The Tumor Suppressor hSNF5/INI1 Modulates Cell Growth and Actin Cytoskeleton Organization

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ABSTRACT

hSNF5/INI1, which encodes a component of the ATP-dependent chromatin remodeling hSWI-SNF complex, is a tumor suppressor gene mutated in malignant rhabdoid tumors. We have developed a tetracycline-based hSNF5/INI1-inducible system in a hSNF5/INI1-deficient malignant rhabdoid tumor cell line and studied time course variation of 22,000 responsive genes/expressed sequence tags upon hSNF5/INI1 induction. A total of 482 genes were identified and further clustered into 9 groups of coregulated genes. Among genes with early and strong inductions, the use of a fusion protein with the hormone-binding domain of the estrogen receptor enabled the identification of a subset of direct targets regulated independently of de novo protein synthesis. We show that the G1 arrest induced by hSNF5/INI1 is reversible and associated with the down-regulation of components of the DNA replication complex. We also identify an unsuspected role of hSNF5/INI1 in cytoskeleton organization. Indeed, induction of hSNF5/INI1 induces dramatic modifications of the cell shape including complete disruption of the actin stress fiber network and disappearance of focal adhesions associated with up-regulation of genes involved in the organization of the actin cytoskeleton. We document a strong decrease of Rho activity upon hSNF5/INI1 expression, suggesting that the regulation of this activity constitutes a crucial step of the hSNF5/INI1-induced reorganization of the actin network. This study identifies hSNF5/INI1 target genes and provides evidence that hSNF5/INI1 may modulate the cell cycle control and cytoskeleton organization through the regulation of the retinoblastoma protein-E2F and Rho pathways.

INTRODUCTION

The hSNF5/INI1 gene is mutated on both alleles in highly aggressive childhood cancers termed malignant rhabdoid tumors, and constitutional mutations are responsible for the highly penetrant rhabdoid predisposition syndrome (1–3). Animal model studies have clearly confirmed this tumor suppressor role: whereas Snf5/Ini1-deficient mice die very early, at the peri-implantation stage; Snf5/Ini1 heterozygous mice develop, in around one-third of cases, tumors resembling malignant rhabdoid tumor that exhibit loss of heterozygosity of the wild-type allele (4–6). Moreover, the conditional somatic inactivation of Snf5/Ini1 results in the rapid development of aggressive lymphoma with a very high penetrance, demonstrating the extremely powerful oncogenic role of SNF5/INI1 loss of function (7).

hSNF5/INI1 encodes a subunit of the SWI-SNF chromatin remodeling complex, which is one of several evolutionarily conserved multifunctional complexes that disrupt nucleosomes in vitro in an ATP-dependent manner.

In addition to the cancer-related mutations of hSNF5/INI1, mounting evidence suggests a role of SWI-SNF complexes in oncogenesis. Indeed, BRG1 and hBRM interact and collaborate with the retinoblastoma (RB) tumor suppressor and histone deacetylase (HDAC) to repress E2F1 activity (8). This functional link between E2F1 and SWI-SNF is further supported by genetic screens in Drosophila that identified members of SWI-SNF complexes as negative regulators of E2F activity (9). Inactivating mutations in BRG1 have also been identified in several human tumor cell lines or tumors, and the heterozygous inactivation of BRG1 is associated with a tumor-prone phenotype in mice (10–12).

Recently, we and others have shown that hSNF5/INI1 is implicated in cell growth control by inhibiting S-phase entry and by promoting a G1 arrest in hSNF5/INI1-deficient cell lines (13–17). This arrest can be reversed by coexpression of adrenoviral E1a but not by RB binding-deficient E1a mutants, strongly suggesting that the presence of a functional RB is required. However, the ability of RB to arrest hSNF5/INI1-deficient rhabdoid cells indicates that hSNF5/INI1, in contrast to BRG1, is not mandatory to RB function. Regarding the G1 arrest induced by hSNF5/INI1, other groups have provided results suggesting that hSNF5/INI1 may participate in the repression of the cyclin D1 promoter or in the induction of INK4a gene expression (16, 17).

