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Transformation of hematopoietic cells by the Ski oncoprotein involves repression of retinoic acid receptor signaling

RICHARD DAHL*†, MATTHIAS KIESLINGER†‡, HARTMUT BEUG‡, AND MICHAEL J. HAYMAN*§

*Department of Molecular Genetics and Microbiology and Institute of Cell and Developmental Biology, State University of New York, Stony Brook, NY 11794-5222; and ‡Institute for Molecular Pathology, Dr. Bohr-gasse 7, A-1030 Vienna, Austria

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ABSTRACT **The Ski oncogene has dramatic effects on the differentiation of several different cell types. It induces the differentiation of quail embryo cells into myoblasts and arrests the differentiation of chicken hematopoietic cells. The mechanism that Ski uses to carry out these disparate biological activities is unknown. However, we were struck by the similarity of these effects to those of certain members of the nuclear hormone receptor family. Both Ski and the thyroid hormone receptor-derived oncogene v-ErbA can arrest the differentiation of avian erythroblasts, and v-Ski-transformed avian multipotent progenitor cells resemble murine hematopoietic cells that express a dominant-negative form of the retinoic acid receptor, RARα. In this paper, we have tested the hypothesis that v-Ski and its cellular homologue c-Ski exert their effects by interfering with nuclear hormone receptor-induced transcription. We demonstrate that Ski associates with the RAR complex and can repress transcription from a retinoic acid response element. The physiological significance of this finding is demonstrated by the ability of high concentrations of a RARα-specific ligand to abolish v-Ski-induced transformation of the multipotent progenitors. These results strongly suggest that the ability of Ski to alter cell differentiation is caused in part by the modulation of RAR signaling pathways.**

The v-Ski oncogene was originally identified as the transforming protein of the SKV avian retroviruses; these retroviruses were isolated by their ability to transform avian fibroblasts *in vitro* (1). The cellular homologue c-Ski has been identified from several species, including human, chicken, and *Xenopus* (2–4); this homologue is the founding member of a family of proteins that includes itself and the Ski-related protein Sno (5). v-Ski is truncated at the amino and carboxyl termini compared with the cellular protooncogene c-Ski. When overexpressed, c-Ski, v-Ski, and Sno all have the ability to transform chicken embryo fibroblasts *in vitro*.

For an oncogene, Ski has the unusual property of being able to affect cellular proliferation and also initiate differentiation. Quail embryo cells infected with retroviruses expressing v-Ski or c-Ski differentiate into myoblasts capable of forming myotubes in culture (6, 7), and transgenic mice overexpressing a truncated c-Ski protein display enlarged skeletal muscle mass compared with their normal littermates (8). Most recently, c-Ski has been shown to induce the differentiation of a secondary neural axis when its mRNA is injected into *Xenopus* embryos (9). However, Ski also has been shown to block the differentiation of avian erythroid cells and can transform avian fibroblasts *in vitro* (10, 11). Little is known about the pathways that Ski modulates to carry out its disparate activities. Ski localizes to the nucleus and is observed to associate with chromatin (3, 12). Because of its nuclear localization and its ability to induce the expression of muscle-specific genes in quail cells (6), Ski has been assumed to be a transcription factor. Consistent with this assumption, c-Ski has been shown to bind DNA *in vitro* with the help of an unknown protein factor (13) and has been shown in muscle cells to enhance transactivation of reporter genes linked either to the myosin light-chain enhancer (14) or to a promoter/enhancer element from the myogenin gene (15). Recently, Ski has been detected in association with the transcription factor nuclear factor 1 [also known as CCAAT-binding transcription factor 1 (CTF1)] and has been observed to potentiate the transactivation activity of nuclear factor 1 (16).

We have shown previously that v-Ski can affect the growth of avian hematopoietic cells. v-Ski can induce the proliferation of a stem cell factor (kit ligand)-dependent myeloid-erythroid multipotential progenitor cell from avian bone marrow (BM) (17), and these multipotential cells spontaneously differentiate along the erythroid, monocytic, and granulocytic lineages. Interestingly, a dominant-negative retinoic acid receptor (RAR) generated a similar multipotent cell phenotype in mouse BM cultures (18). v-*Ski* can also functionally replace a RAR-related protein, the nuclear hormone receptor oncogene v-*erbA*, in cooperation with tyrosine kinase oncoproteins to transform erythroid progenitor cells (10). Interestingly, v-ErbA has also been shown to induce the differentiation of myoblast cells into myotubes, an ability that is similar to the muscle-inducing activity of Ski (19).

