Transfection of melanoma cells with antisense PAX3 oligonucleotides additively complements cisplatin-induced cytotoxicity

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Abstract
Advanced melanoma is difficult to treat, in part because of greater resistance to therapy compared with other cancer types. The mechanisms underlying this resistance are not well-understood. One factor that is reported to be involved in melanoma cell survival is PAX3, a transcription factor normally expressed during embryonic development, and which is critically required for development of neural crest-derivatives, including skin melanocytes. PAX3 expression is deregulated in primary melanomas and most melanoma cell lines. Here we have investigated whether targeting PAX3 expression in melanoma cell lines together with chemotherapeutic treatment increases susceptibility to therapeutic cell death. Using PAX3-specific antisense oligodeoxynucleotides (PAX3-AS) to treat melanoma cell lines in vitro, we showed dose-dependent reduction of proliferation of melanoma cells, and induction of apoptosis compared with control treatments. Induction of apoptosis was accompanied by the induction of active caspase-3 in UACC62 and M14 cells, and p53 protein in UACC62 cells. Treatment of melanoma cells with cisplatin induces DNA damage and cytotoxicity, which is thought to be via p53-dependent and -independent mechanisms. Treatment of either p53 mutant (M14) or wild-type (UACC62) melanoma cells with cisplatin, and varying doses of PAX3-AS, resulted in percentages of cells undergoing apoptosis equivalent to the sum of the individual treatments, irrespective of mutation status [e.g., UACC62, 43.8% (1 μmol/L PAX3-AS), 30.1% (20 μmol/L cisplatin), 69.6% (PAX3-AS + cisplatin); M14, 12.6% (1 μmol/L PAX3-AS), 41.5% (40 μmol/L cisplatin), 50.2% (PAX3-AS + cisplatin)]. These data suggest that treatment of melanoma cells with PAX3-AS complements cytotoxicity induced by cisplatin. [Mol Cancer Ther 2005;4(6):996–1003]

Introduction
Disseminated melanoma is a radiation- and chemotherapyrrefractory neoplasm for which no standard therapy currently exists. Five-year survival rates of patients with advanced melanoma are very poor, with little improvement in recent years despite intensive research. Although melanomas are curable if detected at an early stage, late stage melanomas are notoriously aggressive, which is in part due to resistance to therapy, but the mechanisms of this resistance are not well-understood (1).

Melanoma arises from melanocytes, which are neural crest–derived pigment cells that, during embryogenesis, migrate to the subdermal layer of skin and retina of the eye. Significant efforts have been made to identify critical genes involved in melanoma development (2). One set of developmental genes, which may play a role in this regard, is the PAX genes. The PAX3 gene is essential for normal development of the somitic mesoderm into neural and skeletal muscular tissues in the adult, as well as development of neural crest–derived structures in the inner ear, heart, and skin (melanocytes). PAX3 plays an important role in early embryonic development of the pigmentary system. Mutations in the PAX3 gene in human are associated with type I and type III Waardenburg syndrome, both of which exhibit pigmentation defects and deafness due to abnormalities of neural crest–derived cells and the melanocyte lineage (3, 4). In mouse, spontaneous heterozygous Splotch (Sp+/+) mice have Pax3 mutations and exhibit coat color abnormalities characterized by a white patch on the belly due to defective neural crest–derived melanocyte development. Homozygous Sp/Sp embryos display multiple severe defects and die prior to day 14 of gestation (5, 6). Although the importance of PAX3 has been identified in many embryologic processes including neural crest cell migration (7), neural tube closure, limb muscle formation (8–10), and PAX3/FKHR (forkhead domain gene) fusion protein generated by chromosomal translocations t (2;13) has been well characterized in the pediatric malignant tumor alveolar rhabdomyosarcoma (11–14), the role of PAX3 in melanoma is still not clear, in spite of several reports on deregulation of PAX3 expression in melanomas (15–18). Mature melanocytes do not express PAX3, even though PAX3 is expressed in melanoblasts (16). Overexpression of PAX3 in cutaneous malignant melanoma and melanoma cell lines suggests that increased PAX3 expression is important for tumor cell survival. In addition, reduced survival has been
observed in rhabdomyosarcoma or melanoma cell lines following treatment with PAX3-AS (11, 16). PAX3 is specifically expressed in rapidly proliferating cells and is down-regulated prior to terminal differentiation during embryo development (19).

