Id2 Reverses Cell Cycle Arrest Induced by γ-Irradiation in Human HaCaT Keratinocytes

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Id2 plays a key role in epithelial cells, regulating differentiation, the cell cycle, and proliferation. Because human skin constantly renews itself and is the first target of irradiation, it is of primary interest to evaluate whether such a gene may be regulated in keratinocytes exposed to ionizing radiation. We show here that Id2 is induced in response to γ-irradiation and have investigated the consequence of this regulation on cell fate. Using RNA interference, we observed that Id2 extinction significantly reduces cell growth in human keratinocytes through the control of the G1-S transition of the cell cycle. We have investigated whether the impact of Id2 on the cell cycle may have a physiological role on the cell’s ability to cope with radiative stress. Indeed, when Id2 is down-regulated through interfering RNA, cells are more sensitive to irradiation. Conversely, when Id2 is overexpressed, this somehow protects the cell. We propose that Id2 favors reentering the cell cycle after radiation-induced cell cycle arrest to permit the recovery of keratinocytes exposed to ionizing radiation.

Id proteins are negative regulators that inhibit helix loop helix (HLH) transcription factors from binding to DNA through protein-protein interactions. The HLH family of transcription factors comprises more than 200 members, indicating its major role in the coordination of mammalian cell lineage regulation of gene expression, controlling the cell cycle. An essential role has been established for a number of HLH proteins in hematopoietic, myogenic, pancreatic, and neurogenic cell lineage commitment and cell differentiation. Four main groups of HLH proteins can be distinguished on the basis of the presence or absence of additional functional domains. Members of a distinct subfamily of HLH proteins, the Id proteins, lack a DNA binding region and instead form dimers with other transcriptional regulators, principally those of bHLH type. Such ID-bHLH heterodimers are unable to bind DNA, and hence Id proteins act as dominant negative regulators of bHLH proteins (4). Because most bHLH transcription factors positively regulate sets of genes involved in cell differentiation, the term Id conveniently refers to the ability of these proteins to inhibit both DNA binding and differentiation.

Among Id proteins, Id2 plays a particular role in cell cycle regulation. Indeed, Id2 is also a dominant negative antagonist of pRb. A dual connection links Id2 and pRb: when overexpressed, Id2 overrides the tumor suppressor function of RB, allowing binding of E2F to its target genes, promoting entry into S phase and cell proliferation (5).

Human skin is the first tissue exposed to irradiation, including during radiotherapeutic treatment. It is therefore of primary interest to analyze the consequences of this genotoxic stress on human keratinocytes. Contradictory effects of ionizing radiation on cell differentiation have been described in epithelial cells. For example, γ-rays increased the calcium-induced differentiation of mouse epidermal cells (6), whereas they induced dedifferentiation and proliferation in normal human skin cells and carcinoma cells as well as in mouse skin (7, 8).

Because of its major role in differentiation, we have analyzed whether Id2 protein was involved in the response to ionizing radiation in human keratinocytes. We observed induction of Id2 gene expression. We then further analyzed the biological effect of the overexpression or extinction of Id2 proteins in human keratinocytes and, in response to irradiation, investigated the physiological role of Id2 induction in response to γ-irradiation.

MATERIALS AND METHODS

Cell Culture and Irradiation—HaCaT is a non-tumorigenic, spontaneously transformed human keratinocyte cell line (9) generously provided by N. E. Fuesing (German Cancer Research Center, Heidelberg, Germany). Cells were grown at 37 °C in a 5% CO₂ humidified atmosphere in Dulbecco’s modified Eagle’s medium containing 4.5 g/liter glucose supplemented with 10% fetal calf serum, 100,000 units/liter penicillin, 50 mg/liter streptomycin, and 200 μm glutamine. HaCaT differentiation was induced by cultivating cells seeded in plastic flasks at 10,000 cells/cm² for 10 days as described previously (9). HaCaT cells were irradiated with 2, 6, and 10 Gy from a 60Co source at a dose rate of 0.3 Gy/min. Irradiated and sham-irradiated cells (used as control) were then returned to the incubator until they were harvested for analysis.