Because some of the activities of Ski are similar to those of dominant-negative nuclear hormone receptors, we have tested whether Ski functions by modulating the activities of the RAR. In this study, Ski is shown to repress the transactivation activity from a retinoic acid response element (RARE). By using electrophoretic mobility shift assays (EMSAs) and *in vitro*-binding assays, Ski is shown to interact with the RAR complex through binding to RARα. In addition, high concentrations of RARα-specific ligands can revert the transformation of multipotent cells by Ski.

MATERIALS AND METHODS

Cells and Cell Culture. Ski-transformed multipotential BM cells were grown in colony-forming unit–erythroid (CFU-E) medium plus 100 ng/ml of avian stem cell factor, 5×10^{-7} M estradiol, and 40 ng/ml of human insulin-like growth factor 1 (IGF-1) as described (17). The preparation and testing of retinoid-free sera has been described elsewhere (Bauer *et al.*, unpublished observations). Briefly, 2 g of anion exchange resin AG 1-X8 (Bio-Rad) and 0.8 g of activated charcoal (Norit A,

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Abbreviations: RAR, retinoic acid receptor; RXR, retinoid X receptor; RARE, retinoic acid response element; BM, bone marrow; GST, glutathione *S*-transferase; EMSA, electrophoretic mobility shift assay; TK, thymidine kinase; β-gal, β-galactosidase; GAS, γ-interferon-activated site.

†R.D. and M.K. contributed equally to this work.

§To whom reprint requests should be addressed.

Serva) were mixed, washed two times with sterile distilled water, and added to 45 ml of serum; the mixture was subsequently shaken for 6 h at room temperature. The resin/charcoal mixture was removed by centrifugation, and the serum was treated again overnight with fresh resin/charcoal mixture. Thereafter, the resin/charcoal mixture was removed by centrifugation, and the supernatant was cleared and sterilized by filtration.

Differentiation Induction of Ski BM Cells by Retinoids. Cells were seeded at 2×10^6 cells/ml in CFU-E medium containing retinoid-free fetal calf and chicken serum supplemented with 2% retinoid-free high-titer anemic serum. RARα-specific ligand (RO40-6973) plus RXR-specific ligand (RO25-7386) were added at concentrations between 10^{-7} and 10^{-12} M. The RARα-specific antagonist RO41-5253 was used at 10^{-7} M in combination with 10^{-7} M of the RXR-specific agonist (RO25-7386). Cells were counted daily by using an electronic cell counter (Casy-1, Schaerfe Systems, Reuhingen, Germany) that was maintained at densities between 3 and 6×10^6 cells/ml, and cumulative cell numbers were calculated (17). After 3 and 5 days, differentiation was assessed by cytocentrifugation of cell aliquots onto slides and by histological staining. Images were taken with a charge-coupled device (CCD) camera (Photometrics, Tucson, AZ); a green filter (576 nm) was used to increase contrast. Images were processed with Adobe Photoshop (Adobe Systems, Mountain View, CA). In addition, cell aliquots were stained with acid benzidine, and the number of benzidine-positive cells was determined. More than 300 cells were evaluated from each sample.

***In Vitro* Transcription and Translation.** For *in vitro* transcription, plasmids were linearized with the appropriate restriction enzymes. A linearized template was used in an *in vitro* transcription reaction employing the Riboprobe Combination System (Promega) with T7 polymerase according to the manufacturer's instructions. RNA was *in vitro*-translated by using the nuclease-treated rabbit reticulocyte lysate system (Promega) in the presence of Tran^{35}S-label (ICN) according to the manufacturer's instructions.

Glutathione *S*-Transferase (GST) Pull-Down Assay. For use in an *in vitro* translation reaction, RNA was *in vitro*-translated from either pCMX-RARα (20) or pCMX-RXRα (21), both of which were linearized with *Nhe*I. A total of 50 μl of *in vitro*-translated protein was diluted into 250 μl of incubation buffer [20 mM Hepes (pH 7.6), 200 mM NaCl, 100 μM $ZnCl_2$, and 1 mM DTT].

