RhoA-Dependent Regulation of Cell Migration by the Tumor Suppressor hSNF5/INI1

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Abstract

Malignant rhabdoid tumors (MRT) are extremely aggressive pediatric tumors caused by the inactivation of the hSNF5/INI1 tumor suppressor gene, which encodes a core member of the SWI/SNF chromatin remodeling complex. Roles for hSNF5/INI1 in cell cycle and differentiation have been documented. Based on the observation that MRTs are highly invasive, we investigated a role for hSNF5/INI1 in cell migration. MRT cell lines exhibit high migration properties that are dramatically reduced upon hSNF5/INI1 expression. This effect is associated with the disorganization of the actin stress fiber network and is mediated by the inhibition of the activity of the small GTPase RhoA, through a nuclear, SWI/SNF-dependent transcriptional mechanism. We further show that the knockdown of hSNF5/INI1 in epithelial 293T or MCF7 cells results in increased cell size, loss of cell-cell adhesions, and enhanced migration, associated with an increased RhoA activity. Finally, we show that the SNF5 homology domain is required for hSNF5/INI1-mediated inhibition of migration, and that a missense mutation (S284L) associated with cancer is sufficient to impair hSNF5/INI1 function in migration. We conclude that the inhibition of migration is another crucial tumor suppressor function of hSNF5/INI1, in addition to its previously described functions in proliferation and differentiation, and that its loss-of-function in MRTs may account for the high invasiveness and metastatic potential of these tumors. [Cancer Res 2008;68(15):6154–61]

Introduction

Malignant rhabdoid tumors (MRT) are extremely aggressive pediatric tumors that arise in various localizations, mainly the kidney, the brain (ATRT), and soft tissues. Despite intensive chemotherapy, most children die within 1 year after diagnosis. MRTs are undifferentiated tumors of unknown cellular origin, which are caused by the inactivation of the hSNF5/INI1 tumor suppressor gene (1). This gene encodes a core member of the SWI/SNF chromatin remodeling complex, which regulates transcription and plays crucial roles in the control of cell proliferation and differentiation (2, 3). It also presents genetic characteristics of a bona fide tumor suppressor gene, with constitutional mutations being associated with a strong predisposition to develop MRTs (4, 5). This tumor suppressor function has been confirmed in the mouse. Indeed, whereas Snf5/Ini1 homozygous deletion is embryonic lethal, heterozygous mice develop tumors that closely resemble MRTs and that show loss of heterozygosity of the wild-type allele (6–8).

hSNF5/INI1 exhibits an antiproliferative activity because hSNF5/INI1 expression in MRT cell lines induces a cell cycle arrest in the G1 phase. This arrest is reversible and requires a functional RB/E2F pathway (9–11). hSNF5/INI1 also regulates mitotic checkpoint control, chromosomal stability, and DNA damage signaling (12, 13). Finally, recent data from our laboratory and others indicate that SNF5/INI1 is required for various differentiation pathways, including heptocyte differentiation in vivo (14), and neural (15) or adipocyte differentiation in vitro (16).

Although most MRTs display truncating mutations of hSNF5/INI1, being deletions, nonsense, or frameshift mutations, a few missense mutations have been identified in tumors (4, 17). These include in particular the proline 48 to serine (P48S) and the serine 284 to leucine (S284L) changes, the latter being located within the highly conserved SNF5 homology domain (18). Surprisingly enough, these two point mutants were shown to be functional for G1 arrest (12), suggesting that they may contribute to tumorigenesis through other mechanisms.

