many patients, can relieve local pain or symptoms arising from compression of neighboring tissues. The major risk of surgery is secondary nerve injury and hence, surgeons experienced with nerve-sparing surgery should be involved when considering a schwannoma resection. Anecdotal experience with schwannomatosis patients suggests that surgery can occasionally result in worsening of the global pain symptom. In this setting, repeated surgeries appear to offer less benefit in pain control and may contribute to worsening of the pain syndrome. This experience has raised the hypothesis that circulating factors (e.g., cytokines) that are released during surgery can potentiate painful stimuli. There is clearly a need to better understand surgical outcomes for patients with schwannomatosis.

Radiation—Experience with radiation therapy for management of schwannomatosis-related schwannomas is limited. Over the past two decades, there has been increasing experience with stereotactic radiation for sporadic vestibular schwannomas and spinal schwannomas [Niranjan et al., 2008; Gerszten et al., 2012]. Early results suggest that this modality is safe and effective for individuals without an underlying tumor suppressor syndrome. However, there is a theoretical risk that radiation exposure could increase the risk for malignant transformation in patients with schwannomatosis, as has been reported for NF1 and NF2 [Evans et al., 2006]. To date, there is no available data on the risk of secondary malignant transformation of tumors in schwannomatosis patients. At this time, most experts reserve the use of radiation for patients who require treatment for growing schwannomas that cannot be treated with surgery. The role of radiation for symptomatic (i.e., painful) schwannomas remains unclear.

Chemotherapy options—Currently, there are no approved drugs for treatment of schwannomas. Since SMARCB1 directly represses cyclin D1 and since tumors that arise due to loss of SMARCB1 are dependent on cyclin D1, preclinical studies were conducted to test the efficacy of drugs that target the cyclin D1/Cdk pathway [Zhang et al., 2002; Tsikitis et al., 2005]. Flavopiridol is a pan-cdk inhibitor and it represses cyclin D1 at the transcriptional level [Smith et al., 2008; Smith et al., 2011]. In preclinical models of primary rhabdoid tumors that arise due to loss of *hSNF5*, flavoporidol has shown efficacy in eliminating tumors. In a mouse model with transgenic *SMARCB1* mutation with biallelic somatic inactivation of *NF2*, there is activation of the cyclin D1 and mTOR pathways. Possible additional molecular drug targets are those deregulated due to loss of *SMARCB1*, including mitotic genes, PLK1 and Aurora A that are upregualted/de-repressed in tumors, and interferon pathway [Lee et al., 2011]. Hence, the pathways consisting of cyclin D1, PLK1, Aurora A, p16, and interferons are all possible targets for tumors harboring *SMARCB1* mutations. Finally, in a series of two cases (personal communication, J Blakeley, S Huson and DG Evans), the VEGF inhibitor bevacizumab has shown some benefit in pain control and improvement in function in two patients with life-threatening complications of schwannomatosis. In summary, at this time, there is no drug therapy that can be recommended for the treatment of the tumors in schwannomatosis. However, ongoing preclinical work suggests that early clinical studies (i.e., translational and dose finding studies) should be available soon for schwannomatosis patients.

CONCLUSIONS

There has been considerable progress in our understanding of the molecular and genetic basis of schwannomatosis since 2007. This has led to the development of refined diagnostic critera for schwannomatosis. The participants of the workshop identified priorities for continued research over the next 5 years. These priorities include identifying additional genes that predispose to familial and sporadic schwannomatosis, understanding the molecular basis for instability of chromosome 22 in schwannoma formation, characterizing genotype/phenotype correlations for schwannomatosis/inherited prediposition to rhabdoid tumors, uncovering the mechanism of pain in schwannomatosis, and preclinical testing of drugs effecting in schwannomatosis mouse models. Ultimately, these results will open the door for clinical trials to treat patients who are not good candidates for surgery. In the meantime, the International Schwannomatosis Registry (www.schwannomatosis.org) is prospectively enrolling patients into a database to assist researchers who wish to identify patients for these clinical investigations.

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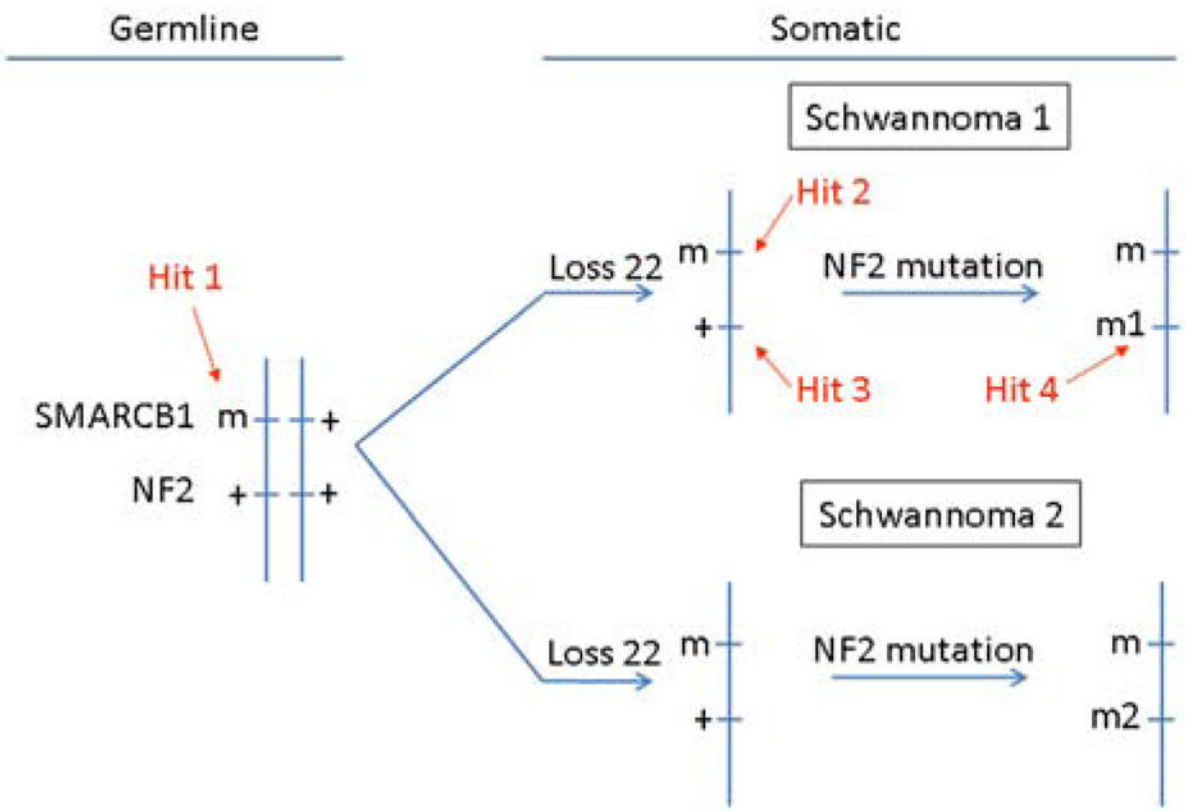

