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Keratinocyte Growth Factor Is an Important Endogenous Mediator of Hair Follicle Growth, Development, and Differentiation

Normalization of the nu/nu *Follicular Differentiation Defect and Amelioration of Chemotherapy-Induced Alopecia*

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The growth and development of hair follicles is influenced by a number of different growth factors and cytokines, particularly members of the fibroblast growth factor (FGF) family. Keratinocyte growth factor (KGF or FGF-7) is a recently identified 28-kd member of the FGF family that induces proliferation of a wide variety of epithelial cells, including keratinocytes within the epidermis and dermal adnexa. Because KGF induces marked proliferation of keratinocytes, and both KGF and KGF receptor (KGFR) mRNA are expressed at high levels in skin, we sought to localize KGF and KGFR in skin by **in situ** ***hybridization. KGFR mRNA was relatively strongly expressed by keratinocytes in the basilar epidermis as well as throughout developing hair follicles of rat embryos and neonates. KGF mRNA was expressed at lower levels than was KGFR but could be localized to follicular dermal papillae in rat embryos and neonates. These results prompted us to investigate the effects of KGF on hair follicles in two distinct murine models of alopecia. In the first model, recombinant KGF (rKGF) induced dose-dependent hair growth over most of the body in*** **nu/nu** ***athymic nude mice when administered intraperitoneally or subcutaneously over 17 to 18 days. When administered subcutaneously, rKGF induced the most extensive hair growth at the sites of injection. Histologically, rKGF induced marked follicular and sebaceous gland hypertrophy, a normalization of the*** **nu/nu** ***follicular keratinization defect, and an increase in follicular keratinocyte proliferation as assessed by bromodeoxyuridine labeling. In the second model, a neonatal rat model of cytosine arabinoside chemotherapy-induced alopecia in which interleukin-1, epidermal growth factor, and acidic FGF have all demonstrated some degree of alopecia cytoprotection, rKGF induced a dose-dependent cytoprotective effect, abrogating as much as 50% of the alopecia in this model when administered beginning 1 day before the onset of chemotherapy. Taken together, these data suggest that KGF is an important endogenous mediator of normal hair follicle growth, development, and differentiation. (Am J Pathol 1995, 147:145–154)***

The growth and development of hair follicles is influenced by a large number of different growth factors and cytokines, including epidermal growth factor (EGF),[1,2] transforming growth factor-α,[3–5] hepatocyte growth factor,[6] parathyroid hormone-related protein (PTHrP),[7] bone morphogenetic protein-4, interleukin

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1 (IL-1),[9,10] and several members of the fibroblast growth factor (FGF) family.[2,11–13] Among the best characterized and possibly most important endogenous mediators of hair follicle growth and development are the members of the FGF family. Acidic FGF (aFGF),[2,14] basic FGF (bFGF),[2,11,14] and FGF-5[12] and their receptors have all been localized within or immediately adjacent to hair follicles, whereas keratinocyte growth factor (KGF or FGF-7) and its receptor have been localized to the skin.[15,16] In addition, these four members of the FGF family have been found to influence normal follicle growth and/or development when administered exogenously (aFGF, bFGF, KGF),[2,11,17] as well as via targeted overexpression in transgenic mice (KGF),[13] via blockade of growth factor activity by targeted expression of a dominant-negative receptor mutant (KGF),[18] or via targeted deletion by homologous recombination (FGF-5).[12]

KGF is a recently identified 28-kd member of the FGF family (alternative designation FGF-7)[15] that specifically binds to the KGF receptor (KGFR), a splice variant of FGF receptor 2 (FGFR-2).[19] KGF has been identified as a paracrine mediator of proliferation in a wide variety of epithelial cells, including hepatocytes and gastrointestinal epithelial cells,[20] type II pneumocytes,[16,21] mammary epithelial cells,[22,23] and keratinocytes.[17,22,24] In the skin, KGF was found to stimulate not only epidermal keratinocytes but also keratinocytes within hair follicles and sebaceous glands.[17] High levels of KGF and KGFR mRNA were found in skin,[15,16] with KGF mRNA localized exclusively to the dermis and FGFR-2, of which KGFR is a splice variant, localized exclusively to the epidermis.[25] These findings suggested that KGF was an important endogenous paracrine mediator of epidermal growth and differentiation. This hypothesis was also supported by recent studies which demonstrated that transgenic mice overexpressing KGF in epidermal basal and follicular outer root sheath keratinocytes, as well as transgenic mice expressing a dominant-negative KGFR mutant in epidermal basal and follicular outer root sheath keratinocytes, had a marked reduction in numbers of hair follicles and exhibited abnormalities in follicular morphology.[13,18] These studies in transgenic mice further suggested that KGF was an important endogenous mediator of normal hair follicle growth and development.