We previously showed that hSNF5/INI1 regulates cell shape and cytoskeleton organization of a MRT cell line (19). This remodeling of the cytoskeleton was associated with the induction of a variety of genes involved in the organization of actin filaments and with a decreased activity of the Rho small GTPase, involved in stress fibers assembly and contractility (19). Given the highly invasive characteristics of MRTs, we have now investigated a role for hSNF5/INI1 in the regulation of cell migration. We show that hSNF5/INI1 can regulate cell migration in different cell models and that this effect is dependent on the regulation of RhoA, but not of Rac1 and Cdc42, two other small GTPases involved in lamellipodia formation at the leading edge, and in filopodia formation and cell polarity, respectively (20). These data suggest that the hSNF5/INI1 chromatin remodeler regulates a complex network of genes, including Guanine nucleotide Exchange Factors (GEF) and GTPase Activating Proteins (GAP), which may control the activity of the RhoA protein and, hence, cytoskeleton architecture and cell migration.

Materials and Methods

Cell culture, transfections, and reagents. MRT and MCF7 cell lines were grown in RPMI supplemented with 10% FCS. The tetracycline-regulated
hSNF5/INI1-inducible cell line I2a was maintained and induced as previously described (19). HEK-293T cells (American Type Culture Collection) were cultured in DMEM supplemented with 10% FCS.

HA-INI1-, HA-INI1:A, HA-INA1:A2, HA-INI1:A3, HA-INI1:48PS, and HA-INI1:284L-pCDNA3 encoding vectors as well as the MRT cells transfection procedure by the Effectene reagent (Qiagen) have been described previously (10). Transfected cells were selected with puromycin (2 μg/mL). pCDNA3-HA-INI1-L266A was constructed by mutating the Leucine residue 266 of hSNF5/INI1 into Alanine, using site-directed mutagenesis procedure with the following oligonucleotides: 5'CGTCATCATCAAGGCCAACATCCATGTGGGA3' and 5'TCCCCACATGGAT-TCCCACTAGGATTTGGCTTTGAGTAGGCG3'. The pB85-Myc vectors encoding Myc-tagged versions of wild-type Rho, RhoN19, and RhoA63 and the pGEX-4T2-RBD and PBD vectors encoding glutathione S-transferase (GST)-RBD and GST-PBD were provided by Jean de Gunzburg (Institut Curie, Paris, France; ref. 21). GST production was performed in BL21 bacteria. pCDNA3-BRG1-K798R was constructed by subcloning the corresponding HA-tagged BRG1-K798R cDNA (5349pb HpaI-fragment) from the pB85 vector (kindly provided by Christian Muchardt, Institut Pasteur, Paris, France), into the pCDNA3.1 vector at EcoRV site.

The ROCK inhibitor Y-27632 (Sigma) was used at a concentration of 5 μM and the C3 Transferase (Tebu Bio) at 1 μg/mL.

Retroviral and lentiviral infections. The retroviral pWZL-IRES-Blast vector encoding HA-hSNF5/INI1 was previously described (16). The Mission TRC shRNA clone n39587 in pLKO.1-puro, specific for hSNF5/INI1, was purchased from Sigma. The production of viral particles in 293T cells was described by Ren and colleague (22), in 20 mmol/L Tris (pH 7.5), 1% triton, 0.1% SDS, 10 mmol/L MgCl₂, proteases, and phosphatases inhibitors, and lysates were incubated with GST-RBD or PBD for 45 min at 4°C, washed thrice, and resolved by SDS-PAGE. The mouse antibodies correspond to HA-tagged BRG1-K798R cDNA (5349pb HpaI-fragment) from the pB85 vector (kindly provided by Christian Muchardt, Institut Pasteur, Paris, France), into the pCDNA3.1 vector at EcoRV site.