Human primary keratinocytes were obtained from human mammary skin biopsy. Briefly, keratinocytes were isolated by overnight trypsinization in 0.5% trypsin (Invitrogen)/5% penicillin/streptomycin (Eurobio) in phosphate-buffered saline at 4 °C, followed by scraping with a scalpel; then cells were cultured in semi-defined KGM2 medium (Clonetics) on flasks coated with collagen type I (Falcon Biocoat) at 37 °C and 5% CO₂. Third passage cultures were used for all experiments (around 12 cell doublings). For the irradiation experiment, cells were seeded at 40,000 cells/cm², reached confluence at day 5, and were further incubated for 3 days after confluence in order to reach a morphological differentiated state. Primary cells were then irradiated with...
2 Gy following the same procedure as mentioned above.

**Microarray Experiments and Analysis—Microarrays were performed as described previously (10). A complete description of the microarrays used in this study including the protocols for production and post-processing of slides has been deposited into the GEO data base (www.ncbi.nlm.nih.gov/geo/). This information is available on request under the following GEO accession numbers: GSE1369, GSE1370, GSM22167-GSM22214, and GSM22117-GSM22140.

RNA Extraction and Real-time Semiquantitative PCR—Total RNA samples (2 μg), prepared with TriPure Isolation Reagent (Boehringer Mannheim, Roche Diagnostics, Meylan, France), were reverse-transcribed with the SuperScript™ II Rnase H reverse transcriptase system (Invitrogen) with a random primer according to the manufacturer’s instructions. Reverse transcription reactions performed in a total volume of 20 μl were diluted to 250 μl, and 5 μl of the diluted cDNA were used as a template for each quantitative PCR. The sequences of specific primers were as follows: Id2, 5′-gct-cag-ctc-ccc-cct-3′ (forward) and 5′-gtt-gtg-cag-cgg-cct-ctc-3′ (reverse); c-myc, 5′-gtc-ggt-agg-aga-cat-ggg-3′ (forward) and 5′-ctc-tga-gac-ggt-tct-ggc-3′ (reverse); and 18S RNA, 5′-cga-tgg-ggc-ggt-att-tct-3′ (forward) and 5′-ctc-gtg-ggc-ctc-tcc-3′ (reverse). Quantitative PCR was carried out with the SYBR Green Jumpstart Taq Ready Mix (Sigma) in the ABI PRISM™ 7700 sequence detection system/real-time quantitative PCR from PerkinElmer Life Sciences. Assays were performed in triplicate. Fold differences were calculated by a mathematical model described by Pfaffl (11).

**Immunoblotting—** Total cellular proteins were extracted using either radioimmune precipitation assay buffer (50 mM Tris-HCl, pH 7.4, 1% Nonidet P-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM NaVO₄, and 1 mM NaF) for 20 min at 4 °C or Laemmli buffer (1×) in being buffer for 10 min. Both lysis buffers were supplemented with the EDTA-free protease inhibitor mixture Complete (Roche Applied Science). Twenty five μg of protein from each cell lysate were run on a 12% polyacrylamide gel, transferred to polyvinylidene fluoride membranes (Bio-Rad), and blocked for 1 h at room temperature in TTBS containing 5% nonfat dry milk and 1% bovine serum albumin. Blots were then incubated overnight at 4 °C in TTBS/3% bovine serum albumin containing either the primary antibody anti-Id2 (C-20), anti-P21 (C-19), anti-P16 (C-20), anti-lamin A/C (636; Santa Cruz Biotechnology, Santa Cruz, CA), or anti-α-tubulin (catalog no. 13-8000; Zymed Laboratories Inc.) at a 1:1000 dilution. After several washes in TTBS, membranes were incubated in TTBS, 5% nonfat dry milk, and 1% bovine serum albumin containing a 1:2500 dilution of horseradish peroxidase-conjugated goat anti-mouse antibody (M32407) (Caltag Laboratories Inc.) at a 1:1000 dilution. Detection was performed with the ECL kit (Amersham Biosciences).