GST fusion proteins were expressed in *Escherichia coli* (DH5) and purified as described (22, 23). For each binding reaction, $\approx$5 μg of GST fusion protein bound to an equivalent amount of glutathione-Sepharose (Pharmacia) was incubated with equal amounts of *in vitro*-translated protein for 2 h at 4°C. Glutathione-agarose/Sepharose-bound proteins were washed extensively in PBS containing 0.5% Triton X-100 or with incubation buffer for *in vitro*-translation products. Proteins were eluted in SDS sample buffer, separated by SDS/PAGE gel, and either transferred onto a nitrocellulose membrane or fixed in 10% acetic acid and 20% methanol for 30 min; the proteins were subsequently incubated in Amplify (Amersham) for 30 min.

Transcriptional Reporter Assays. All transfections were performed by calcium phosphate precipitation (24). QT6 cells were cotransfected with 1 μg of reporter plasmid [DR5–thymidine kinase (TK)–luciferase], 0.5 μg of simian virus 40–β-galactosidase (β-gal), and 5 μg of pMT–v-Ski or pMT–c-Ski. Total plasmid DNA was brought up to 10 μg with the empty expression vector pMT2.

For luciferase assays in QT6 cells, transfection efficiency was normalized by the cotransfection of a simian virus 40–β-gal reporter plasmid. β-gal activity was quantified by using a β-gal assay kit (Promega) according to the manufacturer's instructions. Cell lysates (10–100 μg) were incubated with assay reagent for 15–30 min at 37°C. The absorbancies of the samples were measured at 420 nm.

Luciferase activity was quantified by using a Luciferase Assay kit (Promega) according to the manufacturer's protocol. Cells transiently transfected with reporter plasmid and effector plasmid(s) were harvested with reporter lysis buffer. Cell lysate (2–20 μl) was added to the luciferase reagent, and luminescence was quantified with an Optocomp I (MGM Instruments, Hamden, CT) luminometer. The luciferase reporter plasmids that were used to measure hormone responses were provided by D. D. Moore (Boston).

Electrophoretic Mobility Shift Assays (EMSAs). To determine the DNA-binding ability of RARα and/or complexes containing this receptor, a direct-repeat element with a spacing of five nucleotides (DR5) was used (RARE): 5′-CTTAGAGGTCACCGAAAGGTCAGCCCAG-3′. In control experiments, the γ-interferon-activated site (GAS)-related element IFP-53 was used that preferentially binds STAT5: 5′-GATCAATCACCCAGATTCTCAGAAACACTT-3′. The double-stranded oligonucleotides were ^{32}P-labeled by filling in the ends with Klenow polymerase and ^{32}P-radiolabeled deoxynucleotides. Whole-cell extracts from v-Ski BM cultures were prepared as described (25). The binding reaction was performed in a buffer containing 20 mM Hepes (pH 7.9), 40 mM KCl, 1 mM $MgCl_2$, 0.1 mM EGTA, 0.5 mM DTT, 10% (vol/vol) glycerol, and poly(dI-dC) (250 ng/ml) to which 0.5 ng of ^{32}P-labeled oligonucleotide was added. The protein–DNA complexes that formed after 30 min were analyzed on a 6% acrylamide gel at 4°C by using 0.2× Tris-borate-EDTA as running buffer. For supershift experiments, an anti-gag mAb [1G10 or ascites (26)] or a STAT5 control rabbit antiserum (27) and commercially available antibodies to human RARα and RXRα (Santa Cruz Biotechnology; #sc-551 and #sc-774) were added at the amounts indicated.