Wild-type and cancer-associated missense mutant, disorganizes the actin cytoskeleton and inhibits migration in MRT cell lines. A, I2a cells are derived from the M0N MRT cell line and harbor an inducible hSNF5/INI1 gene, which can be induced upon tetracycline removal. I2a cells incubated with or without tetracycline for 3 d were submitted to transwell assay. Columns, mean obtained from duplicate experiments; bars, SD. B, G401 and KD cells infected with empty pWZL (– INI1) or pWZL-INI1 (+ INI1), selected for 3 d by blasticidin and then subjected to transwell assay. Columns, mean obtained from duplicate experiments; bars, SD. C, G401 and KD cells infected with empty pWZL (– INI1) or pWZL-INI1 (+ INI1) and subjected to 3 d of blasticidin selection were plated on coverslips and stained with phalloidin. D, C and D, M0N cells were transfected with empty vector pCDNA3 (–) or with wild-type (+ INI1), deletion, or point mutants of hSNF5/INI1; selected with puromycin; and either subjected to transwell assay (C) or stained with phalloidin (D). Columns, mean obtained from duplicate experiments; bars, SD. The constructs encode the following amino acid sequences: (10): INI1, 1 to 385; Δ2, 1 to 187; and Δ3, 1 to 329.

Rho-GTP levels were measured by a pulldown assay using GST fused to the Rho-binding domain of Rhotekin (GST-RBD), whereas Rac-GTP and Cdc42-GTP levels were measured using GST fused to the p21 Binding Domain of p21 Activated Kinase PAK1 (GST-PBD). Cells were lysed as described by Ren and colleague (22), in 20 mmol/mL Tris (pH 7.5), 1% triton, 0.1% SDS, 10 mmol/mL MgCl₂, proteases, and phosphatases inhibitors, and lysates were incubated with GST-RBD or PBD for 45 min at 4°C, washed thrice, and resolved by SDS-PAGE. The mouse antibodies used were as follows: RhoA (sc418; Santa-Cruz, Teshu), Rac1, Cdc42, ARHGAP5 and hSNF5/INI1 (BD Biosciences), and HA (Babco). Horseradish peroxidase–conjugated anti-mouse secondary antibody was purchased from Amersham.

Quantitative reverse transcription-PCR analyses. Total RNA was extracted using Trizol Reagent (Invitrogen). cDNAs were synthesized from 1 μg of RNA using the GeneAmp RNA PCR core kit (Applied Biosystem). Quantitative PCR analyses were performed with qPCR Mastermix Plus without UNG, using the following TaqMan Assays-on-demand: ARHGAP5 (Hs00750732_s1) and glyceraldehyde-3-phosphate dehydrogenase (Hs99999905_m1). GAPDH expression, which does not vary with the experimental conditions, was used for normalization.
Results

Inhibition of migration upon hSNF5/INI1 expression in rhabdoid cell lines. We previously showed that expression of hSNF5/INI1 in deficient MON MRT cells induced cell cycle arrest and morphologic changes (19). In particular, MON cells exhibit major cytoskeleton reorganization upon hSNF5/INI1 expression. We further investigated this phenotype by analyzing the migration properties of these cells in transwell assays. We used the MON-derived I2a cell line in which hSNF5/INI1 expression can be regulated by tetracycline (19). To limit the influence of cell proliferation, which is decreased in the presence of hSNF5/INI1, cell migration experiments were allowed to proceed for only 8 hours. hSNF5/INI1-deficient I2a cells exhibited a high motility that was dramatically decreased upon hSNF5/INI1 expression (Fig. 1A).

To investigate whether this was a general property of MRT, we analyzed the actin cytoskeleton structure and motility of G401 and KD, two other MRT cell lines. G401 cells have a polygonal shape similar to that of MON cells, whereas KD cells harbor an elongated morphology. Both displayed a highly organized actin stress fibers network (Fig. 1B). In both instances, this network was no longer observed upon hSNF5/INI1 expression (Fig. 1B). Moreover, this cytoskeleton change was associated with a severely decreased motility (Fig. 1A). Taken as a whole, these data indicate that hSNF5/INI1 expression in MRTs induces a loss of stress fibers, associated with a severe impairment in migratory properties.