In this study, we have taken advantage of the construction of two different conditional systems for hSNF5/INI1 activity in hSNF5/INI1-deficient rhabdoid cells, one based on the tetracycline-inducible system and another based on the fusion between hSNF5/INI1 and the estrogen receptor (ER) hormone binding domain, to perform time course experiments of the gene expression profile and analysis of the cell phenotype after restoration of the hSNF5/INI1 protein.

MATERIALS AND METHODS

Cell Culture. Parental malignant rhabdoid MON cells were maintained in RPMI 1640 supplemented with 10% FCS and 100 units/ml penicillin-streptomycin (Invitrogen, Groningen, the Netherlands). I2A, I2B, and ER4A clones were grown in the same medium supplemented with 300 μg/ml G418 (Geneticin), 50 μg/ml hygromycin B, and 1 μg/ml tetracycline (Invitrogen). Activation of the HA-INI1-ER fusion protein was induced by the addition of 0.5 μM 4-hydroxystrophanoxifen (Sigma-Aldrich, St. Louis, MO). Where specified, cells were exposed to cycloheximide (Sigma) at 10 μg/ml for 4–12 h. Green fluorescent protein staining was observed using Fluorescent microscopy with Zeiss Axioplan 2 Microscope. Cells fixed in 70% ethanol and stained with anti-bromodeoxyuridine antibody (Harlan Sera-Lab, Hilleshog, United Kingdom) and propidium iodide (Sigma-Aldrich) were analyzed on a Becton Dickinson FACScan as described previously (18, 19). Detection of apoptotic cells by flow cytometry was carried out with annexin V-allylphococyanin according to the manufacturer’s instructions (BD Biosciences PharMingen, Torreyana, CA).

Plasmid Construction. To construct the pBI-HA-INI1 vector, the HA-INI1 coding sequence (20) was PCR amplified using forward primer 5′-GCTAGCTTACTGAAGATCTCTCAAGCTCCACCATGGGG-3′ and reverse primer 5′-CATGACATCGGATGCTAGCTAGCGATGGGC-3′ and cloned into pBluescript II (BglII and NheI) restriction sites, respectively. The PCR product was digested by both restriction enzymes and cloned into BglII/NheI linearized tet-responsive reporter plasmid pBIEGFP vector (21). The hormone binding domain of ER (22) was cloned in the XhoI site 3′ to HA-INI1, creating the pCDNA-HA-INI1-ER vector.
Subsequently, the HA-INI1-ER cDNA was cloned in place of luciferase in the XbaI/NheI sites of the pRLTK vector (Promega, Madison, WI). The BamHI/SpeI fragment containing the HA-INI1-ER and globin intron sequences was then cloned by PCR in the BglII/NheI linearized pB1-EGFP vector. Constructs were verified by sequencing.

**Generation of Inducible Rhabdoid Cell Lines.** MON-tTa-expressing clones were obtained by transfection using the Effectene method (Qiagen GmbH) of the human rhabdoid MON cell lines with the tTA-IRES-NEO vector (21). The MON-tTA25 clone, which yielded an optimal induction of luciferase on transient transfection with the pUHC 13.3 reporter plasmid, was then transfected with the PBI-EGFP/HA-INI1 and PBI-EGFP/HA-INI1-ER constructs. Inducible clones were sought by reverse transcription-PCR and Western blot.

