Meeting Report

The Role of INI1 and the SWI/SNF Complex in the Development of Rhabdoid Tumors: Meeting Summary from the Workshop on Childhood Atypical Teratoid/Rhabdoid Tumors


Department of Pediatrics, Children’s Hospital of Philadelphia and University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104 [J. A. B.]; Albert Einstein College of Medicine, Bronx, New York 10461 [G. K.]; University of Cincinnati College of Medicine, Cincinnati, Ohio 45267 [E. S. K.]; Children’s National Medical Center, Washington, D.C. 20010 [R. J. P.]; Dana Farber Cancer Institute, Boston, Massachusetts 02115 [C. W. M. R.]; National Cancer Institute, Bethesda, Maryland 20892 [C. J. T., M. S.]; and University of North Carolina School of Medicine, Chapel Hill, North Carolina 27599 [B. W.]

Abstract

A workshop on childhood atypical teratoid/rhabdoid tumors of the central nervous system, sponsored by the National Cancer Institute and the Pediatric Brain Tumor Foundation of the United States, was held on January 29, 2001 in Bethesda, Maryland. Drs. Malcolm Smith, Jaclyn Biegel, and Roger Packer hosted the meeting. There were 22 participants from 14 institutions. The sessions were designed to review what was known about the biology and clinical behavior of rhabdoid tumors and to begin to develop treatment strategies that might be effective against this clinically aggressive group of malignancies. This report summarizes the biology sessions from the meeting. A detailed summary of the discussions regarding diagnostic studies and the roles for radiation therapy, chemotheraphy, and bone marrow or stem cell transplant in the treatment of rhabdoid tumors will be published separately.

Introduction

CNS3 AT/RT was first described in 1987 by Rorke et al. (1). Histologically, the tumors contain rhabdoid cells, but they may also demonstrate areas of primitive neuroepithelial, mesenchymal, and epithelial tissues. The tumors are often misdiagnosed, usually as medulloblastoma or primitive neuroectodermal tumor but also as choroid plexus carcinoma or germ cell tumor. Rhabdoid tumors in all anatomical sites, including the brain, kidney, and soft tissues, are diagnosed predominantly in very young children, especially those <2 years of age (1–4). In the CNS, 50% of AT/RTs are supratentorial and there is a predilection for the cerebello-pontine angle. Approximately one-third of tumors are disseminated at the time of diagnosis. These factors make surgical resection challenging, and total or near-total resections often cannot be obtained. Approximately 50% of AT/RTs will be, at least transiently, responsive to chemotherapy; however, chemotherapy alone is rarely curative. The use of radiotherapy, and specifically, the dose and volume, has not been standardized. Whereas radiation therapy appears to improve survival, its use is limited in children <3 years of age because of subsequent neurological deficits. The prognosis for infants and children <3 years of age is dire, with two-year survival rates being ≤20% (3, 4).

Two goals of the workshop were to define diagnostic criteria for CNS AT/RTs and to determine whether there was sufficient information on the functional role of the INI1 rhabdoid tumor suppressor gene to be able to incorporate biologically designed therapies into novel treatment strategies. Dr. Jaclyn Biegel (Children’s Hospital of Philadelphia, Philadelphia, PA) opened the biology session of the meeting and provided an update regarding the molecular cytogenetic deletions of chromosome 22q11.2 in rhabdoid tumors and mutation screening of primary biopsy samples. Biegel and others (2) have shown that rhabdoid tumors of the CNS (AT/RT), kidney, and soft tissues are characterized by monosomy 22 or a partial deletion of chromosome band 22q11.2, which contains the hSNF5/INI1 gene (5–7). Homozygous inactivation of INI1 results from the deletion of (on one or both copies) and/or mutation of INI1 (5–7). Alterations of INI1 have been documented in the majority of CNS AT/RTs, as well as rhabdoid tumors from other sites (5–7). Furthermore, the identification of germline INI1 mutations in children with one or more primary tumors, consistent with a “first hit,” predisposes children to the development of malignancy (6, 8). INI1 thus appears to function as a classic tumor suppressor gene.