**Plasmid Constructions for Id2 and Lamin A**

Overexpression or Extinction—For overexpression experiments, the Id2 coding region was amplified by PCR using forward primer 5′-ggg-cag-cat-gag-ctc-3′ and reverse primer 5′-gct-gtt-gtg-aga-cgc-3′. The PCR product was inserted into the HindIII and NotI sites of pPlc/BSV (Invitrogen) to make pPlc-Id2. The Id2 sequence was verified by sequencing. For extinction experiments, short hairpin RNA (shRNA) specific for Id2 and lamin A/C gene was generated as described on-line (sitemaker. umich.edu/dlturner/sequences/files/u6_hairpin_sirma_6_31_02.doc) using the m6Upro vector (12). Briefly, three duplexes of oligonucleotides covering the sequences 5′-tggt-gct-ctt-ctc-3′, 5′-gcc-gtg-agc-cat-ggg-3′, and 5′-ctg-gcc-ttg-cag-ctc-3′, and 5′-aga-acc-agg-gaa-aga-gtc-3′ of the Id2 gene and one duplex of oligonucleotides covering the 5′-aac-ttg-acc-aga-ata-3′ sequence of the lamin A/C gene were cloned into m6Upro vector digested by BbsI and XbaI to construct shRNA Id2A, Id2B, Id2C, and aca-t-3′. The PCR fragment was transformed into expression vector pBluescript II SKⅢ+ (Stratagene) and sequenced by several times.

**Transient Transfections**—Transfections were performed using the JetPEITM transfection reagent (Qbiogene, Carlsbad, CA). The day before transfection, living HaCaT cells were seeded at 20,000 cells/cm² in a 24-well plate. On the day of transfection, 1 μg of plasmid was diluted into 50 μl of 150 mM NaCl and added to another 50 μl of 150 mM NaCl containing 2 μl of JetPEI™. Fifteen minutes later, the mixture was added to the cells together with 900 μl of fresh medium. Cells were incubated for 4 h at 37°C and washed with phosphate-buffered saline, and fresh medium was added.

Hoechst 33342 and Propidium Iodide Staining for Microscopic Analysis of Cell Death—One day after transfection, living HaCaT cells were washed once in phosphate-buffered saline and stained with Hoechst 33342 dye (Sigma-Aldrich) and propidium iodide (Sigma-Aldrich) to final concentrations of 1 and 2.5 μg/ml, respectively. After a 15-min incubation period, cells were washed once again, and coverslips were mounted for fluorescent staining analysis with an Olympus IX70 microscope.

**Cell Cycle Analysis**—Per fraction, 10^5 cells were collected and processed for cell cycle analysis. Cells were fixed and permeabilized in 70% ethanol (−20°C) at 4°C for 20 min, washed with phosphate-buffered saline and 5% fetal calf serum, and treated with 40 μg/ml RNase for 20 min at 37°C. Cells were incubated in 3 μg/ml propidium iodide (Sigma-Aldrich) for 5 min at room temperature and placed on ice before flow analysis in a MoFlo (DakoCytomation).

**Proliferation Assays**—Proliferation assays were performed using the Vialight™ HS test, a bioluminescent assay monitoring ATP present in metabolically active cells (BioWhittaker, Walkersville, MD). One day after transfection, cells were collected and transferred to a 96-well plate at a concentration of 5000 cells/well. Four days later, 100 μl of Nucle-
otide Releasing Reagent were added to each well. After a 5-min incubation, 180 μl of the supernatant were mixed into 20 μl of ATP Monitoring Reagent, and the ATP content present in the sample was immediately quantified in a MicroLumatPlus LB96V luminometer (Berthold Technologies, Bad Wildbad, Germany).

RESULTS

Id2 Is Induced in Response to γ-Irradiation—We investigated global changes in gene expression in response to γ-irradiation in human primary keratinocyte cells. Microarray results revealed a total of 639 probes shown to be modulated in response to the therapeutic dose of 2 Gy. This represents 9.39% of the probes included in the analysis. Among the genes involved in RNA synthesis and modification, Id2 was found to be transiently induced 24 h after irradiation (Fig. 1A). Id2 induction was weak (1.655) but reproducible (p < 0.05). To further challenge and analyze Id2 response to γ-radiations, we investigated Id2 expression and function in the HaCaT cell line as a model of the human keratinocyte. We have monitored Id2 regulation of expression was biphasic. We observed that Id2 transcript levels slightly decreased (25%) in HaCaT cells 3 h after treatment (Fig. 1B) and then increased significantly (up to 2.5-fold) when compared with untreated cells. Id2 messenger induction began 18 h post-irradiation and was still observable 24 h after irradiation. This result was further confirmed at the protein level, with Id2 protein accumulating as soon as 6 h after irradiation (2 Gy) in HaCaT cells (Fig. 1D) and reaching a 2-fold increase after 24 h.

