Large-Scale Molecular Comparison of Human Schwann Cells to Malignant Peripheral Nerve Sheath Tumor Cell Lines and Tissues

Shyra J. Miller, Fatima Rangwala, Jon Williams, Peter Ackerman, Sue Kong, Anil G. Jegga, Sergio Kaiser, Bruce J. Aronow, Silke Frahm, Lan Kluwe, Victor Mautner, Meena Upadhyaya, David Muir, Margaret Wallace, Jussara Hagen, Dawn E. Quelle, Mark A. Watson, Arie Perry, David H. Gutmann, and Nancy Ratner

Abstract

Malignant peripheral nerve sheath tumors (MPNST) are highly invasive soft tissue sarcomas that arise within the peripheral nerve and frequently metastasize. To identify molecular events contributing to malignant transformation in peripheral nerve, we compared eight cell lines derived from MPNSTs and seven normal human Schwann cell samples. We found that MPNST lines are heterogeneous in their in vitro growth rates and exhibit diverse alterations in expression of pRb, p53, p14ARF, and p16INK4A proteins. All MPNST cell lines express the epidermal growth factor receptor and lack S100B protein. Global gene expression profiling using Affymetrix oligonucleotide microarrays identified a 159-gene molecular signature distinguishing MPNST cell lines from normal Schwann cells, which was validated in Affymetrix microarray data generated from 45 primary MPNSTs. Expression of Schwann cell differentiation markers (SOX10, CNP, PMP22, and NGFR) was down-regulated in MPNSTs whereas neural crest stem cell markers, SOX9 and TWIST1, were overexpressed in MPNSTs. Previous studies have implicated TWIST1 in apoptosis inhibition, resistance to chemotherapy, and metastasis. Reducing TWIST1 expression in MPNST cells using small interfering RNA did not affect apoptosis or chemoresistance but inhibited cell chemotaxis. Our results highlight the use of gene expression profiling in identifying genes and molecular pathways that are potential biomarkers and/or therapeutic targets for treatment of MPNST and support the use of the MPNST cell lines as a primary analytic tool. (Cancer Res 2006; 66(5): 2584-91)

Introduction

Malignant peripheral nerve sheath tumors (MPNST) are highly aggressive soft tissue sarcomas with poor prognosis. Excision of the tumor does not always prevent local recurrence, and metastases to the lung, liver, and brain are common. Current therapeutic regimens have limited use because the tumors are generally resistant to standard chemotherapy and radiation (1). Half of all MPNSTs are sporadic in nature; half arise in individuals with neurofibromatosis type 1 (NF1), an autosomal dominant disorder affecting 1 in 2,500 to 3,500 individuals worldwide (2). MPNSTs represent a major cause of mortality in NF1 patients (3). Nearly all NF1 patients develop benign dermal neurofibromas and ~30% of NF1 patients have benign plexiform neurofibromas (4) which, unlike dermal neurofibromas, can undergo malignant transformation to MPNST (5). Greater than 50% of MPNSTs contain cells immunoreactive for S100B protein, suggesting that tumors arise from neoplastic Schwann cells or their precursors (6).

Human tumor cell lines have historically served as experimental models for the study of tumor cell biology and drug development. A small number of cell lines, derived from NF1-associated MPNSTs (7-10) or sporadic MPNSTs (11, 12), have been reported in the literature but have never been directly compared with each other and primary tumors. These cell lines are a valuable resource as primary MPNSTs are difficult to permanently establish in culture. Here, we used MPNST cell lines to identify differences between MPNST cells and normal human Schwann cells (NHSC), the proposed cell of origin for MPNST.

Molecular events contributing to peripheral nerve tumor development are unclear. In the context of NF1, loss of the NF1 protein product is believed to be the earliest event as patients inherit a mutated NF1 allele and lose the second copy in MPNST cells. Loss of NF1 has also been documented in sporadic cases of MPNST (13). Another early alteration in MPNST development is the expression of epidermal growth factor receptor (EGFR). Whereas EGFR is not expressed in NHSCs, the protein is detected in primary MPNSTs, MPNST cell lines, and subpopulations of neurofibroma Schwann cells (14). Additional genetic mutations targeting regulators of the retinoblastoma protein (pRb) and p53 tumor suppressor pathways seem to represent later events in malignant transformation of Schwann cells as they are not observed in neurofibromas (15-17).