Molecular and cytogenetic findings in a series of 76 rhabdoid tumors from different anatomical sites were presented. AT/RTs demonstrate monosomy 22 at a higher frequency than renal or extrarenal rhabdoid tumors, whereas extrarenal rhabdoid tumors have a very high rate of homozygous deletions of the entire INI1 gene. The loss of one allele, with concomitant mutation in the remaining copy of INI1, was observed in 42 of 76 tumors. Compound heterozygous mutations or apparent splicing mutations were detected in 4 tumors. The majority of tumors had single bp nonsense mutations, or frameshift mutations that are predicted to result in the coding of a novel stop codon, and premature truncation of the protein. Approximately 10% of cases do not have coding sequence mutations in any of the nine exons of INI1, and yet have decreased expression levels of INI1 by reverse transcription-PCR analysis or undetectable levels of the protein by Western blot analysis. Approximately 15% of tumors show no alterations of the INI1 gene at the DNA, RNA, or protein levels. One mechanism proposed for the loss of expression of INI1 in tumors without coding sequence mutations is hypermethylation of the INI1 promoter. Bisulfite modification and sequence analysis of DNA from primary rhabdoid tumors did not demonstrate hypermethylation of the INI1 promoter region. This mechanism is therefore not believed to account for decreased expression of INI1 in tumors without coding sequence mutations.

Participants discussed the criteria required for a diagnosis of CNS AT/RT, given that mutations cannot be identified in 100% of cases with classic morphology and immunohistochemical patterns. It was...
agreed that if the histological features and immunostaining were consistent with AT/RT, the absence of an INI1 mutation would not be sufficient to change the diagnosis. Alternatively, the presence of an INI1 mutation, in a tumor with features suggestive of medulloblastoma or primitive neuroectodermal tumor, was sufficient to change the diagnosis to AT/RT and treat the patient accordingly.

The development of CNS AT/RT and other rhabdoid tumors is clearly associated with deletion and mutation of the INI1 gene. To develop targeted biological agents for treatment of rhabdoid tumors, a greater understanding of the functional role of INI1 in normal and tumor cells is needed. INI1 is a member of the SWI/SNF chromatin remodeling complex, and several presentations were given on the role of SWI/SNF proteins in human and murine tumor development.

Ganjam Kalpana (Albert Einstein College of Medicine, Bronx, NY), described her efforts to use cell culture and other genetic systems to understand the function of INI1/hSNF5 in mammalian cells. The regulation of transcription initiation by RNA polymerase II in eukaryotic cells involves a series of protein-protein and protein-DNA interactions at transcriptional regulatory sites. Several classes of proteins have been shown to influence the rate of transcription, such as the general transcription factors, gene-specific regulatory proteins, and accessory proteins (coactivators, corepressors, and mediators; Refs. 9 and 10). Many of these accessory proteins are part of multiprotein complexes and regulate transcription both positively and negatively by modifying chromatin at regulatory sites. The positive regulators include the SWI/SNF and SAGA (Gcn5p HATs) complexes whereas the negative regulators include HDAC complexes. The HAT and HDAC complexes alter chromatin by covalently modifying the NH$_2$ terminus of histones by acetylation or deacetylation, respectively.

In contrast to complexes that covalently modify histones, the SWI/SNF and related complexes such as RSC (Remodel the Structure of Chromatin), ACF (ATP-using Chromatin assembly and remodeling Factor), NuRF (Nucleosome Remodeling Factor), and CHRAC (CHRomatin Accessibility Complex), alter chromatin by reorganizing or repositioning the nucleosomes in a ATP-dependent manner (11–15). The ATPase activity resides within SNF2/SWI2 of the yeast complex (16). In mammalian cells, there are many SWI/SNF complexes containing one of the several SNF2/SWI2 homologues, such as hBRM or hBRG1 (11–17). The ATPase activity of the SNF2/SWI2 homologue is essential for the chromatin remodeling activity of all SWI/SNF complexes. Other components of the SWI/SNF complex are variable with the exception of INI1, a homologue of the yeast SNF5 protein, which is found in all mammalian complexes (18, 19). INI1 was originally isolated by Kalpana et al. (20) by the yeast two-hybrid system through its interaction with HIV-1 Integrase; hence, the name Integrate interactor 1. In vitro reconstitution experiments have demonstrated that along with BRG-1 and BAF (BRG-1-Associated Factor)155 and 170, INI1 forms the functional core of the SWI/SNF complex that is sufficient to remodel the nucleosomes in vitro (21).

