Increased Frequency of Aberrations in the p53/MDM2/p14<sub>ARF</sub> Pathway in Neuroblastoma Cell Lines Established at Relapse

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Abstract

p53 mutations have been reported in cell lines derived from relapsed neuroblastoma tumors. We hypothesize that functional inactivation of p53 by mutation or other mechanisms is common in relapsed neuroblastoma and can contribute to chemoresistance. Our aim was to determine the frequency of p53 mutations, p14<sub>ARF</sub> methylation, or deletion and MDM2 amplification in 23 neuroblastoma cell lines (6 derived at diagnosis and 17 derived at relapse). One cell line was p53 mutant (BE2c) and two cell lines were deleted for p14<sub>ARF</sub> (LAN-6 and SHEP). Two cell lines were methylated for p14<sub>ARF</sub> (GIMEN and PER-108), one of which had low levels of p14<sub>ARF</sub> mRNA expression which increased following demethylation with 5-aza-2-deoxycytidine treatment (GIMEN), and four cell lines were confirmed to be MDM2-amplified. All these cell lines were derived from neuroblastomas at relapse. Inactivation of the p53 pathway was observed in 9 out of 17 neuroblastoma cell lines (53%) established at relapse and in none of the cell lines established from pretreatment tumors. If these data are confirmed in neuroblastoma tumors, this suggests that p53-independent therapy and reactivation of inactive p53 approaches would be useful in the management of relapsed neuroblastoma. (Cancer Res 2006; 66(4): 2138-45)

Introduction

Neuroblastoma is the most common extracranial pediatric solid tumor. Despite intensive treatment, only 25% to 40% of children with widespread disease over the age of 1 year are curable. Initially, most high-risk neuroblastomas respond to cytotoxic therapy and local radiotherapy, however, neuroblastoma frequently relapses displaying high level drug resistance, which often correlates with the intensity of the therapy (1).

An important mechanism of intrinsic chemoresistance in tumor cells is an abnormality in the p53/MDM2/p14<sub>ARF</sub> pathway. The p53 gene, located on the short arm of chromosome 17p13, is the most commonly mutated gene in human cancer, occurring in up to 60% of human cancers (2). p53 is a key regulator of cell cycle checkpoints and apoptosis, which upon activation by cellular stresses, particularly DNA damage, binds DNA in a sequence-specific manner to activate the transcription of a number of genes, including p21, MDM2, PIG-3, and BAX. The activation of p53 results either in cell cycle arrest or apoptosis (reviewed in ref. 3). MDM2 is an important autoregulatory feedback inhibitor of p53. MDM2 expression is induced by p53 and the ubiquitination of p53 by MDM2 targets p53 for proteosome-mediated degradation, hence, forming an autoregulatory feedback loop, to tightly regulate cellular levels of p53 (4). Amplification of MDM2 has been shown in some tumors and can suppress the activity of p53 by increasing its degradation.

The INK4a/ARF locus, located on 9p21-22, encodes two structurally distinct gene products, the cyclin-dependent kinase inhibitor gene p16<sup>INK4a</sup> and p14<sub>ARF</sub>. Both p16<sup>INK4a</sup> and p14<sub>ARF</sub> exert active roles in the retinoblastoma and p53 pathways, respectively, thereby regulating cell proliferation and playing a role in the development of cancer. p14<sub>ARF</sub> and p16<sup>INK4a</sup> share a common coding sequence for exons 2 and 3; however, they have distinct promoter and exon 1 sequences (5). It has been shown that p14<sup>ARF</sup> can activate the p53 pathway by interacting with and inhibiting the ubiquitin ligase activity of MDM2 (6); hence, preventing the polyubiquitination, nuclear export, and cytoplasmic degradation of p53 (7). Inactivation of p14<sub>ARF</sub> by deletion or methylation can increase levels of MDM2 which in turn inactivates p53. There is evidence showing that p53 down-regulates p14<sub>ARF</sub> expression, establishing an autoregulatory loop between p53-DM2-p14<sub>ARF</sub> (8). The p53/MDM2/p14<sub>ARF</sub> pathway is therefore critical for normal cell cycle progression.

In neuroblastoma, p53 mutations at diagnosis are rare, occurring in <2% of cases. We have previously reported the development of an inactivating p53 mutation in a neuroblastoma cell line (BE2c) established at relapse (9). This cell line was shown to be more resistant to doxorubicin, etoposide, cisplatin, and melphalan than the paired p53 wild-type cell line (BE1n) established from the same patient at diagnosis (1). Some neuroblastoma cell lines established after treatment have been found to have alternative mechanisms of p53 functional inactivation such as MDM2 amplification and p14<sub>ARF</sub> deletion (10).