Cisplatin is an important drug in the treatment of cancer, including melanoma (20, 21), although it is not as effective as dacarbazine in clinical treatment of metastatic melanoma (22). However, 80% to 85% of melanomas remain unresponsive to either drug (22). Cisplatin is, however, broadly used in in vitro experiments. In the present study, we investigated whether treatment with PAX3-AS augments cell death induced by cisplatin. Treatment of melanoma cells with varying doses of PAX3-AS combined with cisplatin, resulted in an additive, nonsynergistic cytotoxicity, which suggests that PAX3 is a critical factor contributing to melanoma cell survival, and that its overexpression may contribute to therapy resistance in melanoma.

Materials and Methods

Cell Culture, Transfections, and Oligonucleotides

Human melanoma cell lines, M14 and UACC62, were obtained from the NCI anticancer cell line panel of the National Cancer Institute-Division of Cancer Treatment and Diagnosis repository, National Cancer Institute, Frederick, MD. Cells were grown at 37°C and 5% CO₂ in humidified atmosphere as described (23). Eighty percent confluent cells were transfected with either single-stranded sense or antisense oligonucleotides against human PAX3 at 1 μmol/L (unless otherwise indicated) with lipofectin (Invitrogen, San Diego, CA). Control cell incubations were carried out with lipofectin and with medium only. After 5 hours, the transfection medium was supplemented with serum-containing medium. PAX3-AS and -5 phosphorothioate oligonucleotides were synthesized (Genset, Singapore) corresponding to nucleotides 358 to 376 of human PAX3 mRNA: PAX3-AS (5'-GGCTTGTTGTCATCTGTCGCC-3'), PAX3-S (5'-GCCCTCCAGGATGACCCGC-3'; ref. 11). For detection of the combined effect of PAX3-AS and cisplatin on cell proliferation or apoptosis, the cells were plated and transfected as above, afterwards fresh medium supplemented with cisplatin was added either 5 or 24 hours after transfection. The cells were continuously cultured a further 24 or 72 hours in the cisplatin solution before the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay or apoptosis assays were done. As cisplatin was dissolved in dimethylformamide, dimethylformamide treatment alone was used to test its cytotoxicity and the results were negative.

MTT Assay

Cell proliferation of M14 and UACC62 cell lines was measured by using an MTT cell proliferation kit (Roche). Cells were seeded in 96-well plates, and following transfection, the cells were incubated for 12 or 72 hours. Ten microliters of MTT (5 mg/mL in PBS) was added to each well and incubated for 4 hours at 37°C. The resulting formazan within the cells was dissolved in 100 μL of 10% SDS in 0.01 mol/L HCl. Optical densities were read at 570 nm using a PolarStar Optima micro-plate reader 18 to 24 hours later.

Flow Cytometry

Cells (M14 and UACC62) grown in 24-well plates were assayed by flow cytometry and annexin V staining (Molecular Probes, Eugene, OR) as described (23).

SDS-PAGE and Immunoblotting

At the indicated times, M14 and UACC62 cells were lysed in radioimmunoprecipitation assay buffer [50 mmol/L Tris-Cl (pH 8.0), 150 mmol/L NaCl, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mmol/L phenylmethylsulfonyl fluoride, 1 mmol/L sodium orthovanadate]. Subsequently, protein concentrations were determined using the bicinchoninic acid protein assay reagent kit (Pierce, Rockford, IL) with bovine serum albumin as a standard. Equivalent amounts of protein were loaded in each lane of a 10% SDS-polyacrylamide gel and electrophoresed followed by blotting onto a polyvinylidene difluoride membrane. After blocking with 5% (w/v) fat-free milk powder, 0.1% Tween 20 in PBS, the membrane was incubated for 1 hour at room temperature with anti-PAX3 antibody (1:2,000) or anti-actin antibody (Sigma, St. Louis, MO) diluted into 3% (w/v) fat-free milk powder, 0.1% Tween 20 in PBS, washed and incubated with secondary antibody (Sigma). The membrane was then washed and incubated with Western blot chemiluminescence luminol reagents according to the manufacturer’s directions (Amersham Biosciences, Castle Hill, NSW, Australia). The amplified signals were then detected with Kodak X-OMAT AR film.