In this study, we identify a gene expression profile that distinguishes MPNST cell lines from NHSCs and validate this MPNST cell line gene signature in a large panel of primary MPNSTs. Furthermore, we provide evidence that one candidate gene over-expressed in all MPNST samples, TWIST1, is an attractive therapeutic target for novel MPNST treatment strategies.

Materials and Methods

MPNST tumor acquisition and processing. Tumor specimens and corresponding clinical data were collected and used in accordance with Institutional Review Board–approved protocols. The diagnosis of NF1 was...
established according to published criteria (NIH Consensus Statement), Frozen, archived tumor specimen pathology was reviewed and RNA isolated and then analyzed on Affymetrix U95Av2 GeneChip microarrays as reported (18).

**Cell culture.** T265p21, 90-8, ST88-14, 88-3, and STS26T cell lines were provided by Jeff DeClue (National Cancer Institute, Bethesda, MD). The YST-1 cell line was provided by Yoji Nagashima (University School of Medicine, Yokohama, Japan). Human NF1 cells that express isopropyl-β-D-thiogalactopyranoside (IPTG)–inducible human ADP ribosylation factor (ARF) were provided by Gordon Peters (Imperial Cancer Research Fund, London, United Kingdom). ST88-14, STS26T, ST50, S462, and Narf lines were grown in DMEM (Fisher Scientific, Pittsburgh, PA) supplemented with 10% fetal bovine serum (FBS; Harlan, Indianapolis, IN). 88-3, 90-8, and T265p21 lines were grown in a RPMI 1640–based medium as described (7). The YST-1 line was grown in RPMI 1640 (Fisher Scientific) containing 10% FBS. NHSCs were generated as previously described (19). Analyses were done under standard culture conditions for each cell line. Several assays were not conducted on the 88-3 cells due to difficulties in culturing this line.

**NF1 mutation analysis.** DNA was isolated from frozen STS26T or YST1 cell pellets. The NF1 gene was screened for mutations by denaturing high-performance liquid chromatography–based heteroduplex analysis using the WAVE analysis system (Transgenomic, Omaha, NE) as described. Several primer sequences were redesigned to reduce their homology to the NF1 pseudogenes sequences (20).

3′-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Cells (5 × 10^3) were plated in triplicate on a 24-well plate (day 0). The 3′-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was done on day 1 and day 4 by adding 50 μL of a 5 mg/mL MTT solution (Sigma-Aldrich, St. Louis, MO) to each well. Following incubation at 37°C for 2 h, the formazan precipitate was extracted in isopropanol-HCl and absorbance measured at 540 nm. For Taxol sensitivity assays, the noted concentration of paclitaxel (Sigma-Aldrich) was added to the medium on day 1. Concentration of paclitaxel was based on previous studies (21).

**Viability assay.** Cells were cultured on glass coverslips. Live versus dead cell numbers were determined using a LIVE/DEAD Viability/Cytotoxicity kit (Molecular Probes, Eugene, OR) according to the protocol of the manufacturer. The total number of live and dead cells was counted in five fields per sample and averaged. Assays were done in duplicate.

**Bromodeoxyuridine incorporation.** Twenty-four hours post plating 3 × 10^4 cells onto glass coverslips, cells were labeled for 1 h with bromodeoxyuridine (BrdUrd) labeling reagent (1:1,000; Amersham Biosciences, Piscataway, NJ) to detect DNA synthesis. Cells were fixed with 3.7% formaldehyde (Fisher Scientific), permeabilized in 0.3% Triton X-100 (Sigma-Aldrich), and incubated with anti-BrdUrd antibodies (1:200; Accurate Chemical & Scientific, Westbury, NY) in immunofluorescence buffer [20 mM MgCl2, 50 units DNase I (Roche, Basel, Switzerland)] for 45 minutes at 37°C. Cell nuclei and BrdUrd localization were visualized by incubating in 5 μg/mL bisbenzamide (Sigma-Aldrich) and rhodamine-conjugated donkey anti-Rat secondary antibodies (1:100; Jackson ImmunoResearch, West Grove, PA). Total number of cells and number of BrdUrd-positive cells were counted in five fields per sample and averaged. Assays were done in duplicate.