In this study, we have investigated the role of the p53/MDM2/p14<sub>ARF</sub> pathway in a panel of 23 neuroblastoma cell lines, 6 of which were established at diagnosis, and 17 at relapse. In this context, a relapse cell line is defined as one established from a tumor sample taken after the patient has received prior chemotherapy. We report that 53% of cell lines established at relapse have alterations of the p53 pathway and in some cases this is associated with chemoresistance.

Materials and Methods

Cell lines. A panel of 23 neuroblastoma cell lines established from patients at different stages of therapy was used. These included two matched pairs of cell lines derived from the same tumor at diagnosis and relapse SKNBE1n (BE1n) and SKNBE2c (BE2c; ref. 9), NBL-W and NBL-W-R (11), and one set of cell lines established from the same tumor at diagnosis, relapse, and further relapse, PER-106, PER-107, and PER-108.
(12). Out of a further 16 unmatched cell lines, 3 were established at diagnosis, NB69 (13), IMR-32 (14), NBL-S (15), and 13 at relapse LS (16), TR-14 (17), NB1691 (18), NGP (19), CHP902R (20), SMRSCNR (21), GIMEN (22), SHSYSY, SKNSH, SHEP (23), SNB-1 (24), LAN-6 (25), and SKNRN (1). All cell lines were cultured in RPMI 1640 (Life Technologies, Inc., Paisley, Scotland) supplemented with 10% FCS (Life Technologies) in a 37°C, 5% CO₂ humidified incubator. All cell lines were tested for Mycoplasma on a regular basis and were found to be free from contamination.

p53 gene sequencing. Total cellular DNA was extracted from frozen cell pellets using the NucleoGen method (Scotlab, Strathtyde, Scotland). Exons 4 to 9 of the p53 gene were amplified by PCR, using primers described previously (26), the PCR product was gel-purified and sequenced by an automated capillary electrophoresis method (Beckman CEQ8000).

Fluorescence in situ hybridization. Fluorescence in situ hybridization (FISH) to detect MDM2 amplification was done on cytopsins of cell lines. For interphase nuclei preparation, 50 μl aliquots of cell suspension consisting of 8 x 10⁶ cells were spun onto clean dry slides and fixed using Carnoy’s fixative. A spectrum orange-labeled MDM2 Vysis probe was used in combination with a spectrum green-labeled chromosome 12 centromeric probe (Vysis, Downers Grove, IL) and FISH was carried out according to the manufacturer’s instructions. MDM2 amplification was defined as the presence of more than four copies of the MDM2 signal relative to the chromosome 12 centromeric signal.

To confirm 9p21 deletion in SHEP cells, FISH was carried out on metaphase chromosome spreads using the p16/CEP9 probe set (Vysis). The dual color probe set is a mixture of the p16 probe, spectrum orange, and the CEP 9 probe, which is labeled with spectrum green. This probe spans 190 kb which contains multiple genetic loci including p16INK4a, p14ARF and p15INK4a. Hybridization and posthybridization washes were carried out according to the manufacturer’s instructions and slides were counterstained with 4,6-diamidino-2-phenylindole antifade (Vector Laboratories, Burlingame, CA).

p14ARF gene promoter methylation. The DNA methylation status of the p14ARF gene was determined by methylation-specific PCR (MSP). One microgram of DNA was denatured by sodium hydroxide and modified by sodium bisulfite treatment, which converts unmethylated cytosines to uracil. Bisulfite-treated DNA was amplified using MSP primers specific for either methylated or unmethylated DNA using previously published primers (27, 28). Universally methylated DNA (Chemicon International, Temecula, CA) was used as a positive control and placental DNA was used as a negative control. The PCR products were loaded onto 1.5% agarose gels and separated by gel electrophoresis. Methylation studies were done thrice and consistent results were obtained.