Fig. 2. Effect of Id2 extinction in HaCaT cells. HaCaT cells plated at low density were transfected with three different shRNAs specific for Id2 and one shRNA specific for lamin A/C. A, 3 days after transfection, protein extracts were subjected to immunoblot analysis with anti-Id2 antibodies. The same membrane was re-probed with anti-lamin A/C antibodies for both loading and lamin A/C extinction controls. B, subconfluent HaCaT cells transfected with shRNA-Id2A, shRNA-Id2B, shRNA-Id2C, or shRNA-lamin A/C constructs were seeded in a 96-well plate. Cell number and viability were monitored sequentially at 24, 48, and 72 h in a proliferative assay monitoring ATP content in the cells. Cell number is proportional to the level of ATP expressed in fluorescent units (RLUs). When cell growth was statistically different (p < 0.01) from the control, an asterisk was placed above the bar. C, subconfluent HaCaT cells were transiently transfected with the constructs shRNA-Id2A and shRNA-Id2C, and cellular phenotype was analyzed the next day. Transfected HaCaT cells were incubated with Hoechst 33342 dye (blue cells) and propidium iodide (red cells) to detect cell death. Cell staining was analyzed immediately. Cell mortality was counted and expressed as a percentage. Cells exposed only to the transfection reagent exhibited a mortality of 2.4%. D, 1 day post-transfection, HaCaT cells were fixed, and DNA content was stained with propidium iodide to determine the cellular cell cycle repartition of cells expressing Id2 (shRNA-Id2C) or lacking Id2 (shRNA-Id2A). The different populations sorted (G2, S, and G2-M) are expressed as percentages of the total cell population. Cell cycle analysis of non-transfected proliferating HaCaT cells (NT) is also represented to demonstrate the effect of transfection. Experiments were performed several times, and the most representative one is presented.
Although control of human HaCaT keratinocyte growth, we investigated whether the induction of Id2 was merely a consequence of its up-regulation by transcription factors such as c-myc (5) or whether Id2 induction had a physiological effect in irradiated cells, we knocked down Id2 in human keratinocytes and analyzed their response to irradiation. We blocked Id2 expression by RNA interference in HaCaT cells. We generated three different shRNAs, specific to three regions of the Id2 mRNA sequence, and monitored their gene extinction abilities. As observed in Fig. 2A, the shRNA-Id2A construct was the most effective in silencing Id2 expression, shRNA-Id2B induced only a slight diminution, whereas the plasmid shRNA-Id2C had no effect. This shRNA-Id2C construct was then used as a negative control in subsequent experiments. Expression of lamin A/C was also analyzed to confirm the specificity of the Id2 silencing we observed (Fig. 2A). Delivery of these shRNAs in HaCaT cells in transient transfection experiments resulted in a decrease in cell growth directly proportional to the degree of Id2 extinction (Fig. 2B). Cell growth was diminished by half in shRNA-Id2A-expressing cells. Kinetic experiments highlight a decrease of cell proliferation in HaCaT cells expressing low levels of Id2 when transfected with shRNA-Id2A and weak growth in HaCaT cells expressing shRNA-Id2B in comparison with shRNA-Id2C and shRNA-lamin A/C (Fig. 2B). These results suggest that Id2 is either essential to maintain cell survival or implicated in cell cycle control. To determine the potential origin of reduced growth in cells lacking Id2, we analyzed cell death and the cell cycle. Staining of dying cells was performed in HaCaT cells expressing the different shRNA constructs. HaCaT cells no longer expressing Id2 showed no particular increase in cell death 24 h post-transfection, as demonstrated by propidium iodide staining and quantification of dead cells (Fig. 2C). Therefore, we investigated the cell cycle distribution of transfected cell populations. The experiment showed a major block (>80% of cells) in the G0–G1 phase of the cell cycle when Id2 is knocked down (Fig. 2D). These results indicate that Id2 protein is required for HaCaT cell growth and is a key regulator of human keratinocyte cell cycle progression at the G1-S transition.