**Immunoblotting, Immunoprecipitation, and Immunofluorescence.** The 738 polyclonal antibody was obtained by immunizing rabbits with the RNTGDADQWCPLLET peptide. Antibodies against β-actin (ac-74), BRG1 (2SN-2E12-AS), BAF155/170 (sc 9746/sc 9744) were obtained from Sigma, Euromedex (Montreal, Canada), and Santa Cruz Biotechnology (Santa Cruz, CA), respectively. Western blotting was conducted as described previously (15). For immunoprecipitations, precleared nuclear cell extracts were incubated with protein G-coupled with anti-hemagglutinin [HA (12CA5; Roche Molecular Biochemicals, Indianapolis, IN)] or anti-FLAG antibodies (F3165; Sigma). Protein-antibody complexes were subsequently washed in buffer containing 150 mM NaCl and 0.2% NP40 and analyzed by immunoblotting. Immunofluorescence experiments were conducted with tetramethylrhodamine isothiocyanate isothiocyanate-conjugated phalloidin (Sigma) or a paxillin-specific monoclonal antibody (clone 349; BD Biosciences) on 3% formaldehyde-fixed and 0.2% Triton X-100-permeabilized cells.

**Affinity Precipitation of Cellular GTP-Rho or Measuring Rho-GTP Levels.** Glutathione S-transferase-Rhotekin pull-down assay was performed using the EZ-Detected Rho Activation Kit (Pierce Biotechnology), according to the manufacturer’s instructions. Briefly, lysates from induced or uninduced I2A cells were incubated with glutathione S-transferase-Rhotekin and glutathione-conjugated beads to capture GDP/GTP-bound proteins. Bound proteins were washed several times and eluted with sample buffer before analysis by immunoblot using an anti-Rho antibody.

**Microarray Analysis.** Total RNAs were extracted using the TRIzol reagent (Gibco-BRL) and quality controlled by migration on Agilent Bioanalyzer 2100 (Waldbronn, Germany). The RNA samples were labeled and hybridized on Affymetrix Genechip human U133A (22k) as recommended by the manufacturer. The arrays were scanned using a Hewlett Packard confocal laser scanner, and data were processed with the MICROARRAY SUITE (Mas 5.0; Affymetrix) and dChip softwares (23). We used Rosetta’s algorithm to calculate the baseline signal resulting from the calculation of mean of the three uninduced samples, taking into account the SE of the measurements. The fold change was calculated for each of the seven time points as the ratio of signals from induced or uninduced samples. To identify differentially expressed genes, probe sets were filtered to include only genes responding to the following criteria: (a) at least one value
scored “present” and a signal difference higher than 60 as compared with
the control; and (b) for early time points (28–96 h), the analysis included
genes with a fold change higher than 2 for at least two time points. For the
late time point (10 days), a 3-fold variation was required, given the high
number of genes demonstrating variation at this time. A total of 482 unique
genes met all conditions. We used the logarithm of fold change to cluster
genes with a similar pattern of expression by applying the k-means clus-
tering algorithm using Euclidian distances (Supplementary Data 2).

Quantitative Reverse Transcription-PCR. Total RNAs treated with
DNase I (Invitrogen) were reverse-transcribed with oligo-random hexamers
using the GeneAmpRNA PCR kit (Applied Biosystems) and amplified with
specific primers according to the manufacturer’s instructions. Glyceraldehyde-
3-phosphate dehydrogenase was used for calibration (primer sequences are
indicated in Supplementary Data 1).

RESULTS

Establishment of Inducible hSNF5/INI1 Expression Rhabdoid
Cell Line. To establish an inducible hSNF5/INI1 expression system
in rhabdoid cells, we initially used standard tTA or rtTA vectors. As
reported previously for p53, inducible clones could not be obtained,
probably due to the leakiness of these systems (21). To overcome
these drawbacks, we used the tet-off system modified by Yu et al.
(21), in which the expression of tTA is selected for by G418 through
a Neo-IRES-tTA bicistronic transcription unit. hSNF5/INI1-deficient
cells stably expressing tTA were generated and then transfected with
a vector driving expression of both enhanced green fluorescent protein
(EGFP) and a HA-tagged version of hSNF5/INI1 (HA-INI1) under the
control of a bidirectional rTA-responsive promoter. Clones I2A and
I2B were obtained. They both demonstrated a strong induction of
EGFP and HA-INI1 on withdrawal of tetracycline and an undetectable
level of expression in the presence of this drug (see results for clone
I2A in Fig. 1, A–C). HA-INI1 was detectable 12 h after tetracycline
depletion, and its expression reached a maximum at 30 h. The ex-
pression level was then stable over time and similar to that of the
endogenous protein in non-rhabdoid cell lines (Fig. 1 C). An efficient
integration of HA-INI1 in the SWI-SNF complex was documented by
immunoprecipitation experiments, which showed that in extracts from