**Western blot analysis.** Cells were lysed on ice in 50 mM/L Tris (pH 7.5), 120 mM/L NaCl, 1 mM/L EDTA, 0.5% NP40, 0.1 mM/L sodium vanadate, 1 mM/L sodium fluoride, 5 μg/mL leupeptin, and 30 μM/L phenylmethylsulfonyl fluoride. Lysates were sonicated and clarified by centrifugation. Equivalent amounts of protein (50-100 μg) were separated by electrophoresis on SDS-polyacrylamide gradient gels (7-15% or 4-20%; C. Cell nuclei and BrdUrd localization were visualized by incubating in 5 μg/mL bisbenzamide (Sigma-Aldrich) and rhodamine-conjugated donkey anti-Rat secondary antibodies (1:100; Jackson ImmunoResearch, West Grove, PA). Total number of cells and number of BrdUrd-positive cells were counted in five fields per sample and averaged. Assays were done in duplicate.

**Microarray data analysis.** Microarray gene expression data (MAS *.cel files) were initially processed using Robust Microarray Analysis (RMA; ref. 23). RMA data were loaded into Genespring 6.1 computer software (Silicon Genetics, Redwood City, CA), transformed from a log 2 to linear scale, and normalized per gene to the mean value of its level of expression across seven NHSC samples. Welch t test/ANOVA with Benjamini multiple testing correction was used to identify statistically significant differences in gene expression between groups. Unsupervised hierarchical cluster analysis using Pearson correlation for a distance metric was used to arrange genes according to similar expression patterns. Statistical analysis of functional classification was used to identify altered molecular pathways (OntoExpress; http://vortex.cs.wayne.edu/ontoserver/). For analysis of primary MPNSTs, raw data (*.cel) were RMA-processed, assigned a U133 identification number, and imported into GeneSpring 6.1 for further analysis. To compare the primary MPNST data to the MPNST cell line and NHSC data, an additional per chip normalization to the median was conducted, followed by per gene normalization to the NHSC samples. To identify TWIST1 binding sites (24) in putative gene promoter regions, the pattern search program from GEMS Launcher (http://www.genomatix.de) was used to scan sequences with the IUPAC pattern. Quantitative real-time reverse transcription-PCR. Total RNA was used as a template to synthesize double-stranded cDNA using an oligo(dT) primer with Superscript II reverse transcriptase (Invitrogen). Duplicate reactions omitting reverse transcriptase were conducted to control for genomic DNA contamination. Relative levels of RNA were measured by quantitative real-time PCR using the ABI 7500 Sequence Detection System default settings. For most genes, amplification was conducted in SYBR Green Master Mix (Applied Biosystems, Foster City, CA). See Supplementary Table S1 for primer sequences for individual genes. Cycle threshold values were obtained where fluorescence intensity was in the geometric phase of amplification and averaged for triplicate reactions. Values for individual genes of interest were normalized to values for β-actin and used to calculate fold change in gene expression using ABI software. SOX20 (ABI TaqMan Probe ID Hs00366918_m1) and 18S (ABI TaqMan Probe ID Hs99999901_s1) were amplified using ABI 2X Universal TaqMan Master Mix (Applied Biosystems).

**Small interfering RNA transfection.** MPNST cells were plated in DMEM containing 10% FBS and no antibiotic for transfection the following day. Small interfering RNA (siRNA) molecules (siTWIST1 SMARTpool or siCONTROL Non-Targeting siRNA Pool; Dharmacon, Lafayette, CO) were
diluted in serum-free DMEM and incubated with XtremeGENE siRNA transfection reagent (Roche) at room temperature for 20 minutes before dropping on cells at a final optimal concentration of 20 nmol/L. Cell medium was changed 24 hours posttransfection to minimize toxicity. Cells were incubated 1 to 5 days before analyses. Transfection efficiency was ~90% as determined with a fluorescent siGLO control (Dharmacon).