FIG. 1.

Four-hit, three-step mechanism for *SMARCB1* and *NF2* inactivation in multiple schwannomas of a *SMARCB1*-mutation-positive schwannomatosis patient. Tumorigenesis begins with a germline mutation in *SMARCB1* (hit 1), and is followed by loss of a portion of chromosome 22 that contains the second *SMARCB1* allele and one *NF2* allele (hits 2 and 3), and by mutation of the remaining wild-type *NF2* allele (hit 4).

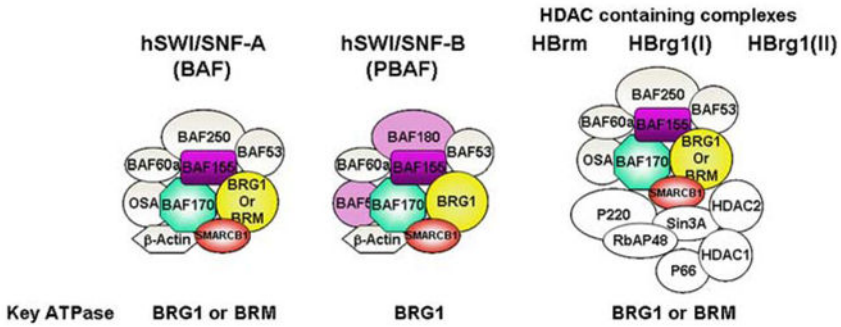

FIG. 2.

Schematic representation of multi-subunit SWI/SNF complexes. SMARCB1 (in shaded red) is present in all complexes.

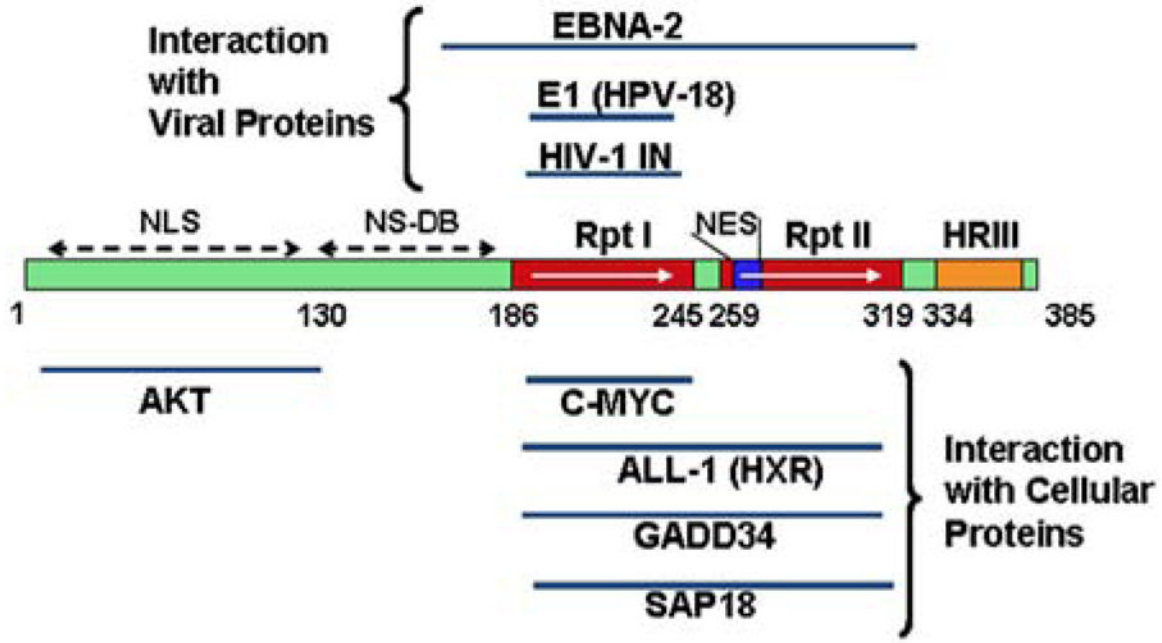

FIG. 3.

Schematic representation of different domains of SMARCB1/hSNF5 and their function. Rpt, repeat; HRIII, homology region III; NES, nuclear export signal; NLS, nuclear localization signal, and NS-DB, nonspecific DNA binding.