Fig. 3. k-means cluster analysis of genes differentially expressed during HA-INI1 time
course induction. A, a total of 482 differentially expressed genes were grouped in nine
clusters (A–I) by the k-means cluster algorithm, according to their profile of expression
across seven time points. Individual genes are indicated vertically, whereas time points are
represented horizontally. Fold change relative to uninduced I2A cells is shown colori-
metrically as indicated at the bottom. B, the relative mean expression level (logarithm
scale) is shown for each cluster in a graphical format. The two horizontal black lines
correspond to an induction or repression by 2-fold.

Fig. 4. Construction of HA-INI1-ER tet-off inducible system and identification of
putative direct targets. A, a schematic representation of HA-INI1-ER tet-off construct
(TRE, tTa-responsive element). Four h after tetracycline depletion, INI1-ER transcript and
protein can be detected by semiquantitative reverse transcription-PCR and immunoblot,
respectively. B, functional characterization of the HA-INI1-ER inducible system. Cells
were grown for 4 days in the presence or absence of tetracycline and 4-hydroxytamoxifen
(4-OHT) and analyzed by fluorescence-activated cell sorting after propidium iodide
staining. Histograms indicate the amount of cells in the different phases of the cell cycle.
C, quantitative reverse transcription-PCR analysis of five early HA-INI1-induced targets
was performed in ER4A cells. Eight to 12 h after tetracycline removal, 4-OHT and
cycloheximide (CHX) were added. Fold change was calculated relative to the uninduced
cells using glyceraldehyde-3-phosphate dehydrogenase for normalization. The absence of
effect of tetracycline or 4-OHT on the expression levels of these five genes was controlled
in parental MON cells. Inductions observed after 4-OHT treatment in the presence or
absence of CHX are shown. For ATP1B1 and Frizzled 7, CHX alone has no effect, and
a full induction by INI1-ER is maintained in the presence of CHX. For pleiotrophin and
HESR1, despite an increase of mRNA levels associated with CHX treatment, induction
linked to 4-OHT activation of INI1-ER is clearly observed. Finally, no induction specific
for INI1-ER is observed for HB-EGF in the presence of CHX.
induced cells, subunits of the complex, including BRG1, BAF155 and BAF170, were brought down by an anti-HA antibody, but not by a control anti-FLAG antibody (Fig. 1D). Therefore, this HA-INI1 inducible tet-off system allows a physiological level of expression of the protein, which is properly integrated in the SWI-SNF complex.

hSNF5/INI1 Induces a Reversible Cell Cycle Arrest in Rhabdoid Cells. As expected, a strong decrease of cell density was observed after HA-IN1 induction as compared with uninduced cells (data not shown). A more precise monitoring of the cell cycle showed a progressive decrease of S phase and an increase of G1, resulting in an almost complete G1 block at 4 days (Fig. 2a). Interestingly, the repression of HA-IN11 expression, through reintroduction of tetracycline in the medium, resulted in the reversion of the cell cycle arrest (Fig. 2b). These data showed that most of the HA-IN11-expressing cells arrested in G1 phase still remained competent to reenter the cell cycle. Accordingly, HA-IN11 expression did not lead to significant apoptosis as monitored by annexinV staining (Fig. 2c) and sub-G1 analysis or to senescence as evaluated by β-galactosidase staining (data not shown).