**Migration assay.** The migratory response of MPNST cells was measured using a modified Boyden chamber assay. Four days posttransfection with siRNA (Dharmacon)XtremeGENE (Roche) complexes or XtremeGENE reagent alone, 4 × 10^4 cells in serum-free DMEM were plated on the upper chamber of a transwell with 8-μm pores (Costar, Corning Inc., Corning, NY). The lower chamber contained 800 μL MPNST conditioned medium. Cells were incubated for 16 hours at 37°C in 10% CO₂. Nonmigrating cells were removed from the upper surface of the membrane with cotton swabs. Membranes were stained with bisbenzimide and mounted onto glass slides. Migration was quantified by counting cells in four fields. Each condition was done in triplicate and the number of migrated cells was normalized to the total number of cells on an unscraped filter to validate the total number of cells plated. Data shown are representative of three independent experiments; values presented are the mean ± SD. Statistical significance was determined by t test using Microsoft Excel software.

**Results**

**A collection of MPNST cell lines.** Eight MPNST cell lines were collected for analysis (Table 1). Six cell lines were derived from NF1 patients. Two cell lines were derived from sporadic MPNSTs. NHSCs were chosen as a control because Schwann cells or their precursors are the most likely cells of origin in MPNSTs. Patient information and histopathology of the primary tumors have previously been documented in the original articles cited in Table 1.

We confirmed that the sporadic MPNST lines STS26T and YST-1 were wild-type at the NF1 locus. All 60 exons of the NF1 gene were screened for mutations using denaturing high-performance liquid chromatography–based heteroduplex analysis. No mutations were detected in the coding region of the NF1 gene in either sporadic MPNST sample (data not shown). Loss of heterozygosity (LOH) at the NF1 locus has previously been confirmed in five of the six NF1-associated MPNST lines (8, 25, 26).

**Cell growth rate varies in MPNST cell lines.** We compared growth properties of the MPNST cell lines by cell accumulation assays (Fig. 1). There was no correlation with NF1 status and growth rate. The fastest growing cell lines were STS26T, a sporadic MPNST line, and T265p21, an MPNST line derived from an NF1 patient (Fig. 1A). The more slowly proliferating cell lines, S520, 90-8, and ST88-14, evidenced density-dependent growth. At higher plating densities, they showed more robust growth but the overall trend in relative rates of cell accumulation remained the same (data not shown). We determined whether different rates of cell cycle progression or cell death accounted for the large variation in cell proliferation among the MPNST cell lines. BrdUrd labeling of the MPNST cell lines showed that their rates of BrdUrd incorporation paralleled their relative rates of proliferation (Fig. 1B). In contrast, the rates of cell death were approximately constant in all of the lines (Fig. 1C). Thus, rates of cell proliferation vary among the MPNST cell lines and do not distinguish the NF1-associated MPNSTs from the sporadic MPNSTs.

**Cell cycle proteins and Schwann cell markers are altered in MPNST cell lines.** Functional disruptions of the pRb tumor suppressor pathway are frequent events in primary MPNSTs.
(17, 27–29) due to inappropriate pRb phosphorylation via dysregulation of upstream regulators (p16INK4A mutation or cyclin overexpression) or loss of the pRb gene itself. pRb immunoreactivity was detected in all of the MPNST lines, suggesting that the RB gene itself is not lost in the tumor cells (Fig. 2A). In comparison with NHSCs, an increase in the inactive or phosphorylated form of pRb was detected in the 90-8, S520, ST88-14, STS26T, and YST-1 cell lines. Overexpression of cyclin-dependent kinase 4 (CDK4) or loss of the p16INK4A tumor suppressor can both result in enhanced pRb phosphorylation. CDK4 was not differentially expressed between the MPNST cell lines and NHSCs (data not shown). In contrast, p16INK4A was expressed only in cells that were wild-type for neurofibromin, NHSCs, and the two sporadic MPNST lines (Fig. 2A). These results suggest that p16INK4A selectively inactivated in the NF1-associated MPNST cell lines.