It is hypothesized that INI1, as a member of the SWI/SNF complex, affects its tumor suppressor function by modulating the transcription of cellular genes. It appears that only a subset of euchromatic genes require the SWI/SNF complex for transcriptional regulation, which is apparently determined by the strength of the promoter and activator and the extent of the chromatin condensation at the regulatory sites of genes (22). The SWI/SNF complex is not known to have the ability to recognize specific DNA sequences (23). Therefore, targeting of this complex to a subset of SWI/SNF-dependent genes could be through its interaction with sequence-specific DNA-binding proteins.

To identify the potential sequence-specific activators that require the SWI/SNF complex for activation, the yeast two-hybrid system was used to identify proteins that directly interact with it. One of the positive IN1-interacting clones isolated was identified as a fragment of c-Myc (9). Mapping the minimal domain of interaction indicated that the interaction involves the COOH-terminal region of c-Myc containing a bHLH-Zip domain and the highly conserved repeat 1 region of INI1. The c-Myc partner, Max, does not interact with INI1.

The specific association of c-Myc with the highly conserved repeat 1 region of INI1 both in vivo and in vitro suggests a functional interaction of the two proteins. c-Myc is a sequence-specific transactivator which, when heterodimerized with Max, binds to a CAC(G/A)TG motif known as the E-box (24). The repression of transcription by c-Myc family members, such as the Max:Mxi1 heterodimers, is mediated by chromatin condensation via the recruitment of HDACs to promoters containing E-box motifs (Refs. 25 and 26; Fig. 1A). Therefore, it is likely that the mechanism of activation by c-Myc might involve chromatin remodeling via the recruitment of the SWI/SNF complex to the E-box, mediated by its interaction with INI1. In support of this hypothesis, Kalpana found that dominant negative inhibitors derived from two different components of the SWI/SNF complex, BRG-1 (BRG-1*) and INI1 (56), inhibited the c-Myc trans-activation function in a dose-dependent manner. The results of this study suggest a unified mechanism, which is mediated by chromatin remodeling, for both repression and activation by the c-Myc family of proteins. Whereas Max:Mxi1 heterodimers repress transcription by recruiting the HDACs, Myc:Max heterodimers could activate the same promoters by recruiting the SWI/SNF complex (Fig. 1B).

The c-Myc protein is an enigmatic protein that is involved in diverse and opposing processes such as cellular transformation, differentiation, and apoptosis (24). It is possible that the interaction between INI1 and c-Myc could facilitate the transcription of only a discrete subset of c-Myc target genes involved specifically in differ-

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**Fig. 1.** Activation and repression of transcription by the c-Myc family of proteins mediated by the recruitment of chromatin remodeling complexes. A, repression of transcription by Max:Mxi heterodimers mediated by the recruitment of the Sin3/HDAC complex. B, activation of transcription by Mxi-Max heterodimers mediated by the recruitment of SWI/SNF complex via the direct interaction of INI1 with c-Myc.
entiation or apoptosis, which is consistent with the tumor suppressor activity of INI1. Furthermore, c-Myc is not the only cellular protein that has been identified as a binding partner for INI1. It has been shown to bind to the EBV trans-activator protein, EBNA-2; the human homologue of Triithorax, ALL1 (also referred to as MLL, HTRX, and HRX); and the replication protein E1 of human papilloma virus and GADD34 (9, 27–30). Of particular interest are MLL and GADD34. Translocations involving MLL are found frequently in leukemias, whereas GADD34 is involved in apoptosis. Although the functional implications of these interactions have not been demonstrated, they could be important in mediating tumor suppression by INI1. In addition, INI1 could interact with additional transactivators and assist in their activity. One emerging hypothesis from these studies is that INI1 may recruit the SWI/SNF complex to specific target sites by its ability to interact with a varied set of proteins to mediate such diverse processes as transcription, replication, integration, and tumor suppression (Fig. 2).