Time Course Expression Profiling on Induction of hSNF5/IN11. We performed a time course analysis of the expression profile of the I2A rhabdoid cell line after induction of HA-IN11. RNAs were isolated at various times and hybridized on 22K, U133A Affymetrix arrays. Stringent criteria enabled the identification of 534 probe sets corresponding to 482 unique genes, which could be further classified into 9 groups by the k-means clustering algorithm based on similarity of expression patterns of genes across the time course (Fig. 3; Supplementary Data 2). The number of genes demonstrating induction was higher (332; 69%) than that of repressed genes (150; 31%). Induced genes could be divided into seven groups (clusters A–G). Early-induced genes were grouped in clusters A (2 genes), B (11 genes), C (71 genes), and D (91 genes) according to their magnitudes of induction. As expected, HA-IN11 was induced very strongly and rapidly and belonged to group A. Genes with intermediate and late induction were classified in clusters F (63 genes) and G (70 genes), respectively. Genes demonstrating a transient induction were clustered in group E (24 genes). Finally, groups H (100 genes) and I (50 genes) included repressed genes at early and late times, respectively.

A total of 12 genes showing a variable degree of induction ranging from 2 to 200 were further evaluated by quantitative PCR. Microarray and quantitative PCR analyses demonstrated highly consistent results, even for low fold changes. Indeed, the Pearson correlation coefficient evaluating the degree of linear dependence between the two approaches was higher than 0.75 for all compared genes (Supplementary Data 3), demonstrating the accuracy of these microarray data. To exclude an effect of tetracycline, the expression levels of these genes were investigated after treatment of parental MON cells with this drug. No significant change was observed, indicating that the modifications observed in I2A cells were linked to hSNF5/IN11 induction and not to tetracycline treatment (data not shown).

Identification of Direct Targets. To identify direct transcriptional targets, we developed a conditional hSNF5/IN11-ER fusion protein comprising hSNF5/IN11 and the ER ligand-binding domain (22). This fusion protein demonstrated a slight residual activity in the absence of 4-hydroxytamoxifen, which precluded the selection of clones with stable expression. To take into account this parameter, we constructed clone ER4A with a tetracycline-based inducible expression of HA-IN11-ER (Fig. 4A). On tetracycline withdrawal, a slight cell cycle effect was observed, consistent with the weak basal activity of the HA-IN11-ER protein. The addition of 4-hydroxytamoxifen resulted in a complete cell cycle arrest, indicating that 4-hydroxytamoxifen restored full activity of the fusion protein (Fig. 4B).

Direct transcriptional targets are expected to demonstrate both an early regulation and an independence toward de novo protein synthesis. Genes from clusters A and B, with early and high inductions after HA-IN11 expression, were therefore further tested with the HA-IN11-ER system. Interestingly, the induction of NaK ATP1B1, pleiotrophin, HESR1, and FRIZZLED 7 was maintained in the presence of cycloheximide, strongly suggesting that these transcripts are
directly controlled by hSNF5/INI1. In contrast, HB-EGF was not induced in the presence of cycloheximide.

**Functional Groups.** Differentially expressed genes were classified in functional clusters according to their known or inferred functions using gene ontology. The classification of genes shown in Table 1 and 2 was based on this analysis, which identified highly significant groups of genes (P < 0.001). The first one corresponds to genes encoding cell cycle regulators including components of the prereplication complex (MCMI, MCM5, MCM10, MCM4, RF-C, CDT1, CDC6, and CDC7) and a number of E2F targets (Table 1; Ref. 24). Within this group, which mainly contains late down-regulated genes, it is worth mentioning the DNA replication licensing factor CDT1, which is essential for the initiation of DNA replication and demonstrates a very early down-regulation. The second main functional group contains numerous genes that participate in cytoskeleton organization and cell-matrix or cell-cell interactions (Table 2; Refs. 25–27).