To study the domain(s) of hSNF5/INI1 required to inhibit migration and its relationship with oncogenesis, we expressed various cancer-associated mutants of hSNF5/INI1 in MRTs and subsequently analyzed cytoskeleton organization and migration. These were three previously described deletion mutants (10), as well as two missense mutants, P48S and S284L. The pathogenicity of these last two mutations, which were both described in MRTs, is likely on the basis of the highly conserved status across species of both residues and on the absence of reported single nucleotide polymorphism at these positions. The somatic occurrence of P48S could be clearly documented (17). MON cells were transfected with these various constructs, selected with puromycin, then subjected to migration assays or plated on coverslips for phalloidin staining 3 days after transfection. The Δ1 and Δ3 mutants, which contain the Rpt1 and Rpt2 regions of the SNF5 homology domain but lack NH2-terminal and COOH-terminal–coiled coil regions, respectively, were as efficient as wild-type hSNF5/INI1 to inhibit migration (Fig. 1C). In contrast, the Δ2 mutant, which lacks the SNF5 homology domain, did not modify the migration. The inhibition of migration was associated with the loss of actin stress fibers that were still observed after expression of Δ2 but not of Δ1 and Δ3 (data not shown). These data indicate that the Rpt1 and Rpt2 regions of the SNF5 homology domain are required for the hSNF5/INI1-mediated remodeling of the actin cytoskeleton and for the inhibition of migration. Strikingly, the S284L mutant was not functional in these assays, affecting neither cytoskeleton organization nor migration, whereas the P48S mutant was as efficient as the wild-type protein in both assays (Fig. 1C and D).

The hSNF5/INI1-induced inhibition of migration correlates with a decreased RhoA activity. Small GTPases of the Rho family are key regulators of actin cytoskeleton organization and cell migration. Their activity can specifically be measured by purification of the GTP-bound form by GST-pulldown experiments with GST-RBD for RhoA and GST-PBD for Rac1 and Cdc42 (22). We previously showed that Rho activity was decreased upon a 7-day induction of hSNF5/INI1 in I2a cells, although the total Rho protein level was not modified (19). Here, we performed a time course experiment at earlier time points (2, 3, and 7 days) and could show that this decreased RhoA activity was observed as early as 2 days after tetracycline removal (Fig. 2A). A similar decreased level of GTP-bound RhoA was observed 3 days after ectopic expression of wild-type hSNF5/INI1 in MON or in G401 cells (Fig. 2B; data not shown). The expression of Δ3, and to a lesser extent, that of Δ1, led to a decreased RhoA activity in MON cells, whereas Δ2 expression had no effect (Fig. 2B, left). These data indicate that the Rpt1 and Rpt2 regions of the SNF5 homology domain are required for hSNF5/INI1-mediated inhibition of RhoA. Interestingly, the S284L mutant did not lead to any inhibition of RhoA (Fig. 2B, right). These results were completely consistent with the effects of the various mutants on actin stress fibers described above, indicating that RhoA inhibition correlated with hSNF5/INI1-mediated cytoskeleton remodeling and migration inhibition.

Finally, the Rac1 and Cdc42 GTPases exhibited high-levels of GTP-bound forms in MON cells, but in contrast to RhoA, these levels were not significantly modified upon hSNF5/INI1 expression (Fig. 2C). These data not only indicate that RhoA, Rac1, and Cdc42
are strongly activated in MRTs, but also that hSNF5/INI1 expression specifically inhibits RhoA activity.

hSNF5/INI1-mediated effects on cytoskeleton and migration require RhoA inhibition. To further address the correlation between the decreased RhoA activity and the hSNF5/INI1-mediated effects on cytoskeleton and migration, we investigated the consequences of modulating RhoA activity in MRT cell lines. A total disappearance of stress fibers was observed when MON cells were transfected with RhoN19, a dominant-negative version of RhoA, thus indicating that Rho inhibition was sufficient to mediate the morphologic changes induced by hSNF5/INI1 (Fig. 3A and B). Similarly, the treatment of MRT cells with the C3 transferase Rho inhibitor, or with Y-27632, an inhibitor of ROCK, the main Rho effector, led to cell motility and actin stress fibers modifications similar to those induced upon hSNF5/INI1 expression (Fig. 3C and D; data not shown). At the opposite, RhoL63, an active form of RhoA with altered GTPase activity and constitutive binding to effectors, prevented hSNF5/INI1-mediated cytoskeleton remodeling (Fig. 3A and B). Taken together, these data show that hSNF5/INI1-mediated effects on cytoskeleton and migration require inhibition of the RhoA-ROCK pathway.