The p53 tumor suppressor gene is frequently deleted, mutated, and/or overexpressed in primary MPNSTs (15–17, 28). Therefore, we assessed the integrity of the p14ARF-HDM2-p53 signaling pathway in the MPNST cell lines in comparison with NHSCs. p14ARF was only expressed in the sporadic MPNST cell line STS26T (Fig. 2A). In addition, all MPNST cell lines showed a significant increase in HDM2 expression, a negative regulator of p53, in comparison with NHSCs (Fig. 2B). Notably, p53 was highly overexpressed in two of the MPNST cell lines, S520 and S462, whereas a lower molecular weight species (likely a truncated form of p53) was overexpressed in the 90-8 line in comparison with NHSCs (Fig. 2B). The remaining MPNST lines, ST88-14 and T265p21, both showed levels of p53 expression that were modestly higher than that in NHSCs. One sporadic MPNST line, YST-1, also exhibited a slight overexpression of p53 in comparison with NHSCs whereas p53 expression was completely absent in the STS26T sporadic MPNST line.

De novo EGFR expression has previously been implicated as an early event in Schwann cell transformation (14, 30). We stained NHSCs and the MPNST cell lines for EGFR and double labeled with the Schwann cell marker S100β protein. NHSCs are S100β-positive and EGFR negative (Fig. 2C). All of the NF1-associated MPNST lines and one sporadic MPNST line, STS26T, were strongly immunoreactive for EGFR in 100% of the cells (Fig. 2E). The sporadic MPNST line YST-1 exhibited moderate EGFR expression in ~50% to 60% of the cells (Fig. 2F). All of the MPNST cell lines currently lack S100β protein expression (Fig. 2E and F) although S100β immunoreactivity was originally reported for ST88-14, T265p21, and YST-1 (9, 12, 31). Thus, MPNST lines aberrantly express EGFR and lack expression of a Schwann cell marker, perhaps reflecting progressive anaplasia or loss of differentiation.

Gene expression profile of MPNST cell lines is consistent in MPNST in vivo. To identify gene expression changes associated with malignant transformation of Schwann cells, we did global gene expression analysis of MPNST cell lines using microarray technology. We analyzed samples from six NF1-associated MPNST lines, two sporadic MPNST cell lines, and seven NHSC samples on Affymetrix GeneChips containing >45,000 probe sets (841 of which were statistically significant (P < 0.01, Welch t test with Benjamini correction). To minimize false positives, we eliminated genes differentially expressed 3-fold in at least one normal reference sample (NHSC), reducing the number of probe sets to 592 consistently expressed among NHSCs and differentially expressed in MPNST cell lines (Fig. 3A).

Statistical analysis of functional classifications of these genes emphasized cell growth and differentiation, cell adhesion/migration, and immune response as key dysregulated cellular systems. Decreased expression of many Schwann cell markers, including S100B, PMP-22, NGFR, CNP, and SOX10, indicates aberrant differentiation in the MPNST cells. However, GFAP and MBP are expressed at similar levels in NHSC and the MPNST cell lines. Alterations in the pRB and p53 pathways are supported by the up-regulation of nine E2F targets (32) and three p53 targets, GADD45 (33), Sema3B (34), and DDAH (35). Genes within the MPNST molecular profile do not overlap with a generalized tumor cell line list generated by microarray analysis of the NCI 60 cancer lines (36) or a list of 24 genes up-regulated in synovial sarcomas and 4 MPNSTs (37).

To validate the data and to assess the utility of MPNST cell lines as a model for MPNST in vivo, the microarray data generated from the MPNST cell lines and NHSC were compared with microarray
data generated from 45 primary MPNST samples hybridized to Affymetrix U95 chips, containing ~12,000 probe sets representing ~10,000 annotated genes (18). Nearly all of the genes represented on the U95 chip were also on the U133 chip, resulting in 9,433 overlapping genes. Unsupervised hierarchical cluster analysis of the 9,433 probe sets across all samples distinguished normal samples, NHSCs, from tumor samples, MPNST cells, and primary MPNSTs (data not shown). When we examined expression of the 592 probe sets differentially expressed in MPNST cell lines in the primary MPNST samples, approximately one third (209 of 592) were represented on both the U133 and U95 chips (Fig. 3A). Therefore, the gene list we refer to as the MPNST molecular signature is not a complete profile of global gene expression. However, the majority (162 of 209) of probe sets that distinguished MPNST cells from NHSCs and were represented on both platforms predicted gene expression changes in solid tumors, validating the use of the MPNST cell lines as an in vitro tumor model system.