5-Aza-2-deoxycytidine treatment and real-time reverse transcription-PCR. Two cell lines, GIMEN and SHSYSY were grown in the presence of 5-aza-Cdr (5 μmol/L, Sigma, St. Louis, MO) for 72 hours. Medium was changed daily. The relationship between gene promoter methylation and gene silencing was investigated using real-time quantitative reverse transcription-PCR. RNA was extracted from cell lines using the Qiagen RNeasy system (Qiagen, Ontario, Canada). Two micrograms of RNA were reverse-transcribed to cDNA using TaqMan reverse transcriptase reagents (Applied Biosystems, Foster City, CA) with random primers. Primers and probes for p14ARF and p16INK4a were designed and synthesized by custom TaqMan gene expression assays. Both primers amplified across the exon 1/exon 2 boundary to select against amplification from any genomic DNA contamination. TaqMan glyceraldehyde-3-phosphate dehydrogenase control reagents (Applied Biosystems) were used for internal reference. Reverse transcription-PCR was done in a total reaction volume of 10 μl containing 5 μl of TaqMan universal PCR master mix, 0.5 μl of primers and probes mix (Applied Biosystems), 2.5 μl cDNA and 2 μl of H₂O. Each sample was set up in triplicate and quantitatively analyzed using the ABI Prism 7900HT sequence detection system (Applied Biosystems).

p14ARF homozygous deletion. Homozygous deletion of the p14ARF locus was investigated using duplex PCR with previously published primer sequences (29). PCR primers were designed to amplify exon 1β which encodes for p14ARF. Primer concentrations used were 1 μmol/L and the sodium channel gene, which maps to chromosome 3p21, served as an internal control.

Protein expression analysis. Whole cells were lysed using previously described methods (9). Fifty micrograms of cell lysate was loaded onto a 4% to 20% Tris-HCl precast SDS polyacrylamide gel (Invitrogen, Paisley, United Kingdom) for electrophoresis with a See Blue prestained standard 1x molecular weight marker (Invitrogen). The proteins were transferred to nitrocellulose membrane (Amersham, United Kingdom) and probed with the following antibodies and dilutions: NCL-p53 DO-7 (Novocastra, Newcastle, United Kingdom) at 1:1,000, p14ARF (Calbiochem, Cambridge, MA) at 1:100, MDM2 (Calbiochem) at 1:100; Actin (Sigma) at 1:1,000. Peroxidase-conjugated goat anti-mouse antibody (Dako, Glostrup, Denmark) was used as a secondary antibody for p53, p14ARF, and MDM2, and goat anti-rabbit (Dako) antibody was used for actin, all at 1:1,000. Protein detection was done using enhanced chemiluminescence as described previously (9).

Results

p53 gene sequencing. In this panel of neuroblastoma cell lines, 22 of 23 were p53 wild-type. The BE2c cell line was p53 mutant, as previously reported (9). Exons 4 to 9 of the p53 gene were sequenced in 10 neuroblastoma cell lines in which p53 status had not previously been reported. This panel of 10 cell lines included a matched pair of cell lines derived from the same tumor (NBL-W and NBL-W-R) and a matched set of cell lines derived from the same tumor (PER-106, PER-107, and PER-108), together with GIMEN, LS, CHP902R, NB69, and TR-14. All 10 cell lines sequenced were p53 wild-type (Table 1).

MDM2 FISH. Four cell lines were shown to be MDM2-amplified confirming previous reports; NB1691 (18), LS, TR-14, and NGP (30). None of the other cell lines were found to be MDM2-amplified. Figure 1 shows MDM2 amplification by FISH results in the NB1691 and LS cell lines and MDM2-nonamplified SKNSH cells. All MDM2-amplified cell lines were established from relapsed tumors. MDM2 amplification was not found in the absence of MYCN amplification.

p14ARF methylation and expression. p14ARF methylation was determined by MSP using previously reported primers (27). Partial p14ARF methylation was observed in 2 out of 23 (9%) of neuroblastoma cell lines, GIMEN and PER-108 (Fig. 2A). To investigate whether methylation was associated with transcriptional silencing of p14ARF in GIMEN and PER-108 cell lines, p14ARF mRNA expression was determined using real-time PCR (Fig. 3A). p14ARF gene methylation was associated with transcriptional silencing in GIMEN cells but not in PER-108 cells, when the latter was compared with the parent cell line PER-107 (Fig. 3A).

p14ARF mRNA expression was significantly higher in MDM2-amplified neuroblastoma cell lines (LS, NB1691, NGP, and TR14) compared with MDM2 nonamplified cell lines (Fig. 3A; P < 0.001, Mann-Whitney test). Two nonneuroblastoma cell lines, one with MDM2 amplification (SJA), and one with MDM2 gain, i.e., eight copies (JAR), had lower levels of p14ARF compared with the MDM2-amplified neuroblastoma cell lines (Fig. 3A). Increased p14ARF expression was also observed in the p53 mutant BE2c cell line compared with the paired p53 wild-type BE1n cell line (Fig. 3A). The relatively higher expression of p14ARF in MDM2-amplified cell lines was also confirmed at the protein level by Western blotting, as was the difference between the BE1n and BE2c paired cell lines (Fig. 3B).