hSNF5/INI1-mediated RhoA inhibition requires a nuclear, SWI/SNF-dependent, transcriptional activity. Different mechanisms may account for the hSNF5/INI1-mediated inhibition of RhoA activity. A direct link between hSNF5/INI1 and RhoA could be hypothesized on the basis of recent observations showing that proteins with nuclear regulatory function on proliferation or transcription, such as p27 (23–25) or EZH2 (26), may also harbor cytoplasmic roles on actin cytoskeleton organization and cell migration. Although hSNF5/INI1 is barely undetectable in the cytoplasm, it harbors a masked nuclear export signal (NES) and can transiently shuttle in this compartment (27). However, our efforts to detect a putative interaction between hSNF5/INI1 and RhoA by coimmunoprecipitation experiments remained unsuccessful.

To determine whether the cytoplasmic shuttling of hSNF5/INI1 was required for the inhibition of RhoA in the cytoplasm, we took advantage of a previously published point mutant of hSNF5/INI1, L266A, which was shown to disrupt the NES and, hence, prevent the nuclear export of the protein (27). When transfected in MON cells, this L266A mutant was as efficient as the wild-type hSNF5/INI1 protein to inhibit RhoA activity and cell migration, showing that hSNF5/INI1 acts through a nuclear mechanism (Fig. 4A and B).

To further test whether a functional SWI/SNF complex was required for the hSNF5/INI1-mediated regulation of RhoA activity, MON cells were cotransfected with hSNF5/INI1 and a dominant negative mutant of BRG1 that encodes a protein is defective for the ATPase activity (28). In the presence of this mutant, hSNF5/INI1 ability to inhibit RhoA and cell migration was severely reduced (Fig. 4A and B). This mutant also prevented hSNF5/INI1-mediated

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**Figure 3.** hSNF5/INI1-mediated effects on cytoskeleton and migration require the specific inhibition of RhoA activity. A and B, MON cells were transfected with empty (−) INI1 or hSNF5/INI1-expressing pCDNA3 vector (+ INI1), together with empty (pRK5) or Myc-RhoN19 or Myc-RhoL63-expressing pRK5 vectors. Cells were either lysed, subjected to GST-RBD pulldown assay and to Western blot analysis with anti-RhoA, anti-HA, and anti-Myc antibodies (A), or submitted to phalloidin staining (B). C and D, MON and KD cells were treated for 30 min with 5 μmol/L of ROCK inhibitor Y-27632, subjected to transwell assay (C) or to phalloidin staining (D). The mean values and SDs obtained from duplicate experiments are indicated.
cell cycle arrest (data not shown). These data show that the SWI/SNF ATP--dependent chromatin remodeling activity is required for hSNF5/INI1-mediated inhibition of RhoA and migration.

We previously described that hSNF5/INI1 expression led to the modification of the expression of many genes involved in cytoskeleton organization and cell-cell adhesion (19). To explore the mechanisms underlying hSNF5/INI1-mediated RhoA inhibition, we focused on GEFs and GAPs proteins. Among these regulator proteins, we found that hSNF5/INI1 activated the expression of ARHGAP5 (p190-B RhoGAP), both at the RNA and at the protein levels (Fig. 4C). Whereas ARHGAP5 was not expressed in I2a cells grown in the presence of tetracycline, it was detected at the RNA level as soon as 2 days (data not shown) and at the protein level 7 days after withdrawal of this antibiotic (Fig. 4C). In KD cells, which express a higher basal level of ARHGAP5, a 10-fold increase was also observed upon hSNF5/INI1 expression (Fig. 4C), indicating that ARHGAP5 activation is a common feature of hSNF5/INI1-expressing MRTs.