Immunofluorescence Microscopy

Both detached and adherent cells were analyzed for active caspase-3 and p53 immunofluorescent staining. Detached (presumed dead) cells were collected separately, washed with PBS, and applied to APES-coated glass slides. Adherent cells were grown on glass coverslips and rinsed in PBS. Both detached and adherent cells on glass slides were fixed in cold methanol for 10 minutes on ice, and permeabilized in a solution of 0.2% Triton X-100, 5% normal goat serum in PBS for 30 minutes, which also served to block nonspecific antibody binding. Slides and coverslips were rinsed with PBS before incubation for 60 minutes at room temperature with the rabbit anti–active caspase-3 antibody (Promega, Madison, WI, 1:250) or mouse anti-p53 antibody (Santa Cruz, Santa Cruz, CA, sc-126, 1:200) diluted in 1.5% normal goat serum in PBS for 30 minutes, which also served to block nonspecific antibody binding. Slides and coverslips were rinsed with PBS for 5 minutes at room temperature with the rabbit anti–active caspase-3 antibody (Promega, Madison, WI, 1:250) or mouse anti-p53 antibody (Santa Cruz, Santa Cruz, CA, sc-126, 1:200) diluted in 1.5% normal goat serum in PBS. After rinsing four to five times with PBS, the slides were subsequently incubated with Alexa Fluor 488 goat anti-rabbit secondary antibody or Alexa Fluor 594 goat anti-mouse secondary antibody (Molecular Probes, 1:200) in 1.5% normal goat serum in PBS for 1 hour at room temperature in the dark, washed with PBS, and mounted with 4',6-diamidino-2-phenylindole–containing fluorescence mounting solution (Vector, Burlingame, CA; H-1200). Negative control incubations using the same secondary antibody, but omitting the primary anti–active caspase-3 antibody or anti-p53 antibody were also carried out. At least
four individual experiments were carried out for each data-point. For each experiment, the images were captured using an Olympus BX50 digital camera. Using 10 randomly selected fields, ~500 cells were counted manually and the positively stained cells were scored (except for analysis of anti-caspase-3 staining in attached UACC62 cells in which ~200 cells were counted).

**Quantitative Real-time PCR**

Real-time PCR was carried out as described (23). Primers used for amplification of p53 were cat. no. 4319446-0207002 (Hup53) from Assays-on-demand (Applied Biosystems Incorporated, Bedford, CA).

**Statistical Analyses**

Student’s *t* test was used for all statistical calculations. The values presented represent the mean ± SDs.

**Results**

**PAX3-AS Treatment of Melanoma Cell Lines Inhibits Cell Proliferation, and Increases Apoptosis**

Using quantitative real-time PCR, we have previously reported that PAX3 mRNA is expressed in all seven investigated melanoma cell lines of the NCI-60 cell line panel (23). In that study we showed that PAX3-AS treatment of M14 and UACC62 cells was associated with induction of apoptosis. To confirm that PAX3 protein levels were reduced by PAX3-AS treatment, Western blot studies were carried out using an anti-PAX3 antibody, and in the first instance, basal levels of PAX3 protein expression were analyzed in M14 (TP53 mutant) and UACC62 (TP53 wild-type) melanoma cell lines. The M14 cell line expressed high levels of PAX3, whereas UACC62 cells expressed much lower levels of PAX3 (Fig. 1A), reflecting the marked variability in PAX3 expression between different melanoma cell lines as previously observed (23). Upon transfection with PAX3-AS, the level of PAX3 protein was reduced in both cell types (Fig. 1B and C).

Morphologic analysis of the cells following PAX3-AS treatment revealed a progressive detachment of the cells from the plate bottom from 12 hours after treatment until the end of the observation period at 72 hours. These detached cells appeared round, and smaller than the attached cells when observed under an inverted microscope. In contrast, the control PAX3-S-treated and lipofectin-treated cells showed only a subtle effect 12 hours after treatment.