RESULTS

v-Ski and c-Ski Can Repress Transcription from RARE. v-Ski and c-Ski were analyzed for their ability to affect the transcription regulated by a RARE. RARs activated by the ligand all-*trans* retinoic acid can recognize direct repeats of the consensus DNA sequence AGTTCA separated by two or five nucleotides (DR2 or DR5 response elements) (28). To measure the responsiveness to all-*trans* retinoic acid, a reporter plasmid containing a single copy of the DR5 element upstream of the luciferase gene linked to a minimal TK promoter (DR5–TK–luciferase) was used. The QT6 cell line was cotransfected with this reporter plasmid and either the v-Ski or the c-Ski expression plasmid. Transfection efficiencies were normalized by measuring the β-gal activity generated from a cotransfected simian virus 40–β-gal plasmid; the average of values obtained from three transfections is shown. Both v-Ski and c-Ski could repress transcription in the presence and absence of exogenously added hormone (Fig. 1). We assume that the transcription observed in the absence of added hormone reflects the presence of ligand in the serum used to grow the cells. Ski had no effect in cotransfection assays using the same reporter plasmid in which the DR5 element was replaced with two copies of an estrogen response element. c-Ski was also able to repress transcription from a reporter construct that contained a single copy of the DR2 RARE in place of the DR5 element and from an E1A promoter-linked chloramphenicol acetyltransferase reporter gene downstream of the RARE from the RARβ gene promoter (data not shown).

v-Ski Associates with the DR5 RARE. These results suggest that Ski can function as a repressor of RAR transactivation. To investigate the nature of this repression, the ability of Ski to associate with a RARE was tested by EMSA. Extracts from

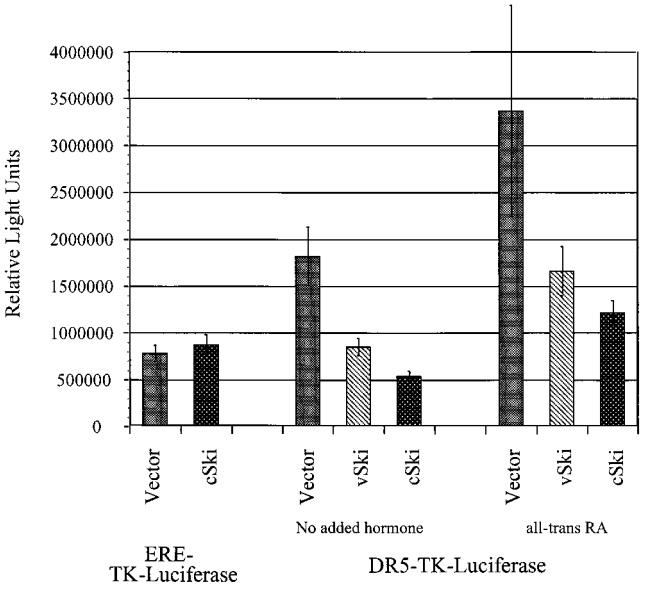

FIG. 1. Inhibition of retinoic acid-induced transcription by Ski in QT6 cells. QT6 cells were transiently transfected with estrogen response element (ERE)–TK–luciferase or DR5–TK–luciferase by using 0 μg or 5 μg of v-Ski or c-Ski expression plasmids as indicated. Cells were treated or mock-treated with 1 μM of all-*trans*-retinoic acid (Sigma) for 30 h as indicated. Extracts were prepared at 48 h posttransfection. The luciferase activity is shown as the relative light units that were obtained from each sample and has been normalized for transfection efficiency as described. Relative light units are an average of the values obtained from three independent transfections. Error bars represent the SD from the mean.