hSNF5/INI1 gene knockdown in HEK-293T or MCF7 cells results in morphologic changes, increased migration, and RhoA activation. To address the potential of hSNF5/INI1 to regulate migration in a different cell model, we knocked down its expression in HEK-293T cells, which are epithelial cells with very limited motility (29). These cells were infected with lentiviruses expressing control or hSNF5/INI1-specific shRNA and established as stable populations. hSNF5/INI1 knockdown was very efficient and was associated with dramatic morphologic changes (Fig. 5A and B). Indeed, compared with control cells that formed epithelial-like structures rich in cell-cell adhesions, hSNF5/INI1-inhibited cells exhibited increased cell size and attachment area (5-fold), with appearance of many cellular extensions (Fig. 5B and C). A loss of cell-cell contacts was also observed as documented by a decreased staining for common cell-cell adhesion molecules such as E-Cadherin and ZO1 (Fig. 5B). However, the actin cytoskeleton network was apparently not modified, 293T being poor in stress fibers with no enrichment upon hSNF5/INI1 knockdown.

The motility was strongly induced by hSNF5/INI1 knockdown. Whereas virtually no control 293T cell could migrate in transwell assays, hSNF5/INI1–knocked down cells exhibited a very potent ability to migrate (Fig. 5D). To determine whether the molecular regulators characterized in MRTs were also involved in 293T cells, we measured the activities of RhoA, Rac1, and Cdc42. No activity of RhoA, Rac1, and Cdc42 was detected in control 293T cells. In KD cells, GTP-bound RhoA could be detected in control 293T cells. In contrast, hSNF5/INI1–knocked down cells exhibited RhoA activation (Fig. 5A). Rac1 and Cdc42 activities were not affected upon hSNF5/INI1 knockdown (Supplementary Data S1). I2a cells were induced for hSNF5/INI1 through tetracycline removal (+ INI1) for 7 d. KD cells were infected with empty pWZL (~) or pWZL-INI1 (+ INI1) and selected with blasticidin for 7 d. RNA and proteins were extracted and subjected to quantitative reverse transcription-PCR (RT-PCR; top) or Western blot (bottom) analyses for ARHGAP5 expression. For quantitative RT-PCR experiments, the level of expression is compared with that of I2a cells.

Figure 4. hSNF5/INI1-mediated RhoA inhibition requires a nuclear, SWI/SNF-dependent, transcriptional activity. A and B, MON cells were transfected with empty (~), wild-type (INI1), or missense (L266A) hSNF5/INI1-expressing pCDNA3 vector (left), either with empty (~) or hSNF5/INI1-expressing pCDNA3 vector (+ INI1) together with empty (~) or BRG1K798R-expressing pCDNA3 vector (right). Cells were either lysed, subjected to GST-RBD pulldown assay and to Western blot analysis with anti-HA and anti-RhoA antibodies (A), or subjected to transwell assays (B). Columns, mean obtained from duplicate experiments; bars, SD. C, I2a cells were induced for hSNF5/INI1 through tetracycline removal (+ INI1) for 7 d. KD cells were infected with empty pWZL (~) or pWZL-INI1 (+ INI1) and selected with blasticidin for 7 d. RNA and proteins were extracted and subjected to quantitative reverse transcription-PCR (RT-PCR; top) or Western blot (bottom) analyses for ARHGAP5 expression. For quantitative RT-PCR experiments, the level of expression is compared with that of I2a cells.
Discussion

In the present study, we show in different cell systems, i.e., MRT cell lines, epithelial 293T and MCF7 cells, that hSNF5/INI1 regulates cell migration in a RhoA-dependent manner. We indeed show an inhibition of cell migration in hSNF5/INI1-expressing MRT cells and, reciprocally, an increased cell migration in hSNF5/INI1–knocked down 293T and MCF7 cells. The regulation of RhoA is achieved at the posttranslational level because the total amount of the protein is not modified by hSNF5/INI1. It is also specific to RhoA because the GTP-bound levels of Rac1 and Cdc42 do not vary. Although hSNF5/INI1 has been previously shown to transiently shuttle in the cytoplasm (27), our data show that the hSNF5/INI1-mediated inhibition of RhoA activity is not achieved through a direct cytoplasmic effect but rather through a nuclear, SWI/SNF-dependent transcriptional mechanism. This suggests that hSNF5/INI1 acts through the transcriptional activation or repression of determinants that regulate RhoA activity.

