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Immunohistochemical Analysis Supports a Role for INI1/SMARCB1 in Hereditary Forms of Schwannomas, But Not in Solitary, Sporadic Schwannomas

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Abstract

The *INI1/SMARCB1* protein product (INI1), a component of a transcription complex, was recently implicated in the pathogenesis of schwannomas in two members of a single family with familial schwannomatosis[1]. Tumors were found to have both constitutional and somatic mutations of the *SMARCB1* gene and showed a mosaic pattern of loss of INI1 expression by immunohistochemistry, suggesting a tumor composition of mixed null and haploinsufficient cells. To determine if this finding could be extended to all tumors arising in familial schwannomatosis, and how it compares to other multiple schwannoma syndromes (sporadic schwannomatosis and neurofibromatosis 2) as well as to sporadic, solitary schwannomas, we performed an immunohistochemistry analysis on 45 schwannomas from patients with multiple schwannoma syndromes and on 38 solitary, sporadic schwannomas from non-syndromic patients. A mosaic pattern of INI1 expression was seen in 93% of tumors from familial schwannomatosis patients, 55% of tumors from sporadic schwannomatosis, 83% of NF2-associated tumors and only 5% of solitary, sporadic schwannomas. These results confirm a role for *INI1/SMARCB1* in multiple schwannoma syndromes and suggest that a different pathway of tumorigenesis occurs in solitary, sporadic tumors.

The *SMARCB1* (also known as *INI1*, *hSNF5* and *BAF47*) is a tumor suppressor gene that maps to chromosome band 22q11.2. Biallelic inactivation of *SMARCB1* is frequent in atypical teratoid/rhabdoid tumors (AT/RT) and malignant rhabdoid tumors, aggressive malignant tumors of the central nervous system and kidneys in children. Constitutional mutations of *SMARCB1* can be seen in rare familial cases of AT/RT[2]. The protein encoded by *SMARCB*, the INI1 protein, is a subunit of the SWI/SNF ATP-dependent chromatin-remodeling complex and is ubiquitously expressed in all cell types examined[3]. AT/RT occurring both sporadically

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and in the context of a tumor suppressor gene syndrome, show diffuse loss of expression of INI1 by immunohistochemistry, a feature often used in the pathological diagnosis of these tumors[4].

SMARCB1 lies in the candidate region for familial schwannomatosis, a form of neurofibromatosis characterized by multiple schwannomas without vestibular nerve involvement[5,6]. In a recent report[1], constitutional and somatic mutations of the *SMARCB1*gene were found in tumors from a single kindred with familial schwannomatosis. Loss of nuclear INI1 protein expression by immunohistochemistry was seen in four separate tumors from two members of this family. However, in contrast to the immunostaining pattern of INI1 in AT/RT, loss of INI1 expression was seen in only a subset of tumor cells suggesting a mosaic makeup of null and haploinsufficent cells. Furthermore, several previous studies have identified somatically acquired mutations in the *NF2* gene in schwannomatosis tumors[6],,[7], but in tumors from this family no molecular evidence of *NF2* involvement was seen, raising the question of how representative this family may be. Here we report an expansion of these results to other familial schwannomatosis kindreds as well as analysis of the INI expression pattern in tumors associated with other multiple schwannoma syndromes (sporadic schwannomatosis and NF2) and in solitary, sporadic schwannomas.

We analyzed INI1 expression in 83 schwannomas representing the four distinct clinical subgroups: familial schwannomatosis (15 tumors from 10 patients in 5 families), sporadic schwannomatosis (18 tumors from 11 patients), NF2-associated schwannomas (12 tumors from 12 patients) and solitary, sporadic schwannomas (38 tumors from 38 patients). All the schwannomas included in the study were non-vestibular, in order to eliminate possible site-related bias. Diagnosis of patients was established by review of the medical record in accordance with published guidelines[5,8]. The study was approved by the institutional review board (IRB).

Briefly, formalin-fixed, paraffin-embedded tissue sections, were immunostained using a commercial INI1 antibody (BD Transduction Laboratories, Franklin Lakes, NJ) along with appropriate controls; AT/RT as negative control and medulloblastoma and normal cortex as positive controls. Antigen retrieval was achieved by microwaving, steaming in a Borg Decloaker RTU for 48 min (primary body antibody concentration 1:50) or by using the Ventana BenchMark XT Autostainer (Ventana Medical Systems Inc., Tucson, AZ), with the Ventana 'Ultra View Universal DAB Detection Kit' and Heat Induced Epitope Retrieval (HIER) (primary antibody concentration of 1:25).