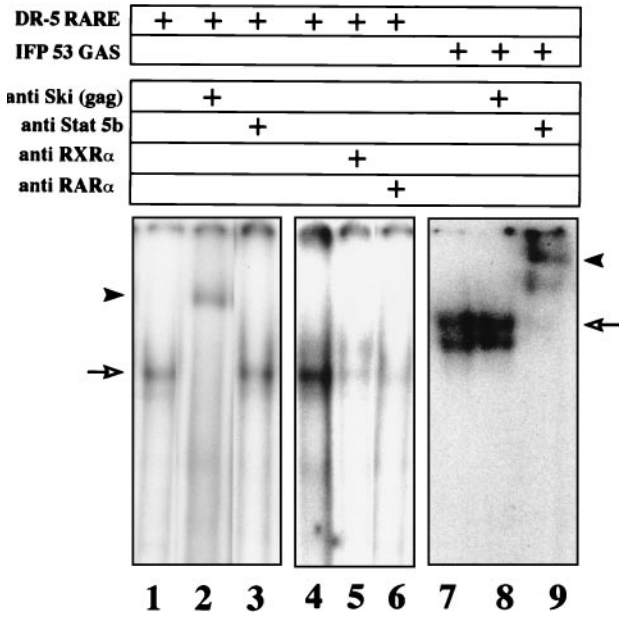

FIG. 2. Ski is localized in a protein complex binding to a DR5 RARE. Cell extracts prepared from Ski-transformed BM cells were analyzed by EMSA by using oligonucleotides that contained a DR5 RARE (lanes 1–6) or an IFP-53 GAS element (lanes 7–9). The DR5 RARE and IFP-53 GAS complexes were incubated with 0.5 μl of an anti-gag mAb [(anti-gag-Ski); MC IG-10 ascites, prediluted to 1/10, lanes 2 and 8], 1 μl of a rabbit antiserum that was reactive with chicken STAT5b (anti-STAT5b, prediluted to 1/10, lanes 3 and 9), and 2 μl of antibodies to RARα and RXRα (lanes 5 and 6) as described. The arrows indicate the positions of the mobility-shifted complexes; the arrowheads indicate the positions of the DR5 RARE (*Left*) and IFP-53 GAS (*Right*) complexes that were supershifted by the anti-gag-Ski antibody and anti-STAT5 antibody, respectively.

v-Ski-transformed hematopoietic cells were tested for the presence of RARE-binding activity by using a DR5 element. As shown in Fig. 2, lanes 1 and 4, the RARE DNA-binding activity that was inhibited by the addition of antibodies to either RXRα (lane 5) or RARα (lane 6) could be identified in these extracts. The v-Ski gene product is a fusion of gag and Ski sequences; consequently, an antibody specific to the gag portion of the viral Ski protein was included in the binding reaction to test whether Ski was present in this DNA complex. As shown in lane 2, the addition of this anti-gag antibody supershifted the complex that had formed on the DR5 element. The anti-gag antibody had no effect on the STAT–DNA binding complex that formed on an IFP-53 GAS element (lane 4). In contrast, an antibody to the transcription factor STAT5 that was unable to supershift the DR5-binding activity (lane 3) was able to supershift a complex formed on an IFP-53 GAS element (lane 9). These data indicate that v-Ski is present in the DNA-bound RAR complex.

Ski Binds to RARα *in Vitro*. Recently, several nuclear hormone receptor corepressors have been identified (SMRT, N-CoR) that bind directly to thyroid hormone receptor and RAR in the absence of hormone and are displaced upon ligand binding (29, 30). By using a GST–c-Ski fusion protein in a pull-down assay, the ability of Ski to bind to *in vitro*-translated RARα and RXRα was tested in the presence and absence of the respective hormones of these receptors. Approximately equal amounts of RARα were pulled down by the Ski fusion protein in the presence or absence of added hormone (Fig. 3). In contrast, no RXRα was detected that was bound to c-Ski, regardless of the presence or absence of hormone (Fig. 3) and even following longer exposures of the gel (data not shown). GST protein alone did not bind to either receptor (Fig. 3). GST–v-Ski was also shown to interact with RARα (data not shown). In this assay, GST–c-Ski was able to bind to RARα but not to RXRα, regardless of the addition of exogenous hormone. However, it is possible that there is sufficient endogenous hormone present in the *in vitro* translation reaction to maintain the RARα in a ligand-bound conformation. Although one cannot definitively conclude that Ski can bind to the receptor in the absence of hormone, these results do suggest that Ski differs from the corepressors SMRT and N-CoR in that it can bind to RAR in the presence of hormone.