Hierarchical cluster analysis of the 209 probe sets differentially expressed in MPNST cell lines and represented in the microarray data from 45 primary MPNSTs. Cluster analysis reveals three distinct clusters: I, genes up-regulated in MPNST cell lines but down-regulated in primary MPNSTs (SMC4, RFC4, MKI67, BUB1) promote cell cycle progression; II, genes up-regulated in all MPNSTs (putative oncogenes TM4SF1, RGS2, ETV1, TWIST1, ADA, BASP1, SOX9, HMGA2); III, genes down-regulated in all MPNSTs (Schwann cell markers SOX10, CNP, NGFR, PMP22). See Supplementary Table S2 for complete list. *, genes previously reported as aberrantly expressed in other tumor systems, putative oncogenes, or tumor suppressor genes. Bold italicized genes contain the TWIST1-specific E-box (24) in their upstream sequence, potential transcriptional targets of TWIST1.
samples. The protein products of most of these genes are involved in cell cycle progression. It is reasonable to believe that cell lines growing in tissue culture medium may be exposed to nonphysiologic growth-promoting conditions in vitro. Many genes in cluster II have been implicated in other tumor systems. Genes in cluster III include Schwann cell differentiation markers, validating the notion that MPNST cells do not exhibit properties of differentiated Schwann cells.

**TWIST1 overexpression is necessary for MPNST cell migration but not apoptosis or chemoresistance.** TWIST1 gene expression was up-regulated in all MPNST cell lines and all primary MPNSTs as compared with normal Schwann cells. Differential expression of TWIST1 was confirmed at the RNA level by quantitative real-time reverse transcription-PCR (Fig. 4A) and at the protein level by Western blotting (Fig. 4B). To study the functional significance of TWIST1 overexpression in MPNST cells, we inhibited TWIST1 expression using siRNA molecules. We reduced TWIST1 expression at both the RNA and protein levels to ~10% to 20% compared with cells transfected with a nontargeting siRNA control (Fig. 4C). Putative TWIST1 transcriptional targets (n = 27) were identified by promoter analysis. Differential expression of 8 of 10 targets was validated by quantitative real-time PCR but the expression of none was altered by siRNA exposure for 5 days (data not shown). Furthermore, expression of known TWIST1 targets (38–40) was not significantly different in MPNST samples as compared with NHSCs.

TWIST1 can cause tumor cell resistance to microtubule-stabilizing drugs such as Taxol (21), an interesting finding given the relative chemoresistance of MPNSTs. We ascertained Taxol resistance of MPNST cell lines. All of the MPNST cell lines follow the survival curve of the TWIST1-expressing positive control cell line, PC3, indicating that they are relatively resistant to Taxol-induced cell death (Fig. 5A). IC_{50} values for individual MPNST cell lines were statistically different from the LNCaP TWIST1 negative control (P < 0.001; data not shown). However, reducing TWIST1 expression in MPNST cells by transient transfection with TWIST1 siRNA did not affect chemosensitivity (Fig. 5B). TWIST1 has also been shown to inhibit apoptosis (38). Neither cell growth nor apoptosis was altered in MPNST cells transfected with TWIST1 siRNA (data not shown).

TWIST1 can promote tumor cell migration, invasion (41), and metastasis (39). To examine TWIST1 function in MPNST migration, we tested whether MPNST cells were migratory in an in vitro migration assay. Interestingly, the MPNST cells did not have a haptotactic phenotype; they did not migrate in the serum-free or serum-containing medium. Because EGFR is overexpressed in MPNST cells, we added EGF to the standard serum-containing medium but observed minimal migration. MPNST conditioned medium was required for significant chemotactic migration (data not shown). We tested whether MPNST cells had a nontargeting control (Fig. 5B). TWIST1 has also been shown to inhibit apoptosis (38). Neither cell growth nor apoptosis was altered in MPNST cells transfected with TWIST1 siRNA (data not shown).