The INI1 immunostaining was interpreted as showing either a diffuse positive nuclear staining consistent with retained expression (figure 1A) or a mosaic pattern of mixed positive and negative nuclei, consistent with loss of expression in a subset of tumor cells (figure 1B). In cases with a mosaic pattern, there was both considerable intertumoral and intratumoral variability, ranging from <10% to >50% immunonegative nuclei. A diffuse immunonegative pattern, as typically observed in AT/RT (figure 1C), was not seen in any of the schwannoma samples. In most mosaic cases the negative and positive cells were intimately intermixed. The mosaic pattern was observed in most cases of familial schwannomatosis (14/15; 93%) and NF2-associated schwannomas (10/12; 83%), in some cases of sporadic schwannomatosis (10/18; 55%) but was found only in a few of the solitary, sporadic schwannomas (2/38; 5%). To adjust for correlation due to multiple tumors within the same subject and/or family, we used generalized estimating equations methodology to calculate standard errors and 95% confidence intervals for the proportions with INI1 positive in each of the groups (figure 2). The estimates and 95% confidence intervals for positive, diffuse INI1 staining were: 7% for familial schwannomatosis (1%, 37%), 55 % for sporadic schwannomatosis (38%, 72%), 17% for NF2 (4%, 48%) and 92% for solitary sporadic schwannomas (78%, 97%). For mosaic pattern of

INI1 staining the estimates and 95% confidence intervals were: 93% for familial schwannomatosis, 45% for sporadic schwannomatosis; 83% for NF2 and 8% for solitary, sporadic schwannomas. Schwannomatosis is a recently recognized, third form of neurofibromatosis, characterized by the presence of multiple non-vestibular schwannomas, often associated with pain typically without vestibular involvement [5]. Over two thirds of schwannomatosis patients have no family history (sporadic schwannomatosis) [5]. Non-vestibular schwannomas may occur also in the context of neurofibromatosis 2 (NF2) and as sporadic, solitary tumors in the general population. Clinical distinction of schwannomatosis from early forms of NF2 or from sporadic, solitary cases may be challenging. While biallelic inactivation of *NF2* occurs in all forms of schwannomas[10], only NF2 patients harbor constitutional *NF2* mutations in non-neoplastic tissues. Our previous work has defined a candidate region for schwannomatosis locus in close proximity to the *NF2* gene on chromosome 22q; a region that includes the *SMARCB1/INI1* locus.

Our study of INI1 expression in 83 syndromic and solitary, sporadic schwannomas confirms and expands the findings reported by Hulsebos and colleagues[1]. We found a mosaic pattern of INI1 expression in most familial schwannomatosis schwannomas, confirming its involvement in the pathogenesis of these tumors. The mosaic pattern is suggestive of two populations of cells intermixed within the tumor mass. Surprisingly, this pattern was not limited to schwannomatosis, but was also found in NF2-associated cases, suggesting that INI1 loss may also play a role in the pathogenesis of NF2-associated schwannomas. In contrast, solitary sporadic schwannomas retained INI expression, implicating different pathways of tumorigenesis in hereditary syndromes and in solitary tumors. Sporadic schwannomatosis-associated tumors appear to be heterogeneous; some show INI1 loss of expression; similar to the familial schwannomatosis tumors, while others retain INI1 expression; similar to sporadic solitary tumors. Further work is needed to determine if multiple tumors (or multiple samples from the same tumor) in sporadic schwannomatosis have the same INI expression pattern. Molecular analysis of the tumors to elucidate the underlying molecular alterations in all types of schwannomas is warranted and additional efforts are needed to correlate expression patterns of INI1 protein to underlying *SMARCB1/INI1* molecular alterations as well as to analyze possible co-interaction of *NF2* and *SMARCB1/INI1* that may explain the observed similarity of INI1 expression pattern in NF2 and familial schwannomatosis tumors.

From a clinical standpoint, the different patterns of INI1 staining may be helpful in distinguishing familial and sporadic schwannomatosis; especially while definitive molecular diagnostics is not yet available. Although the mosaic pattern of INI1 staining is not definitive for a familial form of the disease, a diffuse, positive INI1 staining is suggestive that the patient will not become a founder the familial form of the disease.

In summary, this is the first study to report alterations of INI1 expression in a large series of schwannomatosis and NF2-associated tumors and while it elucidates the role of *SMARCB1* in various forms of schwannoma, it also raises many additional questions. The underlying molecular mechanism and sequence of events that lead to the formation of tumors with mixed population of cells also remains unclear. Further studies are necessary to correlate the underlying molecular changes in *SMARCB1* to the INI1 expression patterns.