Reversal of Ski Transformation by Retinoids. In v-ErbA-transformed cells, activation of the endogenous thyroid hormone receptor α/c-ErbA or RARα by their respective ligands partially overcame the v-ErbA-induced differentiation arrest (26). Similarly, the acute promyelocytic leukemia caused by the t(15;17) chromosomal translocation generating a PML–RARα fusion protein can be successfully treated by retinoic acid (31, 32). As shown in Fig. 1, the addition of retinoic acid could increase transcription even in the presence of Ski, although not to the levels that were induced in the absence of Ski. This finding indicated that Ski repression can be overcome to some extent by the addition of a RARα agonist. Therefore, if Ski transformation involves a repression of RARα transcription, then the above precedents indicate that it should be possible to overcome the transformation of Ski-transformed multipotential progenitors by inducing their differentiation with RARα ligands. For these experiments, Ski-transformed BM cells were grown in medium containing T3/retinoid-depleted calf sera supplemented with retinoid-free anemic chicken serum (see *Materials and Methods*). These conditions, which allow the exponential growth of Ski-transformed multipotential cells in retinoid-free medium, were used to test the effect of the RAR ligands. Pilot experiments demonstrated that the cells were most sensitive to equimolar mixtures of a RAR-specific agonist (RO40-6973) and a RXR-specific agonist (RO25-7386). Consequently, the cells were treated with these ligand mixtures at various concentrations and analyzed for

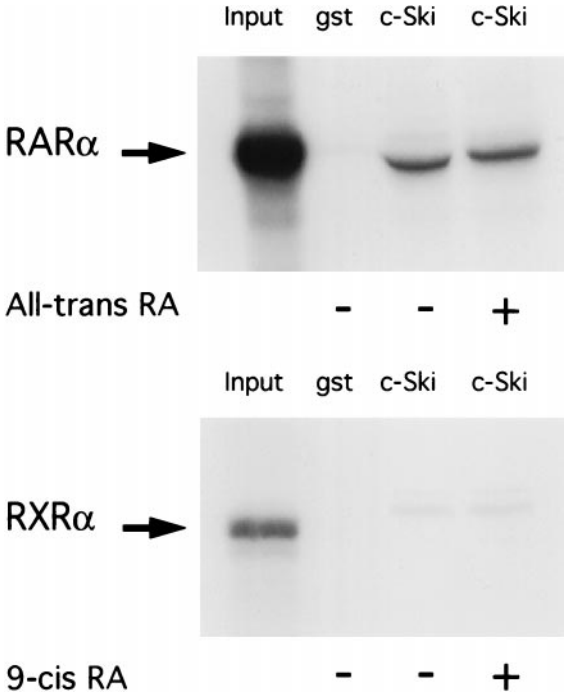

FIG. 3. *In vitro* binding of RARα and RXRα to c-Ski. *In vitro*-translated [^{35}S]methionine-labeled RARα or RXRα were incubated with either GST or a c-Ski–GST fusion protein that was bound to glutathione-Sepharose. Bound proteins were eluted and then separated by SDS/PAGE. The binding reactions were performed in the presence or absence of 10 μM of all-*trans* or 9-*cis* retinoic acid (Sigma) as indicated.

differentiation into the erythroid and myeloid lineages. It has been shown previously that differentiation involves a slow down in proliferation coupled with the appearance of mature erythroid cells and macrophages in the cultures. Therefore, differentiation was monitored by staining for hemoglobin by using benzidine and by cytocentrifugation to identify macrophages. Fig. 4*A* demonstrates that RAR/RXR agonists at 10^{-7} M and 10^{-8} M arrested proliferation after 4 days. An evaluation of the cytospins (Fig. 4*C*) showed that the cells had differentiated into both mature macrophages and erythroblast-like cells after 5 days, with essentially undetectable amounts of undifferentiated cells. Differentiation of the multipotent cells along the erythroid lineage was also evident from the observed increase in benzidine-positive cells (Fig. 4*B*). Lower concentrations of the agonists had less noticeable effects; for example, RAR/RXR ligands had only weak effects at 10^{-9} M, and concentrations of 10^{-10} M or lower were essentially inactive (Fig. 4 *A* and *B* and data not shown). Our observation that a RARα-specific antagonist (RO41-5253) had no detectable effects on the cells (Fig. 4 *B* and *C*) confirms that our retinoid-depleted sera was indeed free of biologically active RAR/retinoid X receptor (RXR) ligands. In conclusion, our results therefore indicate that Ski most likely cooperates with the RAR in its nonliganded configuration.