In particular, this last group contains regulators of the actin cytoskeleton including Arp2, WAVE 3, N-WASP, cortactin, RhoGAP4, and RhoE, which regulate the Arp2/3 complex and the activity of RhoA protein. Most genes from this group demonstrate intermediate or late modulations, suggesting that the effect of hSNF5/INI1 is indirect. The third group comprises genes encoding proteins involved in signal transduction including cytokines, growth factors, receptors, and others.
and intracellular transducers (Table 2). Interestingly, most genes from this group are regulated early after HA-IN1 induction and are therefore susceptible to mediate the late effects of HA-IN1 expression. Finally, in light of the essential role of hSNF5/INI1 orthology during early steps of development, it is worth mentioning the early variation of a set of genes involved in developmental processes (Table 2; Refs. 4–6, 28, and 29).

**hSNF5/INI1 Regulates Actin Cytoskeleton Organization.** The induction of hSNF5/INI1 expression was associated with dramatic modification of cell shape, including rounding of the cell body and a strong decrease of the cell size (Fig. 5A). The well-organized actin stress fiber network of uninduced cells, detected by phalloidin staining, was completely modified on HA-IN1 induction with loss of stress fibers and concomitant appearance of a strong cortical actin staining (Fig. 5B). The modifications of cell shape and actin organization were also associated with a strong tendency of cells to detach from the dish as documented by time-lapse videomicroscopy (data not shown), suggesting a decreased adhesion of cells to the extracellular matrix. In support of this hypothesis, a complete disappearance of paxillin-stained focal adhesions was observed (Fig. 5C). This reorganization occurred with constant amounts of β-actin and paxillin, as indicated by immunoblots showing similar levels of these proteins in induced and uninduced cells (data not shown).

**hSNF5/INI1 Expression Is Associated with Decreased Rho Activity.** The expression of HA-IN1 results in the induction of numerous genes involved in cytoskeleton organization and, in particular, regulators of Rho GTPases (Table 2), together with dramatic modifications of the actin cytoskeleton. The critical role of the activity of members of the Rho family of small GTPases in the regulation of actin stress fibers and focal adhesion formations (25) prompted us to analyze Rho activity in HA-IN1 induced and uninduced cells. We used the glutathione S-transferase-Rhotekin Rho binding domain to specifically pull down the active, GTP-bound Rho proteins. Fig. 6 shows that whereas the overall level of Rho protein is similar in induced and uninduced cells, the GTP-bound fraction is strongly decreased after HA-IN1 expression.

**DISCUSSION**

To more precisely define the function of hSNF5/INI1, we have constructed two conditional systems to control the activity of the hSNF5/INI1 protein. In both systems, the induction of hSNF5/INI1 activity leads to a complete G1 arrest. This block is obtained with levels of hSNF5/INI1 similar to that of the endogenous protein in non-rhabdoid cell lines. Interestingly, this arrest is not associated with subsequent irreversible events because arrested cells can reenter the cell cycle on switching-off the expression of hSNF5/INI1.

Time course gene expression profiling indicates that this arrest is associated with the down-regulation of a high number of genes involved in cell cycle progression. This includes different MCM proteins (CDC6 and CDT1, which are essential members of the DNA replication complex). Numerous E2F target genes are retrieved among down-regulated genes (24). This supports the hypothesis of hSNF5/INI1, together with BRG1, being involved in RB-mediated corepression of E2F (8). Whereas most of these genes demonstrate a relatively late down-regulation, concomitant with the cell cycle arrest, the decreased expression of the DNA replication licensing factor CDT1 occurs much earlier, which may suggest a causal role in the inhibition of DNA replication.