Absence of Id2 Impairs Recovery of Human Keratinocytes Exposed to Ionizing Radiation—Because Id2 is implicated in the control of human HaCaT keratinocyte growth, we investigated the importance of Id2 expression following exposure to γ-rays. HaCaT cells expressing shRNA-Id2A or shRNA-Id2C as a control were exposed to 2 and 10 Gy, and cell number was monitored for 8 days. Counting experiments revealed that the lack of Id2 was deleterious when HaCaT were exposed to γ-irradiation. Indeed, cells lacking Id2 grew much slower after 2 and 10 Gy of irradiation compared with control-irradiated cells (Fig. 3A). After 8 days, the interfering RNA was no longer efficient, and non-irradiated cells were able to proliferate. These cells finally reached a cell number equal to that of cells expressing Id2. This further confirmed that the absence of Id2 induced cell cycle arrest, but not cell death. However, irradiated cells were unable to recover from radiative stress and grew normally in the absence of Id2, even after 8 days. Analysis of cells expressing shRNA-Id2A and shRNA-Id2C 4 days after a range of irradiation doses showed that the deleterious effect of the absence of Id2 is proportional to the delivered dose (Fig. 3B) and to the fraction of cells blocked in G0–G1 or G2–M of the cell cycle (Fig. 4). These data suggest that Id2 expression could be important to permit the exit from the cell cycle pause induced by γ-irradiation.

Id2 Overexpression Reverses Irradiation-induced Cell Cycle Arrest—Given that Id2 seems to be implicated in the recovery of cells that have been exposed to ionizing radiation, we next tested the impact of Id2 overexpression on cell growth in response to γ irradiation. HaCaT cells were transfected with a pRC-Id2 construct leading to overexpression of Id2 in human keratinocytes or with the empty vector, pRC, as control (Fig. 5). Cell growth experiments show that Id2 overexpression stimulates the growth rate of HaCaT human keratinocytes. We then tested the effects of ionizing radiation on these cells. A prolif-
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Fig. 5. Effect of constitutive Id2 expression on HaCaT human keratinocyte proliferation. Representative growth curve of HaCaT cells transfected with either the expression vector pRcRSV encoding Id2 protein (pRc-Id2) or the empty vector (pRc). The total number of viable cells was determined 2, 4, 6, and 8 days post-transfection by trypan blue dye exclusion. Each point represents the mean ± S.D. of triplicate samples. Inset, an immunoblot analysis of Id2 expression 48 h post-transfection shows endogenous and Id2 protein level in HaCaT cells transfected with pRc and pRc-Id2. The Id2 protein-specific signal is indicated by an arrow.

Fig. 6. Analysis of radioresistance in HaCaT cells overexpressing Id2. Human keratinocyte HaCaT cells transfected with either pRc or pRc-Id2 for 24 h were plated in a 96-well plate and exposed the next day to 0, 2, 6, and 10 Gy of irradiation. To monitor cell proliferation after exposure to ionizing radiation, the number of viable cells was analyzed after 1 and 5 days using the ATP monitoring test Vialight™.

To further investigate the mechanism involved, we monitored G1 arrest induced by ionizing radiation in human keratinocytes. To our knowledge, this is the first report of such induction of Id2 in response to ionizing radiation. This induction is not part of the immediate response to irradiation because Id2 expression decreased slightly at 3 and 6 h after irradiation. We had to wait until 18 h and up to 24 h after 2 Gy of irradiation to measure a 2.5-fold induction of Id2 transcripts. This pattern of expression in response to irradiation is consistent with expression of the Id2 gene that has been described following, for instance, mitogenic stimulation. In these studies, Id2 expression is rapidly induced (within 1–2 h) as part of early response genes, followed by a decline in expression sustained throughout the G1 phase of the cell cycle and further up-regulation as cells enter S phase (13, 14).

This induction of Id2 could be a direct consequence of the earlier induction in response to γ-irradiation of genes such as c-myc. Indeed, myc is induced in response to γ-irradiation in numerous cells (15–17) and is known to promote Id2 expression (5). It has been demonstrated that Id2 is a direct target of Myc, and there is good correlation between the expression of N-Myc and Id2 in neuroblastoma-derived cells (5). This hypothesis is supported in our model by the observation that Id2 follows the pattern of c-myc expression in response to γ-rays.