The concentrations of agonists required to induce differentiation of Ski-transformed cells are relatively high. If this requirement reflects a necessity to overcome a Ski-induced repression of retinoic acid signals, then one would predict that normal progenitors would be induced to differentiate by much lower concentrations of RAR/RXR agonists. To test this possibility, normal avian myeloid progenitors were seeded in retinoid-free medium supplemented with 10^{-7}–10^{-11} M RAR- and RXR-specific agonists. Concentrations of the above retinoids down to 10^{-10} M caused complete differentiation into macrophage-like cells within 3–4 days, and even at 10^{-11} M, a partial differentiation-inducing effect was observed (data not shown). This observation is in contrast to the 10^{-7} M and 10^{-8} M required to induce myeloid differentiation of Ski-transformed progenitors (Fig. 4). Thus, Ski-transformed progenitors are about 100-fold more resistant to retinoid-induced differentiation than are normal myeloid progenitors.

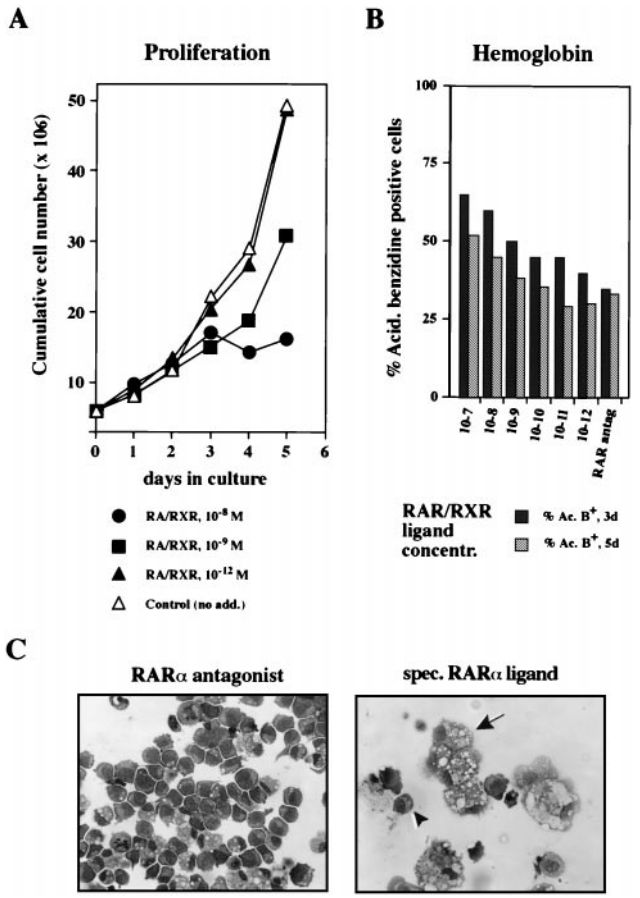

FIG. 4. Reversal of v-Ski transformation by retinoids. (*A*) Ski BM cells were cultivated in medium containing thyroid/retinoid-free serum plus retinoid-free anemic serum, stem cell factor, insulin-like growth factor 1, estradiol, and the indicated amounts of an equimolar mixture of RARα- and RXR-specific agonists. Cells were maintained at densities between 3 and 6 × 10^6 cells/ml by daily dilution or by the addition of fresh medium plus all factors; they were counted daily. The cumulative cell numbers as calculated according to ref. 17 are shown. (*B*) At 3 days (filled bars) or 5 days (hatched bars) after retinoid addition, aliquots of the cultures were stained with acid benzidine, and the number of benzidine-positive cells was determined (17). The values shown are the mean of four independent determinations. (*C*) Five days after retinoid additions, aliquots from the culture containing 10^{-7} M RARα antagonist plus 10^{-7} M RXR agonist (*Left*) or 10^{-8} M RARα agonist plus 10^{-8} M RXR agonist (*Right*) were cytocentrifuged onto slides, stained with neutral benzidine plus histological dyes (17), and photographed under green light to reveal histological details. Arrow, mature macrophage; arrowhead, benzidine-positive, partially mature erythroid cell.