Main regulators of this activity are GEF with activating functions, or GAP and Guanine nucleotide Dissociation Inhibitors (GDI), which are inhibitors of RhoA. Although there are only 3 GDIs, as many as 80 GEFs and GAPs have been identified, a number of which are specific for RhoA (32, 33). Among the most relevant regulators of RhoA, the expression of p115-RhoGEF or p190 RhoGAP was not modified (data not shown), whereas the expression of ARHGAP5 (p190-B RhoGAP) was consistently up-regulated upon hSNF5/INI1 induction. Because this GAP may account for as much as 50% of GAP activity toward RhoA in fibroblasts (34), it constitutes a strong candidate to link hSNF5/INI1 induction with decreased RhoA activity by facilitating GTP hydrolysis and consequent return to the inactive GDP-bound form. However, our preliminary results indicate that the ectopic expression of ARHGAP5 in MON cells is not sufficient by itself to recapitulate the effects of RhoA inhibition on cell migration, and up to now, the level of inhibition of ARHGAP-5 that was obtained with shRNA expressing lentiviruses was not sufficient to conclude whether the induction of this gene was absolutely required for hSNF5/INI1-induced migration arrest in MON cells. Previously published transcriptional analyses allowed the identification of numerous genes, including RhoE, ARP2, WAVE 3, N-WASP, or cortactin (19), which participate in cytoskeleton organization and cell-matrix or cell-cell interactions, all processes that influence cell migration. This suggests that hSNF5/INI1-mediated inhibition of RhoA may not be achieved through a single target but rather through the combined effects of hSNF5/INI1 on different pathways. A more systematic screen of the expression of GEFs, GAPs, and GDIs in MRTs, and as a consequence of hSNF5/INI1 expression, together with transcriptome analyses of hSNF5/INI1–knocked down 293T cells are under progress to identify other relevant regulators.

Another important result is that this hSNF5/INI1-mediated regulation of RhoA activity is dependent on the SWI/SNF complex. In that respect, it is worth mentioning that the SWI/SNF ATPase subunit has already been involved in the regulation of cell morphology and cytoskeleton organization (35, 36). Indeed, NIH-3T3 cells expressing dominant-negative ATPase-deficient BRG1 exhibit a larger size and attachment area (35), an observation in agreement with the increased size of hSNF5/INI1-deficient MRT, 293T, or MCF7 cells. Similarly, pancreatic adenocarcinoma cells, in which the expression of BRG1 was inhibited, exhibit a change in...
morphology and alterations of the actin cytoskeleton organization (36). Although they were not studied in the aforementioned reports, it will be of strong interest to document whether RhoA activation and altered cell motility are common features of the various systems with loss-of-function alterations of subunits of the SWI/SNF complex. Concerning MRTs, the observation that three different cell lines exhibit the same phenotype as regards to RhoA and cell migration further suggests that the activation of this Rho-family protein is a common characteristic of MRTs and that it plays a critical role in their development. Given the observed motility phenotype, it may be hypothesized that the aberrant activation of RhoA, as a result of hSNF5/INI1 loss-of-function, accounts for the extremely high invasive and metastatic potential of MRTs. Aberrant Rho signaling has been recurrently associated with metastasis in vivo (37–39). This also raises the question of activated RhoA influencing other aspects of MRT development, including survival or proliferation, as suggested by the various oncogenic effects of RhoA documented in mouse models of tumorigenesis (40).