![Figure 1](https://example.com/f1.png)

**Figure 1.** PAX3-AS treatment of two melanoma cell lines; effect on PAX3 protein level and cellular morphology. **A**, Western blot analysis of basal PAX3 protein expression in UACC62 and M14 cell lines. Cell lysates (50 μg of protein) were electrophoresed and probed with anti-PAX3 and anti-actin antibodies. Anti-actin was used to verify equivalent protein loading in each lane. **B** and **C**, Western blot analysis of the effect of PAX3-AS (1 μmol/L) and PAX3-S (1 μmol/L) treatment on PAX3 protein expression in (B) M14 and (C) UACC62 cell lines. Cells were cultured with medium only (C), lipofectin/PAX3-AS (AS), or lipofectin/PAX3-S (S) for 72 h (M14) or 24 h (UACC62), and 20 μg of protein was loaded in each lane. After being probed with anti-PAX3 antibody, the blots were reprobed with anti-actin antibody to verify equivalent loading. The numbers immediately below the PAX3 signal in each lane show the relative intensity of the signal measured by densitometry, where the ratio of the densitometric readings for PAX3 and actin signals were normalized relative to the control lane of each cell line, which was standardized at 1.0. **D** and **E**, morphologic changes in (D) M14 cells and (E) UACC62 cells were observed by phase contrast microscopy 48 h after treatment. Bar, 200 μmol/L.
after treatment, and quickly recovered by 48 hours after treatment (Fig. 1D and E). The morphologic changes in the cells treated with the PAX3-AS were consistent with induction of cell death.

Annexin V staining was used to investigate whether apoptosis had occurred following PAX3-AS treatment of melanoma cells. Treatment of M14 and UACC62 cells with PAX3-AS led to significantly increased annexin V staining, confirming morphologic data suggesting cell death. Annexin V-positive staining was detected in 39.7% ± 0.5% of UACC-62 cells and 16.8% ± 1.7% of M14 cells 24 hours after PAX3-AS treatment, but in only 13.4% ± 0.5% and 13.7% ± 2.8% of PAX3-S treated cells, respectively (Fig. 2A and B). Annexin V staining was detected in the PAX3-AS treated UACC62 melanoma cells as early as 12 hours after treatment (data not shown). These results suggest that 1 μmol/L PAX3-AS induces apoptosis in M14 and significantly more apoptosis in UACC62 melanoma cells.

Active Caspase-3 and p53 Protein Were Induced in Melanoma Cells following PAX3-AS Treatment, but TP53 mRNA Levels Were Not Altered

Programmed cell death is associated with activation of caspase-3. We used indirect immunofluorescence staining with anti–active caspase-3 antibody as an independent semiquantitative method to determine whether treatment with PAX3-AS induces cell death via apoptosis in melanoma cell lines. More active caspase-3-positive cells were detected in PAX3-AS-treated than in control cells (Table 1; Fig. 3A), and this was especially true in the detached population of cells, suggesting that the likely cause of detachment of the PAX3-AS-treated cells was apoptosis via activation of caspase-3.

We hypothesized that expression of PAX3 leads to cell survival by repressing p53, in which case one possible outcome is that the function of p53 protein might be activated following PAX3-AS treatment of cells. To investigate this, we carried out immunofluorescence staining with an anti-p53 antibody to determine whether treatment with PAX3-AS leads to increased immunoreactive p53 protein in UACC62 melanoma cells carrying a wild-type p53 gene. This analysis revealed more p53-positive cells in the PAX3-AS-treated cells than in control cells (Table 1). Positive immunoreactivity with p53 was frequently independent of active caspase-3 immunoreactivity, and was observed in both attached and detached cells, whereas active caspase-3 staining was predominantly observed in the detached cells, suggesting that p53 activation had occurred prior to activation of caspase-3 (Table 1; Fig. 3B).

To investigate whether an increased steady-state level of mRNA for TP53 might explain the increased percentage of p53-positive cells, thereby implicating a possible role for PAX3 in transcriptionally repressing TP53, quantitative real-time PCR analysis was done on mRNA isolated from the UACC62 melanoma cell line following PAX3-AS treatment. At 8 and 24 hours after treatment, no difference in the level of TP53 mRNA was detected, despite clear evidence of reduced proliferation and induction of apoptosis in the treated cells (data not shown). Only p53 wild-type UACC62 cells were used for this analysis because these cells showed clear induction of the wild-type p53 protein, and a relatively strong induction of annexin V staining with significant inhibition of cell growth following PAX3-AS treatment, as compared with M14 cells.