DISCUSSION

Some of the biological activities of Ski resemble those of the dominant-negative versions of the nuclear hormone receptors of thyroid hormone receptor α/c-ErbA and RARα. Therefore, we thought that Ski may antagonize the transactivation activity of nuclear hormone receptors. Data were obtained to support this conclusion for RAR. A repression of reporter gene transcription that was dependent upon a RARE was observed in the presence of both c-Ski and v-Ski. To address the mechanism by which Ski repressed RAR-dependent trans-

activation, Ski was tested for its ability to associate with a RARE. An EMSA demonstrated that v-Ski from transformed avian multipotent hematopoietic cells was present in the complex that forms on a DR5 response element. To determine whether association with this complex was mediated by direct interaction with RARs, an *in vitro*-binding assay was employed to determine whether Ski could bind directly to RARα or to its heterodimeric partner RXRα. By using a GST–c-Ski fusion protein, binding was detected between Ski and *in vitro*-translated RARα but not between Ski and RXRα.

One mechanism for the actions of Ski would be that Ski acts autonomously as a repressor when tethered to promoter regions by interaction with RARα. This model would be consistent with recent data that demonstrated that Ski could repress transcription when tethered to a promoter through a heterologous DNA-binding domain (33). Alternatively, Ski may repress transcription by stabilizing RARα in its nonliganded conformation and consequently allow its interaction with corepressors even in the presence of physiological hormone levels. This idea is consistent with our finding that a RARα antagonist did not interfere with transformation by Ski, whereas high concentrations of RARα-specific ligand did induce terminal differentiation in Ski-transformed, multipotent BM cells. It is possible that high ligand levels force the RARα to switch to a ligand-activated conformation despite the presence of Ski. Recently, leukemic transformation by the chimeric oncogenes PML–RARα and PLZF–RARα has been postulated to be caused by the greater affinity of these oncogenes for the corepressors SMRT and N-CoR compared with wild-type RARα (34–36).

Although this work was initiated because of similarities between the phenotypes of the hematopoietic cells expressing Ski and the dominant-negative versions of RARα and thyroid hormone receptor (v-ErbA), the recent description of *ski*-deficient mice also suggests a connection between Ski and retinoic acid signaling (37). The major defects seen in the *ski* null mice are craniofacial defects and a failure of the neural tube to close, which results in the mice dying of exencephally. Craniofacial defects have been reported in the offspring of women who ingested synthetic retinoids during pregnancy (38), and retinoid treatment of the outbred mouse strain CD-1 results in offspring that suffer from craniofacial defects and die of exencephally (39). These observations suggest a model of Ski action in which the deletion of Ski results in an up-regulation of retinoid signaling that mimics exogenous retinoid treatment.

A repression of retinoid signaling may also play a role in the ability of Ski to influence muscle differentiation. Several studies have implicated retinoic acid in the differentiation of muscle tissue. Retinoic acid-induced muscle differentiation was observed in C2 myoblasts (40), rhabdomyosarcoma cells (41), and primary adult chicken satellite cells (42). Although it is currently not clear how nuclear receptors regulate muscle development, the data do show that nuclear hormone receptors are involved in myogenic differentiation and that perturbation of hormone signaling potentially may result in the phenotypes observed with Ski.

Our data suggest that Ski can transform hematopoietic cells by interfering with a pathway that has been shown to be disrupted by other leukemic oncogenes. The human acute promyelocytic leukemia oncogenes PML–RARα and PLZF–RARα and the avian erythroleukemia oncogene v-ErbA have been shown to repress hormone-induced transcription (31, 43–45). A nonnuclear hormone receptor oncogene *myb-ets* has also been demonstrated to repress thyroid hormone and retinoic acid signaling, and this activity is thought to be involved in the ability of this oncogene to induce avian myeloblastosis (46). The recognition that Ski potentially acts as an oncogene by disrupting hormone signaling has interesting implications for the study of leukemia and other neoplastic diseases. Although $\approx$100 oncogenic proteins have been identified, the transformation of a cell by oncogenes may involve the disruption of a much smaller number of cellular pathways. We would postulate that the nuclear hormone receptor pathway is a common target for disruption by several leukemic oncogenes. These observations have implications for the treatment of cancer. Novel therapeutic agents would not have to target the numerous different genetic changes that occur in malignant cells, but instead could target a finite number of growth and differentiation pathways that are disrupted in cancerous tissue. Further study promises to reveal the pathways that are exploited by oncogenes and thus identify the pathways that are crucial for therapeutic intervention.

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