p14ARF mRNA expression was not detected in the negative control cell line DAOY, a medulloblastoma cell line homozygously deleted for chromosome 9p (31). p14ARF was not detected at the mRNA level in four cell lines, SMSKCNR, SHEP, SJNB-1, and LAN-6 (Fig. 3A).

mRNA expression of p14ARF was examined in GIMEN and SHSYSY before and after treatment with the demethylating agent
5-aza-CdR (Fig. 3C). p14ARF expression was restored in GIMEN cells following treatment for 72 hours with 5-aza-CdR, whereas the control unmethylated SHSY5Y cell line showed no increase in p14ARF expression following 5-aza-CdR treatment. This was also confirmed at the protein level by Western blotting (Fig. 3D). In the GIMEN cell line, using the sulforhodamine B assay, we did not observe any significant change in sensitivity to cisplatin following re-expression of p14ARF. The growth inhibition of 50% of cells (GI50) value was 2.5 μmol/L in cells treated with 5-aza-CdR as compared with 3.4 μmol/L in control GIMEN cells treated with PBS (data not shown). The proliferation rate of the GIMEN cell line was reduced following 72-hour treatment with 5 μmol/L 5-aza-CdR. The doubling time in the control cells treated with 5 μmol/L PBS was 19 hours and the treated GIMEN cells were growth-arrested for 5 to 6 days before resuming growth (data not shown).

### Table 1. p53 pathway status of neuroblastoma cell lines established at different stages of therapy

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Abbreviations: WT, wild-type; JM8, carboplatin; CPA, cyclophosphamide; CDDP, cisplatin; RT, radiotherapy; DBZ, dacarbazine; DAUN, daunorubicin; DOX, doxorubicin; ETOP, etoposide; HD L-PAM, high-dose melphalan; LD-RT, low-dose radiotherapy; VM26, Teniposide; VCR, vincristine; PCH, peptichemio.

*Results from this study.

5-aza-CdR (Fig. 3C). p14ARF expression was restored in GIMEN cells following treatment for 72 hours with 5-aza-CdR, whereas the control unmethylated SHSY5Y cell line showed no increase in p14ARF expression following 5-aza-CdR treatment. This was also confirmed at the protein level by Western blotting (Fig. 3D). In the GIMEN cell line, using the sulforhodamine B assay, we did not observe any significant change in sensitivity to cisplatin following re-expression of p14ARF. The growth inhibition of 50% of cells (GI50) value was 2.5 μmol/L in cells treated with 5-aza-CdR as compared with 3.4 μmol/L in control GIMEN cells treated with PBS (data not shown). The proliferation rate of the GIMEN cell line was reduced following 72-hour treatment with 5 μmol/L 5-aza-CdR. The doubling time in the control cells treated with 5 μmol/L PBS was 19 hours and the treated GIMEN cells were growth-arrested for 5 to 6 days before resuming growth (data not shown).

### Figure 1. MDM2 amplification by FISH.

A. NB1691 cell line showing amplification of MDM2 as seen by more than two signals for MDM2 (red) and trisomy 12, indicated by three centromeric signals (green). B. MDM2-amplified LS cells. C. MDM2-nonamplified SKNSH cells.
Four cell lines were MDM2-amplified (NGP, TR14, NB1691, and LS) as previously reported (18, 30, 37). All were established from neuroblastomas at relapse and NB1691 has shown to be more chemoresistant than other wild-type p53 neuroblastoma cell lines (18). A further MDM2-amplified neuroblastoma cell line which is chemoresistant has also been reported, CHLA134 (1). No MDM2-amplified neuroblastoma cell lines have been reported to date without co-amplification of MYCN, supporting the view that MYCN amplification is an early event in neuroblastoma development (38), and that MDM2 amplification is possibly selected for or induced by chemotherapy. Attenuation of p53 function in MDM2-amplified cell lines has been shown by lower levels of the p53 target gene p21 being induced after irradiation in NGP (39), LS, and TR-14 and CHLA-134 (1), compared with non–MDM2-amplified cell lines.