DISCUSSION

A moderate but reproducible induction of the Id2 gene and a similar accumulation of Id2 proteins were observed in human keratinocytes in response to ionizing radiation. To our knowledge, this is the first report of such induction of Id2 in response to γ-irradiation. This induction is not part of the immediate response to irradiation because Id2 expression decreased slightly at 3 and 6 h after irradiation. We had to wait until 18 h and up to 24 h after 2 Gy of irradiation to measure a 2.5-fold induction of Id2 transcripts. This pattern of expression in response to irradiation is consistent with expression of the Id2 gene that has been described following, for instance, mitogenic stimulation. In these studies, Id2 expression is rapidly induced (within 1–2 h) as part of early response genes, followed by a decline in expression sustained throughout the G1 phase of the cell cycle and further up-regulation as cells enter S phase (13, 14).

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REFERENCES
1. Yokota, Y., and Mori, S. (2002) J. Cell. Physiol. 190, 21–28
2. Norton, J. D. (2000) J. Cell Sci. 113, Pt 22, 3897–3905
3. Massari, M. E., and Murre, C. (2000) Mol. Cell. Biol. 20, 429–440
4. Benezra, R., Davis, R. L., Lockshon, D., Turner, D. L., and Weintraub, H. (1990) Cell 61, 49–59
5. Lasorella, A., Noeda, M., Beyna, M., Yokota, Y., and Iavarone, A. (2000) Nature 407, 592–598
6. Song, H. J., Cho, C. K., Yoo, S. Y., Park, K. S., and Lee, Y. S. (1996) Int. J. Radiat. Oncol. Biol. Phys. 41, 897–904
7. Liu, K., Kasper, M., and Trotz, K. R. (1996) Int. J. Radiat. Biol. 69, 763–769
8. Schmidt-Ullrich, R. K., Mikkelsen, R. B., Dent, P., Todd, D. G., Valerie, K., Kavanagh, J., Horner, W. K., and Chen, P. R. (1997) Oncogene 15, 1191–1197
9. Boukamp, P., Petrussevska, R. T., Breitkreutz, D., Hornung, J., Markham, A., and Fusenig, N. E. (1988) J. Cell Biol. 106, 761–771
10. Lemaitre, G., Lamartine, J., Pitaval, A., Vaigot, P., Garin, J., Bouet, S., Petat, C., Soulard, P., Gidrol, X., Martin, M. T., and Waksman, G. (2004) J. Cell. Biochem. 93, 1048–1062
11. Piffi, M. W. (2003) Nucleic Acids Res. 29, e45
12. Yu, J. Y., DeRuiter, S. L., and Turner, D. L. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 6047–6052
13. Barone, M. V., Pepperkok, R., Pevecali, F. A., and Philipson, L. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 4985–4988
14. Hara, K., Yamaguchi, T., Nojima, H., Ide, T., Campisi, J., Okayama, H., and Oda, K. (1994) J. Biol. Chem. 269, 2139–2145
15. Shung, B., Miyakoshi, J., and Takebe, H. (1994) Mutat. Res. 307, 43–51
16. Prasad, A. V., Mohan, N., Chandrasekar, B., and Meltz, M. L. (1995) Radiat. Res. 143, 263–272
17. Sullivan, N. F., and Willis, A. E. (1989) Oncogene 4, 1497–1502
18. Hammond, S. M., Caudy, A. A., and Hannon, G. J. (2001) Nat. Rev. Genet. 2, 110–119
19. Elbashir, S. M., Harborth, J., Lendeckel, W., Yalcin, A., Weber, K., and Tuschl, T. (2001) Nature 411, 494–498
20. Lasorella, A., Iavarone, A., and Israel, M. A. (1996) Mol. Cell. Biol. 16, 2570–2578
21. Mori, S., Nishikawa, S. I., and Yokota, Y. (2000) EMBO J. 19, 5772–5781
22. Platt, P. M., Price, J. O., Shaw, A., and Pietempsel, J. A. (1998) Cell Growth Differ. 9, 535–543
23. Felscher, D. W., and Bishop, J. M. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 3940–3944
24. Karlsson, A., Deb-Basu, D., Cherry, A., Turner, S., Ford, J., and Felscher, D. W. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 9974–9979