To further understand the transcriptional activity of INI1 and its relation to tumor suppressor function, Kalpana has developed a system to study the transactivation function of INI1. The ability of a GAL4 DNA binding-domain/INI1 fusion protein to regulate transcription in various human cell lines was tested using a reporter construct containing the upstream activating sequences. Unpublished data from her group suggests that whereas INI1 could activate transcription in a SWI/SNF-dependent manner, it could also repress transcription in a cell type-specific manner. Several investigators are using gene expression arrays to identify the spectrum of target genes regulated by INI1 and the SWI/SNF complex. This analysis will provide insight into the normal function of INI1; the mechanism of tumor suppression by INI1; drug efficacy during the treatment of rhabdoid tumors; and the genes required for the reversal of the malignant phenotype.

In addition to the in vitro studies of INI1, Charles Roberts (Dana Farber Cancer Institute, Boston, MA) described the use of murine knockout models to study Inil function. Two groups have reported recently “knockouts” of murine Inil/Snf5 (31, 32). Murine and human INI1 are closely related with the predicted protein sequences differing by a single amino acid (31). Whereas Inil is widely expressed during embryogenesis, there are areas of consistent high-level expression in the first branchial arch, brain, and hindlimb bud (31). Mice deficient in Inil/Snf5 die early in embryonic development, prior to the onset of gastrulation (31, 32). Inil/Snf5+/− mice are born at the expected frequency and appear normal (31, 32). However, beginning as early as 5 weeks of age and with a median onset of 7 months, a subset of the mice develop tumors (31, 32). In one Inil/Snf5+/− strain, tumors arise predominantly on the face, but have not been observed in brain (31). In contrast, Klochendler-Yeivin et al. (32) report that 30% of tumors that occur in Inil/Snf5+/− mice are ‘intra-cranial’. In neither case do tumors arise in kidney. It is not clear whether the difference in tumor location is attributable to differences in strain background or a difference in targeting constructs.

On histological examination, all tumors present a consistent appearance (31, 32). Each is composed of areas containing atypical spindle cells and areas of poorly differentiated small round blue cells. These regions are admixed with areas containing variable numbers (5–20%) of cells with prominent hyaline cytoplasmic (“rhabdoid”) inclusions, large eccentric vesicular nuclei, and large nucleoli (31, 32). As is the case in human tumors, classic rhabdoid cells constitute a small minority population in most tumors. However, in some cases the murine tumors consist of a uniform sheet of rhabdoid cells (31). Ultrastructural examination demonstrates paranuclear whorls of intermediate filaments, a key feature of human rhabdoid cells (31). Immunohistochemical and Southern analyses indicate that the remaining allele of Inil/Snf5 has been lost in the tumors (31, 32). Each of these features is entirely consistent with rhabdoid tumors arising in children. These findings indicate that Inil/Snf5 is an essential gene, that its tumor suppressor function is evolutionarily conserved, and that the microscopic appearance of the murine tumors is remarkably similar to that of human tumors.

Given the shared genetic basis, Inil/Snf5 haploinsufficient mice may provide a useful model of a rhabdoid predisposition syndrome. However, two potential challenges exist. First, the overall incidence of tumors in Inil/Snf5+/− mice is somewhat low, presumably in part because spontaneous inactivation of the normal allele is required. Second, the location of the murine tumors differs significantly from those seen in children. For instance, whereas kidney is the most frequent site of malignant rhabdoid tumors in children, none of the mice have developed renal tumors. The generation of conditionally targeted Inil/Snf5 mice, in which both alleles of Inil/Snf5 are deleted specifically only in the brain or kidney, may now be undertaken. These mice have the potential to develop rhabdoid tumors at a high frequency in these sites and thus, offer an attractive model in which to test novel therapies.