Methylation of p14<sup>ARF</sup> was detected in two cell lines, GIMEN and PER-108 (Table 1), both derived from relapsed tumors. Previous studies showed no evidence of p14<sup>ARF</sup> methylation in 10 neuroblastoma cell lines including BE2c, IMR-32, and SHEP (40) as confirmed here. In our panel of 23 neuroblastoma cell lines, methylation of the p14<sup>ARF</sup> gene promoter was observed independently of p16<sup>INK4a</sup> methylation (data not shown). This has also been observed in some neuroblastoma and medulloblastoma tumors (41, 42). Of the two p14<sup>ARF</sup> gene methylated cell lines, low p14<sup>ARF</sup> mRNA expression was observed in GIMEN cells, but not in PER-108 cells (Fig. 3A). The coexistence of p14<sup>ARF</sup> methylation and expression in PER-108 cells may be a reflection of cell heterogeneity, in which a proportion of cells contain methylated p14<sup>ARF</sup> alleles and loss of p14<sup>ARF</sup> expression occurs. It is also possible that there is a threshold at which the degree of gene methylation affects gene expression. In p14<sup>ARF</sup> methylated GIMEN cells, p53 function is compromised, shown by reduced p21 induction following irradiation, compared with other p53 functional cell lines.4

p14<sup>ARF</sup> expression was restored in GIMEN cells following treatment with 5 μM of 5-aza-CdR for 72 hours, however, this did not alter sensitivity to cisplatin in the sulforhodamine B assay. In contrast, previous studies manipulating p53 status by transfection of E6 vectors to degrade p53 have shown increased drug resistance in neuroblastoma cell lines (1). Also, p53 mutant BE2c cells have been shown to be more resistant to a variety of chemotherapeutic agents compared with wild-type BE1n (1, 9).

Increased p14<sup>ARF</sup> expression was also observed in the p53 mutant BE2c cell line compared with the p53 wild-type BE1n cell line. It has previously been reported that wild-type p53 down-regulates p14<sup>ARF</sup> (8), hence, p53 mutant cells might be expected to show increased p14<sup>ARF</sup> expression. An interesting observation was the increased p14<sup>ARF</sup> expression in all four MDM2 neuroblastoma–amplified cell lines (P < 0.001, Mann-Whitney test; Fig. 3A). The current study shows that when p53 is inactivated via MDM2 amplification, levels of p14<sup>ARF</sup> mRNA and protein are increased in MDM2 amplified neuroblastoma cell lines compared with MDM2 nonamplified cell lines. Interestingly, nonneuroblastoma cell lines with MDM2 gain or amplification express lower levels of p14<sup>ARF</sup> both at the RNA and protein level.

4 E. Bell, J. Carr, X. Lu, R. Premkumar, P.E. Lovat, J. Lunec, and D.A. Tweddle. MYCN overexpression is not responsible for the failure of MYCN amplified neuroblastoma cell lines to G1 arrest after DNA damage.
than MDM2-amplified neuroblastoma cell lines. Because MYCN is coamplified in these neuroblastoma cell lines, it is possible that MYCN and MDM2 amplification and overexpression may have a cooperative effect on the expression of p14ARF. MDM2-mediated inhibition of p53 is an important pathway in the progression of neuroblastoma, and it has recently been reported that MDM2 is a direct transcriptional target of MYCN in neuroblastoma (43).

Low p14ARF mRNA expression has been reported in 2 out of 16 (12.5%) advanced neuroblastoma tumors (stages 3 and 4) using reverse transcription-PCR (44) and 1 out of 18 (5%) unfavorable neuroblastoma tumors (stages C and D) based on Pediatric Oncology Group staging criteria (45).

In the current study, p14ARF mRNA expression was not detectable in SHEP, SJNB-1, SMSKCNR, and LAN-6 cells (Fig. 3A). Homozygous deletion of p14ARF was shown in LAN-6 and SHEP by duplex PCR (Fig. 4A). Homozygous deletion of p14ARF in LAN-6 cells has previously been reported (20), and they have also been found to be resistant to the cytotoxic agents, melphalan, carboplatin, and etoposide (1). SHEP cells have previously been reported to undergo lower levels of apoptosis, are more resistant, and have an attenuated p53 response after irradiation, compared with SHSY5Y and SKNSH cells (39). SHSY5Y and SHEP cells, both subclones of the neuroblastoma cell line SKNSH, showed a similar decrease in cell clonogenicity in response to cisplatin; however, the SHEP cell line showed lower levels of apoptosis (20%) as compared with 65% of SHSY5Y (46). This may be due to the p14ARF deletion affecting p53-mediated induction of apoptosis.