Treatment of Melanoma Cell Lines with PAX3-AS Enhances Cytotoxicity in Combination with Cisplatin in an Additive Fashion

Cells were transfected with PAX3-AS and incubated for 24 hours, following which the cells were treated with different concentrations of cisplatin for a further 24 hours,
and the effect of the combined treatment on the extent of cellular proliferation was measured by MTT assay. The results showed that treatment with cisplatin alone inhibited the growth of both cell lines in a dose-dependent manner. Treatment with PAX3-AS alone inhibited the growth of both cell lines down to 66.7% (UACC62) and 87.4% (M14) of the control treatment using culture medium alone (Fig. 4A and B). Combined cisplatin treatment and PAX3-AS transfection enhanced the inhibition for both UACC62 and M14 cell lines. The inhibition on proliferation of cisplatin and PAX3-AS treatment did not seem to be synergistic, but complementary and additive. This was especially evident for M14, where the combined effect of treatment was modestly better than cisplatin alone, but closely paralleled that of cisplatin, and suggested that the effect of the combined treatment on cell proliferation was essentially a summation of the results obtained for each treatment on its own (Fig. 4A and B).

Following the above cell proliferation studies, the effect of PAX3-AS treatment in combination with cisplatin on the induction of apoptosis in the melanoma cell lines was investigated. UACC62 and M14 melanoma cells were treated with PAX3-AS for 24 hours, followed by addition of fresh complete medium containing 20 and 40 μmol/L cisplatin in UACC62 and M14 cells, respectively, for a further 24 hours. At 48 hours, the cells were analyzed for apoptosis by annexin V staining and flow cytometry. In similar fashion to the results of the cellular proliferation assays, the combined treatment of PAX3-AS and cisplatin was equivalent to a summation of the effects of the single treatments on their own [e.g., 43.8% (PAX3-AS), 30.1% (CDDP), and 69.6% (PAX3-AS + CDDP) for UACC62; 12.6% (PAX3-AS), 41.5% (CDDP), and 50.2% (PAX3-AS + CDDP) for M14; Fig. 4C and D].

It is possible that cell death was induced by PAX3-AS treatment within the first 24 hours, and that due to the

Table 1. Semiquantitative analysis of melanoma cells in vitro expressing either active caspase-3 or p53 following transfection with PAX3-AS or PAX3-S

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Cell line</th>
<th>Cell status</th>
<th>Percentage of cells showing immunoreactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Medium control</td>
</tr>
<tr>
<td>Anti–active caspase-3</td>
<td>UACC62</td>
<td>attached cells</td>
<td>0.42 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>M14</td>
<td>attached cells</td>
<td>3.82 ± 0.73</td>
</tr>
<tr>
<td></td>
<td>M14</td>
<td>detached cells</td>
<td>0.51 ± 0.24</td>
</tr>
<tr>
<td>Anti-p53</td>
<td>UACC62</td>
<td>attached and detached</td>
<td>4.31 ± 1.09</td>
</tr>
</tbody>
</table>

*P < 0.01 compared with the corresponding medium control.
addition of cisplatin at 24 hours, was simply the cumulative effect of two separate treatments, whereas addition of cisplatin at an earlier time after transfection, prior to any decrease in PAX3 protein level or induction of apoptosis, led to induction of cell death simultaneously due to PAX3-AS treatment and cisplatin. We added cisplatin at 5 hours after transfection, culturing the cells continuously in cisplatin-containing medium until 72 hours after transfection without changing the medium. The combination treatment induced larger amounts of apoptosis than the medium control or cisplatin alone (89.2% versus 11.2% or versus 44.9%; Fig. 5A). Treatment with cisplatin (20 μmol/L) alone strongly inhibited UACC62 cell proliferation, whereas the combined application of PAX3-AS treatment and cisplatin even more strongly inhibited UACC62 proliferation (Fig. 5B). A similar effect was found in the M14 cell line with the addition of cisplatin 5 hours after transfection (Fig. 5C).

We also examined whether combined cytotoxicity was additive in the presence of lower doses of both PAX3-AS and cisplatin, which was added 5 hours after transfection. The results suggested that at lower doses, PAX3-AS treatment still additively complemented cisplatin-induced cytotoxicity (data not shown).