**Discussion**

We compared primary human Schwann cells to 8 MPNST cell lines and 45 solid MPNSTs. Rates of proliferation and gene expression profiles of the MPNST cell lines were variable, mirroring the heterogeneity of these tumors in vivo. Nevertheless, our microarray gene expression analyses of Schwann cells and MPNST lines resulted in the identification of a specific 159-gene profile for MPNST that was validated in a large panel of primary MPNSTs. The genes in the MPNST signature, many of which have been implicated in other types of cancer, represent potential regulators of MPNST pathogenesis.

Disruption of cell cycle regulation was apparent in the MPNST cell lines but consistent alterations in neither pRb-related nor p53-related proteins were observed. The p16_{INK4a} protein was absent in five of five NF1-associated MPNST cell lines whereas both sporadic lines and NHSCs retained p16_{INK4a} expression. Homozygous deletion of p16_{INK4a} has been detected in both NF1-associated and sporadic MPNSTs, but not in benign neurofibromas, suggesting that its loss contributes to malignant transformation.
TWIST1 expression in MPNST microarray data; secretion of HGF and signaling through c-Met may not be apparent at the transcriptional level (46). We confirmed that all MPNST cell lines, but not NHSCs, were positive for EGFR protein. Our previous analysis of the MPNST primary tumors revealed a statistically significant difference in expression profiles of EGFR-positive versus EGFR-negative MPNSTs (18). These observations suggest that aberrant expression of these genes may not be necessary for Schwann cell transformation and/or MPNSTs expressing these genes may represent a distinct subclass. Alternatively, these genes may be expressed in different populations of cells within each primary tumor.

Genes that are down-regulated in the MPNST molecular profile include several markers of Schwann cell differentiation, suggesting that MPNST cells have dedifferentiated or represent a Schwann cell precursor that has acquired mutations leading to tumor formation. SOX10 expression is down-regulated in MPNSTs and normally regulates expression of genes promoting Schwann cell differentiation (47). A recent study by Levy et al. (48) reports reduced levels of SOX10 and other Schwann cell markers in 9 MPNST samples compared with 14 benign neurofibroma samples by quantitative real-time PCR. SOX9 (49) and TWIST1 (24) are expressed in neural crest stem cells, Schwann cell precursors, and are overexpressed in MPNSTs compared with NHSCs. Although Schwann cells are the proposed cell of origin in MPNSTs, comparisons to additional tumors and normal tissues may reveal other genes relevant to MPNST formation or progression. The idea that tumor cells resemble progenitor cells is supported in other tumor models (50).

We identified TWIST1 as a previously unrecognized cancer-related gene in MPNST. TWIST1 gene expression is up-regulated in both MPNST cell lines and primary tumors. TWIST1 is also overexpressed in other tumors, including sarcomas (21, 38, 39). TWIST1 is normally expressed early in development and regulates cell type determination and differentiation of mesodermal tissues (reviewed in ref. 24). TWIST1 inhibits expression of ARF, as observed in seven of eight of the MPNST cell lines, which results in a bypass of p53-induced apoptosis (38). TWIST1 can inhibit apoptosis and promote cell transformation (38). However, we did not detect a significant change in apoptosis on reducing TWIST1 expression in MPNST cells. In several human carcinoma cell lines, TWIST1 can prevent cell death in the presence of microtubule-stabilizing chemotherapeutic agents (21) and stable suppression of TWIST1 increases sensitivity to Taxol-induced cell death (41). MPNSTs are poorly responsive to standard chemotherapeutic regimens and we showed that MPNST cell lines are resistant to Taxol. However, transiently reducing TWIST1 expression did not result in Taxol sensitivity in our MPNST cell accumulation assay.

TWIST1 is necessary and sufficient for prostate cancer cell migration and invasion in vitro (41). Suppression of TWIST1 inhibits metastasis in a murine breast tumor model (39). MPNSTs are highly invasive tumors that commonly metastasize. Reducing TWIST1 expression in MPNST cells inhibited chemotaxis, a key component of the metastatic process. Although many of the genes that are overexpressed in MPNST cells compared with NHSCs are implicated in cell adhesion, migration, or invasion, none are known chemotacticants. It is possible that hypersecretion and not transcriptional overexpression of a chemoattractant may promote MPNST cell migration.