The cell of origin of MRT is a matter of debate. In particular, it is not clear whether these tumors, whatever their localization in the body, arise from a single progenitor or if the loss-of-function of hSNF5/INI1 may transform a variety of cells with different potentials. We have recently shown that hSNF5/INI1 can induce specific adipogenic differentiation processes in MRT cells, pointing out a putative mesenchymal origin for MRTs or at least for a subset of MRTs (16). In that respect, it is worth mentioning that RhoA is a critical regulator of mesenchymal stem cell commitment and differentiation toward the myogenic, osteogenic, and adipogenic lineages (41). More precisely, Rho activity is decreased during adipogenesis, whereas it is increased during osteogenesis and myogenesis, ARHGAP5 being an activator of adipogenesis (34). In addition to its coactivator function on PPARγ2 and CAAT/enhancer binding protein β (16), two key transcription factors of adipogenic differentiation, hSNF5/INI1, may hence also participate in this process through the regulation of RhoA. In addition, the morphologic changes observed in hSNF5/INI1–knocked down 293T and MCF7 cells are highly reminiscent of an epithelial to mesenchymal transition (EMT), a phenomenon frequently associated with a metastatic progression of epithelial tumors (42). It is also noteworthy that SNF5/INI1 knockout in the developing mouse liver impairs epithelial organization by deregulating cell-cell and cell–extracellular matrix adhesions, including ZO-1 (tight junctions) and E-Cadherin (adherens junctions; ref. 14).

To analyze a putative EMT in 293T cells, we investigated the levels of E- and N-cadherin, the down- and up-regulation of which are characteristic features of an EMT, respectively. However, neither E- nor N-cadherins, nor Twist, Snail, or Slug transcription factor mRNA levels were modified upon hSNF5/INI1 induction (data not shown). Nevertheless, we show that E-cadherin protein localization at cell–cell contacts is severely affected. A role for hSNF5/INI1 in the regulation of the mesenchymal-amoeboid transition, another transition in the type of migration, remains to be investigated (43).

Finally, our data clearly document that, in addition to the control of the G1-S transition through the retinoblastoma protein and to its roles in the mitotic checkpoint and in differentiation, hSNF5/INI1 also controls cell motility by regulating the GTP-bound amount of RhoA. Interestingly, the analysis of different mutants of the hSNF5/INI1 protein indicates that these various functions can be dissociated. In particular, the S284L cancer-associated mutant is deficient for the control of the mitotic checkpoint (12) and for the regulation of cytoskeleton organization and subsequent control of motility but show normal functions in other assays. Because a single amino acid substitution is sufficient to impair the function of hSNF5/INI1 in cell motility, it will be of strong interest to determine whether this serine residue is posttranslationally modified, in particular, through phosphorylation, or/and is required for specific protein-protein interactions. Our study also questions the oncogenic role of the P48S mutation in tumor development. Indeed, this mutant is neither deficient for the regulation of S phase entry (2), nor, as shown here, for cytoskeleton remodeling or migration regulation. The only presently known abnormal function of this mutant is its relative inability to revert the chromosomal instability induced by hSNF5/INI1 deficiency (12). This may suggest that abnormal migration and abnormal S phase regulation are not required for the cell transformation process induced by hSNF5/INI1 loss-of-function. Alternatively, and more probably, the P48S mutation is not the only oncogenic event in the MRT that presented this particular change. The observation that a subset of bona fide MRT do not harbor any hSNF5/INI1 mutation (44) indicates that gene alterations, different from hSNF5/INI1 inactivation, remain to be found in MRT.

Integrative functions that include the control of cell motility have been recently reported for other genes playing a critical role in oncogenesis, including the cyclin-dependent kinase inhibitor CDKN1B (25) or the tumor suppressor proteins TP53 (45), PTEN (46) or LKB1 (47). Our data indicate that the hSNF5/INI1 tumor suppressor function is not limited to its antiproliferative activity but may also rely on its combined effects in cell cycle, differentiation, and migration.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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