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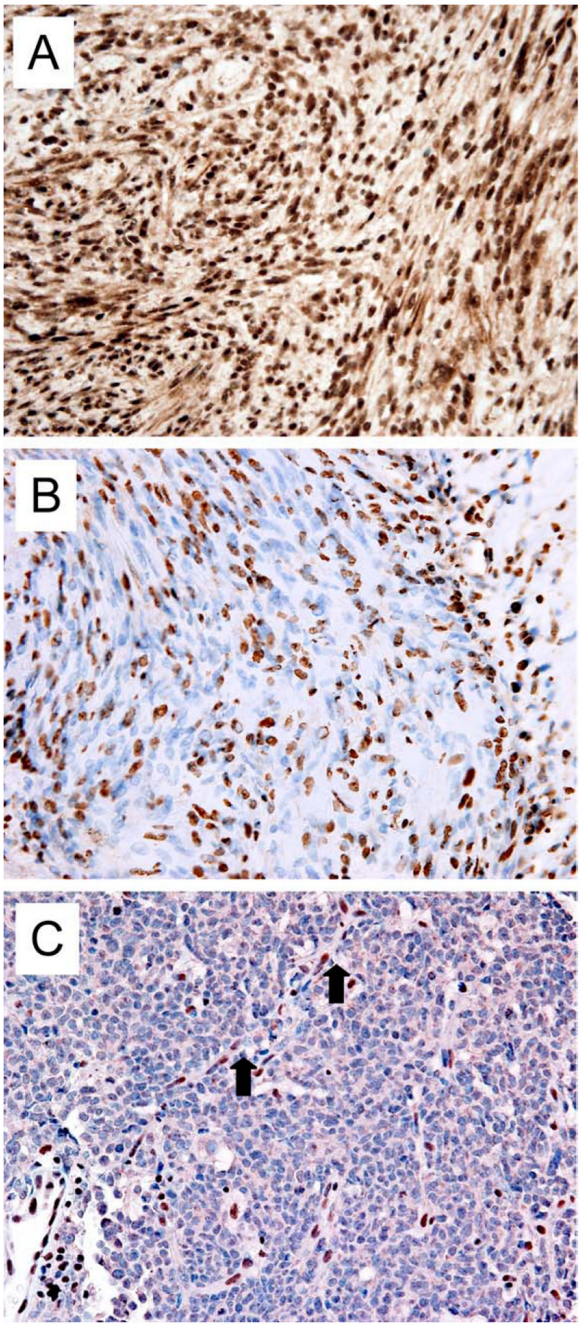

Figure 1. Immunohistochemical staining of INI1 in schwannomas

A, Solitary, sporadic schwannoma showing diffuse immunopositive staining. B, Schwannoma from a familial schwannomatosis patient showing a mosaic pattern with a subset of immunonegative tumor cells intimately intermixed with immunopositive nuclei. C, Negative control AT/RT shows diffuse immunonegative tumor; immunopositive capillary endothelial cell nuclei provide an internal positive control (*arrows*).

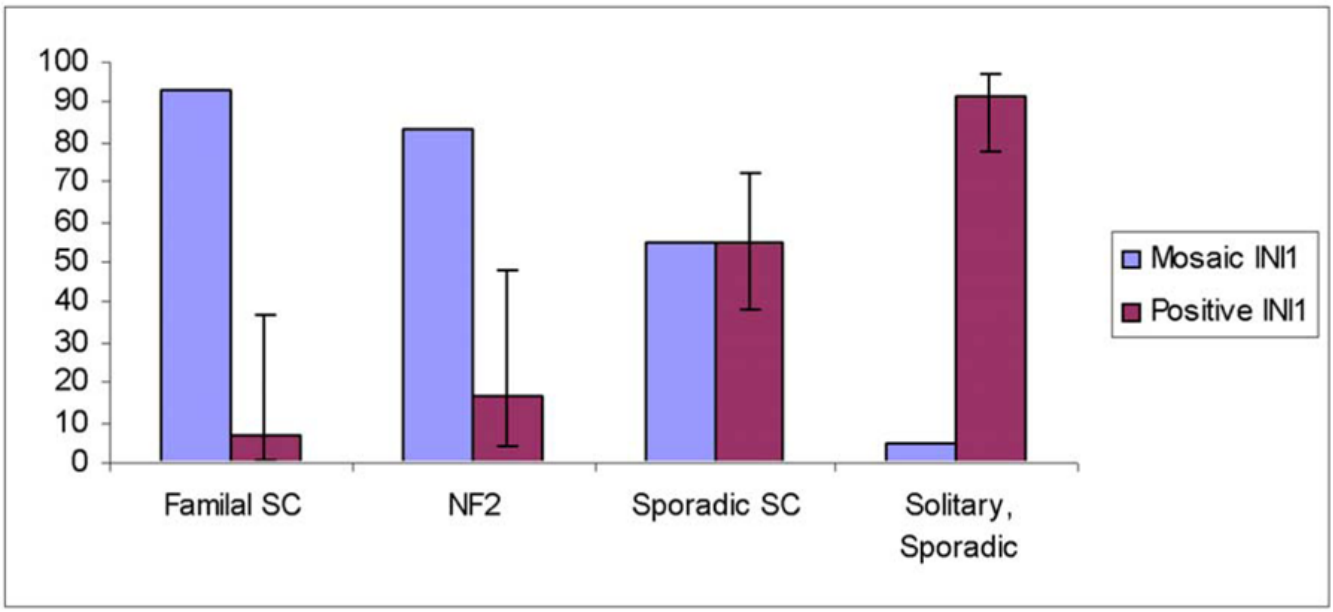

Figure 2. Distribution of patterns of INI1 Expression in Schwannomas Clinical Subtypes

Most of the schwannomas of hereditary type, NF2 and familial schwannomatosis (familial SC) have a mosaic pattern of staining, in contrast to the diffuse pattern of solitary, sporadic schwannomas. Sporadic schwannomatosis (sporadic SC) tumors show both patterns of staining.