Although INI1 may have independent functions distinct from its role as a component of the SWI/SNF complex, the SWI/SNF complex is a critical regulator of transcription that is targeted in specific tumor types. Studies of other members of the complex may also elucidate the tumor suppressor function of INI1. For example, in a random sampling of tumor cell lines, ~10% of the lines studied showed mutations in the core ATPase subunit, BRG-1 (33). Furthermore, heterozygous inactivation of Brg-1 in mice leads to tumor predisposition (34), supporting the role of Brg-1 (similar to INI1) in tumor suppression.

Erik Knudsen (University of Cincinnati College of Medicine, Cincinnati, OH) has been focusing on the action of BRG-1 as a tumor suppressor. Because BRG-1 is a core component of the SWI/SNF complex, it is possible that those phenotypes arising because of a loss of BRG-1 are consistent with the loss of other chromatin remodeling activities, particularly the loss of INI1. Three specific phenotypes associated with the loss of BRG-1 that have clear impact on tumorigenesis were discussed.

Initial studies from the Knudsen laboratory focused on the role of SWI/SNF in signaling by the RB tumor suppressor protein. In early G1, dephosphorylated/active RB functions to inhibit cell cycle progression by assembling repressor complexes that prevent cell cycle progression (35, 36). Mitogens stimulate RB inactivation via induction of cdk/cyclin complexes (37, 38). RB inactivation is required universally for progression through the mitotic cell cycle. Not sur-
prisngly, the RB protein is targeted at high frequency in human tumors through a variety of mechanisms (37, 38). Recently, members of Knudsen’s laboratory (39, 40) identified a distinct mechanism by which RB is inactivated in tumor cells. Specifically, they showed that BRG-1 is required for RB to exert its antimitogenic potential. For these studies, Knudsen’s laboratory initially designed and characterized a constitutively active allele of RB, PSM-RB, which is resistant to inactivation by endogenous cdk/cyclin activity (41). As expected, PSM-RB induced cell cycle arrest in all primary and immortalized cell lines tested and also arrested most tumor cell lines (42). Surprisingly, they identified two cell lines, C33A and SW13, which were resistant to the PSM-RB-mediated cell cycle arrest. Because PSM-RB cannot be inactivated by phosphorylation, this suggested that these tumor cell lines harbored a novel lesion that disrupted signaling downstream of RB. This idea was supported by the finding that whereas PSM-RB down-regulates cyclin A and cdk2-associated kinase activity to mediate cell cycle inhibition in arrested (sensitive) cells, PSM-RB failed to signal to repress cyclin A expression or inhibit cdk2-activity in C33A or SW13 cells (39, 40). Subsequent cell fusion analyses indicated that C33A cells lack a trans-acting factor that is required for RB to repress cyclin A expression and prevent cell cycle progression (39). Prior evidence in the literature indicated that both C33A and SW13 cells are BRG-1 deficient, suggesting that loss of BRG-1 was responsible for the failure in signaling (43, 44). By specifically restoring BRG-1 expression in C33A or SW13 cells, RB-mediated cell cycle inhibition was restored (39, 40). Conversely, a dominant negative BRG-1 allele converted cells to a PSM-RB-resistant phenotype (39). These and additional studies carried out in independent laboratories demonstrate that SWI/SNF activity is required for RB-mediated transcriptional repression and subsequent inhibition of proliferation (33, 39, 45).

Knudsen and others (46, 47) have shown that RB is a critical mediator of the cellular response to chemotherapeutic drugs, such as CDDP. In normal cells, CDDP damage leads to checkpoints that block DNA replication. These effects are dependent on RB because in RB-deficient cells, cell cycle progression continues in the presence of severe DNA damage. These results have clear implications for the treatment of RB-deficient tumors with chemotherapeutic agents (46, 47). To assess the action of BRG-1 in the response to chemotherapeutic drugs, expression of dominant negative BRG-1 was used to ablate BRG-1 function. Under these conditions, the inhibition of replication that is observed upon CDDP damage was overcome, as cells expressing dominant negative BRG-1 continued to progress through the cell cycle in the presence of damage (39). Ongoing studies with a number of BRG-1 deficient tumor cell lines recapitulate this result, suggesting that the loss of BRG-1 during tumorigenesis results in a loss of appropriate DNA-damage checkpoints.