Interestingly, FISH analysis of SHEP cells shows that 66% of cells have lost one copy of the 9p21 region, so in the remaining allele, a smaller 9p deletion specifically affecting the p14ARF locus may be present to explain the absence of a genomic DNA PCR product. Such a deletion, which does not affect the entire 190 kb

Figure 3. A, mRNA expression levels of p14ARF in neuroblastoma cell lines showing significantly elevated p14ARF mRNA expression in MDM2-amplified cell lines (P < 0.001, Mann-Whitney), compared with MDM2-nonamplified cell lines and p53 mutant BE2c cells compared with p53 wild-type BE1n, by real-time reverse transcription-PCR. Columns, mean; bars, SE (n = 3). B, Western blots of p14ARF, p53, and MDM2 in a selection of cell lines. C, p14ARF mRNA expression in GIMEN and SHSY5Y cells before and after 72 hours of treatment with 5 μmol/L 5-aza-CdR. Control cells were treated with PBS. D, p14ARF protein expression in GIMEN cells treated with 5 μmol/L 5-aza-CdR and untreated control GIMEN cells, with actin loading control.
probe target, would not be detected by FISH. In the 34% of cells which have two copies of 9p21, it is likely that both alleles also contain a smaller deletion not detected by our FISH probe. Overall, it would seem that the SHEP cell line is homozygously deleted for a small region of 9p21 which incorporates the p14ARF and p16INK4a locus.

As previously mentioned, SHEP is a subclone of SKNSH; cytogenetically, the only difference between SHEP and SKNSH is that 75% of SHEP cells have an additional isochromosome 1q (23).

It is possible that the 9p21 deletion observed in SHEP and not SKNSH has occurred in vitro and has led to this cell line being more resistant than SKNSH. *In vitro* selection of p53 abnormalities in other cell lines could have occurred, however in three neuroblastoma cell lines, CHLA-174, CHLA-101, and LS in which the primary tumor was examined, the corresponding p53 abnormality was present in the original tumor (20, 30).

It is possible that the lack of p14ARF expression observed in the SJNB-1 and SMSCCNR cell lines may be due to an inactivating
mutation in the p14ARF gene region as both methylation of the p14ARF gene promoter and deletion have been ruled out as possible mechanisms of gene silencing.

In the remaining 8 of 17 cell lines established at relapse, it is possible that other abnormalities of the p53-dependent growth inhibition and apoptosis pathways may also contribute to drug resistance. Abnormalities of genes involved in apoptosis may also exert a role in drug resistance via the p53 pathway (Fig. 5). Bax, a transcriptional target of p53, is a member of a large family of genes which include antiapoptotic members such as BCL-2 and BCL-XL and proapoptotic members including BAX, BAK, NOXA, and PUMA. Overexpression of exogenous BCL-2 or BCL-XL can inhibit p53-induced apoptosis in response to stress signals, conversely down-regulation of BCL-2 can enhance apoptosis (reviewed in ref. 47).

PTEN (phosphatase and tensin homologue located at chromosome 10) acts as a tumor suppressor by negatively regulating the phosphatidylinositol 3’ kinase-Akt pathway, which promotes the phosphorylation and movement of MDM2 into the nucleus where it down-regulates p53. Deletion of PTEN at 10q23.3 has been reported at relapse, it may be appropriate to include p53-independent therapies such as taxol, temozolamide, and arsenic trioxide in the management of relapsed neuroblastoma. Also, reactivation of p53 in cancer cells may be a strategy to explore in the future to increase treatment response rates in relapsed neuroblastoma. Finally, up front therapies for neuroblastoma should exploit p53-mediated apoptotic pathways, while they are still effective.

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5 J. Carr, N. Bown, A. G. Hall, M. Case, J. Lunec and D. A. Tweddel, unpublished observations.

A schematic representation of the p53 pathway. p53 is activated in response to DNA damage and activates the transcription of a large number of genes, including the CDK inhibitor p21WAF1/CIP1, which blocks G1 to S phase cell cycle progression as well as genes such as BAX, p53-induced gene 3 (PIG3), and PUMA, which are involved in apoptosis. MDM2 is the master regulator of p53 and the two form an autoregulatory feedback loop, p14ARF is activated in response to oncogenes such as MYC and E2F and stabilizes p53 by inhibition of MDM2. Also, PTEN negatively regulates the phosphatidylinositol 3’ kinase-Akt pathway kinase promoting the phosphorylation and movement of MDM2 into the nucleus where it down-regulates p53.


Increased Frequency of Aberrations in the p53/MDM2/p14 ARF Pathway in Neuroblastoma Cell Lines Established at Relapse

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