We conclude that whereas MPNST cell lines are heterogeneous in cellular growth, a common gene expression profile in MPNST cell lines and primary MPNSTs distinguishes them from normal Schwann cells. The MPNST cell lines thus provide a valuable resource for future investigations of MPNST biology.
resource for generating in vitro evidence to support preclinical and clinical trials. Overexpression of TWIST1 is constant across all tested MPNST cell lines and primary tumors and is necessary for cell migration. Further investigation into the functional roles of TWIST1 and its transcriptional targets may help to uncover novel biomarkers or drug targets for improved diagnosis and treatment of MPNST.

Acknowledgments

Received 10/12/2005, revised 11/22/2005; accepted 1/5/2006. This work was supported by National Institutes of Health grants DAMD-17-01-0704 and W81XWH-04-1-0273 (N. Ratterer); National Neurofibromatosis Foundation Young Investigator Award and NINDS Translational Neuroscience Award K01-NS049191-01A1 (S.J. Miller); Deutsche Krebshilfe grant 70-2635, 70-2794 (V. Mautner) and NCI Investigator Award and NINDS Translational Neuroscience Award K01-NS049191-02 (S.J. Miller). We thank Dr. Erik Kruysen (University of Cincinnati) for helpful discussions; Yoji Nagashima and Jeff DeClue for providing MPNST cell lines; Jennifer O’Malley for generating quanta; S. Sue Griffo and Claudia Consoli for technical assistance with NFI mutation analysis; the University of Miami organ procurement team (Les Olson, director) for procurement of normal human nerves; Dr. Patrick Wood of the Miami Project to Cure Paralysis; the Laboratory of Immunohistochemistry and Medical Pathology at the University of Maryland Medical Center (Dr. Sue Heffelfinger, Director) for the S100 b and EGFR immunohistochemistry; Cincinnati Children’s Hospital Affymetrix gene chip core (Dr. Steve Potter and Richard Prayson (University of Cincinnati) for providing anti-TWIST1 primary antibody. We thank all the investigators who contributed MPNST cases including Tair Kish (University of California, San Francisco/formerly Johns Hopkins), Richard Prayson (Cleveland Clinic), Abijit Guha (University of Toronto), Julia Bridge (University of Nebraska) and Rosaline Ferrer (King’s College, London).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Dr. Erik Kruysen (University of Cincinnati) for helpful discussions; Yoji Nagashima and Jeff DeClue for providing MPNST cell lines; Jennifer O’Malley for generating quanta; S. Sue Griffo and Claudia Consoli for technical assistance with NFI mutation analysis; the University of Miami organ procurement team (Les Olson, director) for procurement of normal human nerves; Dr. Patrick Wood of the Miami Project to Cure Paralysis; the Laboratory of Immunohistochemistry and Medical Pathology at the University of Maryland Medical Center (Dr. Sue Heffelfinger, Director) for the S100 b and EGFR immunohistochemistry; Cincinnati Children’s Hospital Affymetrix gene chip core (Dr. Steve Potter and Richard Prayson (University of Cincinnati) for providing anti-TWIST1 primary antibody. We thank all the investigators who contributed MPNST cases including Tair Kish (University of California, San Francisco/formerly Johns Hopkins), Richard Prayson (Cleveland Clinic), Abijit Guha (University of Toronto), Julia Bridge (University of Nebraska) and Rosaline Ferrer (King’s College, London).

References

17. Mawrin C, Kirches E, Bolzec G, Dietzmann K, Roessner M. Molecular Comparison of MPNST
29. Prayson (Cleveland Clinic), Abijit Guha (University of Toronto), Julia Bridge (University of Nebraska) and Rosaline Ferrer (King’s College, London).

Molecular Comparison of MPNST
Large-Scale Molecular Comparison of Human Schwann Cells to Malignant Peripheral Nerve Sheath Tumor Cell Lines and Tissues

Shyra J. Miller, Fatima Rangwala, Jon Williams, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/66/5/2584

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2006/03/07/66.5.2584.DC1

Cited articles
This article cites 49 articles, 9 of which you can access for free at:
http://cancerres.aacrjournals.org/content/66/5/2584.full#ref-list-1

Citing articles
This article has been cited by 35 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/66/5/2584.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/66/5/2584.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.