While studying BRG-1 signaling, Knudsen’s laboratory discovered that BRG-1 is a positive regulator of the cell surface protein CD44 (48). The CD44 protein is used widely as a marker for metastasis, invasion, and tumor progression (49–52). In fact, loss of CD44 expression is an indicator of poor prognosis in specific tumor types. In the absence of functional BRG-1, CD44 protein and RNA are virtually undetectable (48). Transduction of wild-type BRG-1 into BRG-1-deficient cell lines results in a massive induction of CD44 RNA and protein. Conversely, expression of dominant negative BRG-1 results in the attenuation of CD44 expression. This effect of BRG-1 is independent of its cell cycle function, as it is readily observed in cells that are RB-deficient. These results provide the impetus for evaluating the role of BRG-1 and chromatin remodeling factors not only in cell cycle control but also in the regulation of cell migration and metastasis.

Together, these studies make specific predictions about the behavior of BRG-1-deficient tumor cells and provide targets that may be dysregulated by the loss of other chromatin remodeling activities. Specifically, whether INI1 loss in AT/RT results in resistance to RB, loss of CDDP checkpoint, and loss of CD44 expression remains to be determined explicitly. Should loss of INI1 fail to influence these signaling programs, it would indicate that disparate lesions of the SWI/SNF complex act upon different pathways to suppress tumor formation. This interesting possibility may in part provide an explanation for the distinct distribution of tumors exhibiting INI1 or BRG-1 loss.

Bernard Weissman (University of North Carolina at Chapel Hill, Chapel Hill, NC) presented studies from his group comparing and contrasting the effects of BRG-1/BRM loss with INI1 loss in human tumor cell lines. In yeast, the inactivation of any component of the SWI/SNF complex yields a nearly identical phenotype. Therefore, Dr. Weissman’s group tested whether inactivation of INI1 led to the same alterations in human tumors as BRG-1/BRM loss. As Dr. Knudsen discussed, BRG-1/BRM loss in human tumor cell lines results in the abrogation of RB-mediated cell cycle arrest. Therefore, Dr. Weissman’s group assessed the inactivation of INI1 on RB function. Three rhabdoid cell lines and one undifferentiated sarcoma cell line that lack INI1 activity were transfected with a constitutively active RB or p16INK4D gene. In all cases, both genes efficiently inhibited growth of the cell lines. A second result of BRG-1/BRM inactivation is the loss of CD44 expression. However, at least one INI1-deficient cell line expressed high levels of this protein. Finally, attempts to isolate cell lines that re-express INI1 in a stable fashion did not prove successful. Infection of three different INI1-deficient cell lines with an INI1 retrovirus yielded small colonies with limited proliferation. After reaching sizes of 2–100 cells, they took on a flat, enlarged morphology with abundant stress fibers. This phenomenon closely resembles that observed with BRG-1/BRM-deficient cells after transfection with a BRG-1 expression vector. Overall, these results indicate that the effects of inactivation of the INI1 gene in human tumors resemble some of those observed after BRG-1/BRM loss. However, other alterations clearly do not overlap. These findings indicate that loss of INI1 may abrogate only a subset of SWI/SNF complex functions. However, they may also suggest a role in cellular functions independent of its inclusion in the SWI/SNF complex.