**Discussion**

Despite intensive research and numerous clinical trials on adjuvant treatment of patients with high-risk cutaneous melanoma, there is no effective treatment for disseminated disease. In the present study, we have investigated whether PAX3 expression could be a contributing factor to resistance to therapy in melanoma. Our results support the contention that PAX3 plays an important role in the survival of human melanoma cells (16), and show that induction of apoptosis using a combination of PAX3-AS transfection and cisplatin treatment in melanoma cells exceeds that of either reagent by an amount equivalent to the sum of the two treatments. Apoptosis induction using the combination of PAX3-AS and cisplatin was unlike BCL-XL antisense treatment (24), or C-MYC antisense treatment (25), where the effects were synergistic.

The exact role of PAX3 expression in tumor and normal cells is not clear. PAX3 is expressed in rapidly proliferating cells and is down-regulated prior to terminal differentiation. Scholl et al. (16) reported that PAX3 expression was unambiguously confined to tumor cells and not detected in surrounding normal tissue, normal skin sections, or sections of benign lesions by real-time PCR. Vachtenheim et al. (17) also reported that in a panel of 17 human melanoma cell lines, PAX3 mRNA was expressed in all cell lines, even in those that had repressed the MITF-M gene and were amelanotic. Our previous studies showed that PAX3 mRNA was expressed in seven melanoma cell lines (23), and preliminary studies carried out on primary melanomas indicated that PAX3 protein is frequently but not always expressed in primary melanoma.3 PAX3 expression in melanoma correlates strongly with a dedifferentiated and proliferative status, just as in alveolar rhabdomyosarcomas (26), where specific down-regulation of PAX3/FKHR in alveolar rhabdomyosarcoma cells by PAX3-AS resulted in reduced cellular viability due to induction of apoptosis (11).

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3 Unpublished data.
In PAX3-deficient Sp/Sp embryos, apoptosis is observed in somites, as well as in the neural tube, of which the latter can be rescued by p53 deficiency, implicating a role for p53-dependent apoptosis during development (27). In the neural tube of Sp/Sp mice, apoptosis was associated with increased p53 protein levels resulting from Pax3 deficiency. We have shown that apoptosis in melanoma cells occurs whether or not a p53 mutation is present. In the present study, we observed that TP53 mRNA levels were not altered by PAX3-AS treatment in p53 wild-type UACC62 melanoma cells. However, greater numbers of UACC62 cells showed positive p53 staining upon treatment with PAX3-AS, than with either control or PAX3-S treatment, suggesting that a p53-dependent mechanism of apoptosis is involved. However, PAX3-AS treatment apparently also leads to p53-independent mechanisms of apoptosis in M14 (p53 mutant) melanoma cells.

The mutation status of p53 may have an effect on apoptosis induced by PAX3-AS treatment. In this regard, although expression of PAX3 protein was inhibited more in M14 cells than UACC62 cells, apoptosis and proliferation assays showed a smaller effect in M14 cells than in UACC62 cells. UACC62 cells had lower basal PAX3 protein levels than M14 cells, but the induction of apoptosis was greater, suggesting that mutant p53 may confer resistance to apoptosis induced by PAX3-AS.

PAX3-AS may induce apoptosis via both p53-dependent and -independent pathways. This is supported by our observations that cisplatin and PAX3-AS treatments did not enhance or diminish each other’s effects, yet were additive in the M14 (p53 mutant) cells, despite having a modest effect. Cytotoxicity due to cisplatin and PAX3-AS was additive in UACC62 and M14 cells no matter whether the agents were added to the cells 5 or 24 hours apart, or whether the cytotoxic dose or length of time of exposure was varied.

With respect to antisense therapies, down-regulation of BCL-2 and/or BCL-XL by antisense treatment has been widely studied to treat melanoma and other tumors (28, 29). Indeed, many members of the BCL-2 family and several antiapoptotic members of other gene families are universally expressed in human melanoma, and most, if not all, are important for the survival of other normal cells (30). However, as PAX3 has a more restricted tissue expression pattern in adults than BCL-2 or BCL-XL, PAX3 gene products might be highly specific targets for antisense therapy of melanoma. The present study suggests that further research on PAX3 is warranted, and could lead to anticancer drugs that significantly improve outcome for patients with malignant melanoma.

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References


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