We identify four genes, which constitute excellent candidates for being direct transcriptional targets of hSNF5/INI1. These genes exhibit strong and early increases after hSNF5/INI1 induction without requirement for de novo protein synthesis. ATP1B1 encodes the β1 subunit of the Na/K-ATPase, an ATP-dependent sodium and potassium transporter across the plasma membrane, and pleiotrophin is a cytokine with diverse functions in growth, apoptosis, angiogenesis, and cell differentiation (30). HESR1 is a bHLH transcription factor related to the hairy/enhancer of split (HES) family of downstream targets of the Notch signaling pathway involved in cell fate commitment in a broad range of developmental processes (31). Interestingly, recent results have shown that BRM and hSNF5/INI1 are recruited to the HES1 and HES5 promoters (32). Taken together, these data strongly suggest that hSNF5/INI1 is involved in transcriptional regulation of targets of the Notch pathway. Finally, FRIZZLED 7 is a receptor of the Wnt signaling pathway. Previous experiments have
indicated that BRG1 cooperates with β-catenin to activate TCF target genes (33). Our work suggests that SWI-SNF could also contribute to the activation of Wnt signaling through increased expression of the FRIZZLED 7 receptor. Hence, through the direct induction of ATF1P1B1, pleiotrophin, HESR1, and FRIZZLED 7, hSNF5/INI1 may directly regulate diverse cellular processes including ion homeostasis, cytokine signaling, and the Notch and Wnt pathway. One may speculate that this direct involvement in various pathways may account for the extremely detrimental effect of hSNF5/INI1 loss of function during development and adult life.

The reexpression of hSNF5/INI1 protein in hSNF5/INI1-deficient cells induces dramatic modifications of the cell shape associated with a complete reorganization of the actin cytoskeleton. These cellular phenotypic changes occur concomitantly with the up-regulation of genes involved in the polymerization and organization of the actin cytoskeleton. These genes include Arp2, N-WASP, WAVE 3, cortactin, Rho-GAP4, and RhoE. Arp2 is a core component of the Arp2/3 complex, which is involved in the nucleation of actin filaments and regulated by N-WASP, WAVE 3, and cortactin (34). Previous reports have shown that WASP and WAVE family proteins play a critical role in the organization of cortical actin filaments (35). When bound to GTP, Rho GTPase proteins increase the stability of actin-based structures such as stress fibers and focal adhesions. They therefore constitute major regulators of the actin cytoskeleton. The present microarray experiments suggest that hSNF5/INI1 expression could result in the inhibition of this function through induction of Rho-GAP4, which inhibits Rho functions by increasing its GDP-bound form, and RhoE, which antagonizes some effects of the RhoA protein and inhibits the assembly of actin stress fibers and the formation of focal adhesions in fibroblasts and Madin-Darby canine kidney cells (36). The analysis of the activity of Rho protein in I2A rhabdoid cells reveals that the induction of hSNF5/INI1 leads to a dramatic decrease of the amount of active GTP-Rho proteins. These data indicate that the modulation of Rho activity may constitute an essential step of the cytoskeleton modifications induced by hSNF5/INI1. Interestingly, recent results also implicate BRG1 in the modulation of the actin cytoskeleton. In SW13 BRG1-deficient cells, restoring BRG1 activity results in enhanced formation of actin stress fibers (37). The opposite effects of BRG1 and hSNF5/INI1, two members of the SWI-SNF complex, on the organization of the actin stress fiber network strongly suggest that the effect of SWI-SNF on cytoskeleton organization may be highly dependent on the cellular context. Strikingly, highly tumorigenic MON cells demonstrate a well-organized network of stress fibers and focal adhesions that are altered on reversion of the transformed phenotype associated with hSNF5/INI1 reexpression. This pattern is somehow paradoxical, given the disruption of actin filaments and decrease of focal adhesions commonly observed after transformation by various oncogenes (38). However, because much of our knowledge of cytoskeleton modifications during cell transformation is derived from experiments performed with fibroblasts, it is likely that this apparent discrepancy reflects cell type specificity (39–41). Importantly, we observe that Rho activity is decreased when hSNF5/INI1 is induced, an observation consistent with the previously documented role of Rho in various transformation processes (39).

Alternatively, this study correlates temporal changes in the gene expression profile with phenotypic modifications, including cell cycle arrest and remodeling of actin cytoskeleton. These phenomena are particularly tightly regulated during development and differentiation and altered in oncogenic processes. This work also allows the identification of putative direct targets, which should prove critical to decipher the transcriptional mechanisms of action of hSNF5/INI1.

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