On the basis of the finding that the primary genetic lesion in rhabdoid tumors is loss of a member of the SWI/SNF chromatin remodeling complex, Carol Thiele, James Zweibel, and Anthony Murgo (National Cancer Institute, Bethesda, MD) discussed the use of chromatin remodeling agents as potential agents for treating patients with rhabdoid tumors. Gene expression is controlled in part through transcription factors that possess DNA-binding domains that recognize sequence-specific regions in DNA and transactivation domains that recruit coactivator or corepressor proteins that regulate transcription through modification of the local chromatin environment. Coactivator proteins may possess intrinsic HAT activity or recruit other proteins with HAT activity (53). SWI/SNF can be recruited by transcriptional activator complexes to specific promoters, suggesting that SWI/SNF assists activators in binding to nucleosomal sites as an early step in transcriptional activation (54). Corepressors possess or recruit proteins with HDAC activity leading to histone hypoacetylation and gene silencing (55). Recent reports provide evidence for interactions between nuclear receptor corepressor complexes containing HDACs and SWI/SNF complexes (56, 57), an observation that could explain the apparent role of SWI/SNF in the repression of the transcription of some genes (58).
The HDAC inhibitors represent a new class of anticancer agents and are the only agents targeted directly at chromatin remodelling currently in clinical trials (59). The anticancer activity of these agents is presumed to be through the transcriptional activation of genes involved in regulating cell growth (e.g., p21 and transforming growth factor β type II receptor; Refs. 60 and 61) and genes involved in mediating apoptosis (62, 63). In acute promyelocytic leukemia, HDAC inhibitors such as trichostatin A and phenylbutyrate have been found to relieve the HDAC-mediated transcriptional repression imposed by the chimeric APL/RAR transcription factor and to stimulate differentiation and apoptosis of leukemic cells (64).

There is too little understanding of the mechanism by which INI1 deficiency leads to AT/RT to confidently predict whether HDAC inhibitors will be active against AT/RT. Several mechanisms can be proposed in support of a potentially beneficial role for HDAC inhibitors. For example, just as mutations in the major core histones relieve transcriptional repression and bypass the requirement for SWI/SNF in yeast (65–67), so might the drug-induced acetylation of histones circumvent the need for INI1 for transcription of key genes involved in repressing tumor development and growth in humans. Alternatively, if loss of INI1-facilitated c-Myc transactivation is a key step in tumorigenesis (which could imply an imbalance in tumor cells between HAT activity associated with c-Myc transcriptional activation and HDAC activity associated with Mad family member-mediated transcriptional repression), then HDAC inhibitors might be active against AT/RT by normalizing this imbalance (68). However, if tumorigenesis is related to the loss of the transcriptional repression activities of SWI/SNF complexes (which could be attributable to SWI/SNF association with HDAC complexes), then there is no obvious direct mechanism by which HDAC inhibitors would have antitu-

cmor activity. MS-27–275 is a synthetic benzamide derivative that is a potent HDAC inhibitor and has marked antitumor activity in preclinical studies in adult tumor cell lines (69). In a study of pediatric solid tumor cell lines, MS-27–275 had marked antitumor activity against a number of pediatric tumor cell lines in vitro.6 In a malignant rhabdoid cell line, G401, in which INI1 is deleted, MS-27–275 decreased cell growth. Like most HDAC inhibitors, MS-27–275 stimulated an increase in cell cycle inhibitor p21, and this was accompanied by an accumulation of G401 cells in the G1 phase of the cell cycle. Further in vitro and in vivo preclinical studies will be necessary to determine whether this class of drugs is likely to be active in malignant rhabdoid tumors and whether they reverse the transcriptional alterations caused by mutation or deletion of INI1.

Summary

Participants at the workshop were able to define criteria needed for the diagnosis of atypical teratoid tumors, based in large part on the identification of deletions and mutations of the INI1 tumor suppressor gene. However, at the present time, there is not enough known about the function of INI1, either as an independent modulator of gene expression or through its association with the SWI/SNF complex, to be able to use specific targeted biological agents for treatment. As shown for the potential use of HDAC inhibitors in rhabdoid tumors, however, further interactions between basic scientists and clinicians will be beneficial for developing the next phase of investigative protocols for treatment of children with rhabdoid tumors.

References


6 C. Thiele, unpublished data.


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