To further investigate the hypothesis that KGF is an important endogenous mediator of hair follicle growth and development, we first sought to localize KGF and KGFR in the skin of rat embryos and neonates by *in situ* hybridization. Once we had determined that KGFR mRNA was expressed at high levels in hair follicles and that KGF was expressed in follicular dermal papillae, we then investigated the effects of exogenously administered KGF on two distinct murine models of alopecia: *nu/nu* athymic nude mice and a neonatal rat model of cytosine arabinoside chemotherapy-induced alopecia. The *nu/nu* mouse model was chosen because these mice have a genetically determined defect in follicular keratin differentiation that manifests as a follicular dystrophy and alopecia,[26] and rKGF stimulates not only keratinocyte proliferation but also up-regulates keratinocyte differentiation *in vitro*.[24] The neonatal rat model of cytosine arabinoside chemotherapy-induced alopecia was chosen because three growth factors/cytokines (EGF, aFGF, and IL-1) had previously been shown to have cytoprotective effects in this model.[27–29]

Materials and Methods

In Situ *Hybridization for KGF and KGFR*

Rat embryos from timed pregnant Sprague-Dawley rats were harvested at 17.5 and 20.5 days post-coitus after euthanasia of the dams. Rat neonates were harvested at 2 days of age. After harvest, rat embryos were immediately immersion fixed in 4% paraformaldehyde buffered with phosphate-buffered saline (PBS) overnight, followed by cryoprotection by immersion in 30% sucrose until the tissues sank. Tissues were embedded in OCT media (Miles Laboratories, Elkhart, IN) and then snap-frozen in isopentane chilled to its freezing point in liquid nitrogen. Frozen sections of embryos were cut at 9 μm on a Jung CM3000 cryostat (Leica, Deerfield, IL) and placed on clean positively charged glass slides (Superfrost Plus, Fisher Scientific, Pittsburgh, PA). Neonatal rats were paraffin embedded after overnight fixation in 4% buffered paraformaldehyde, cut at 5 μm, and also placed on clean positively charged glass slides as above (Superfrost Plus, Fisher Scientific).

KGF and KGFR *in situ* hybridization was performed with previously described riboprobes[16] labeled with [^{33}P]rUTP (New England Nuclear, Boston, MA). The KGFR antisense riboprobe was specific for bases 1270 to 1417 of the murine KGFR splice variant, which is identical in both mouse and rat (GenBank accession number M63503) and does not cross-hybridize with FGFR-2.[16] Sense strands of both probes were transcribed and used as negative controls. Sections were hybridized and washed according to the protocol of Yan et al.[30] After the slides dried, they were dipped in Kodak NTB-2 (Rochester, NY) emulsion, exposed for 21 days, and then developed and counterstained with hematoxylin and eosin (H&E).

Recombinant KGF

Unglycosylated recombinant human KGF (rKGF; 18.9 kd) was produced in *Escherichia coli*, refolded and purified to homogeneity by conventional chromatography techniques, and tested endotoxin free. rKGF was assayed in the BALB/MK keratinocyte line (American Type Culture Collection, Rockville, MD), and stimulated half-maximal proliferation at approximately 19 ng/ml.[17]

Animal Models of Alopecia

Athymic nude mice (*nu/nu*) on an outbred CD1 background were obtained from Charles River Laboratories (Wilmington, MA) and were housed and handled under aseptic conditions. Mice were injected intraperitoneally (ip) or subcutaneously (sc) with specified concentrations of rKGF in PBS or PBS vehicle control. On day 17 or 18, mice were evaluated for hair density and hair coverage by three independent observers blinded to the treatment groups who scored each mouse on a scale of 0 to 4 for hair density and percent of body covered by hair (0 = complete alopecia, to 4 = normal hair density and 100% hair coverage). The four treatment groups were statistically analyzed for differences in both hair density and percent hair coverage by the Kruskal-Wallis test of one-way nonparametric analysis of variance at a 5% significance level (Statview 4.02; Abacus Concepts, Berkeley, CA). Also at this time, gross photographs were taken and skin sections from each mouse were harvested and fixed in buffered zinc formalin (Anatech, Battle Creek, MI) for routine H&E-stained histology and bromodeoxyuridine (BrdU) immunohistochemistry.

Timed pregnant Sprague-Dawley rats were also obtained from Charles River Laboratories. Litters of rat pups were split such that each experimental group consisted of eight pups from at least three different litters. The 8-day-old pups were injected ip with either 50 µg/g body weight cyclophosphamide (Mead Johnson, Evansville, IN) once on experimental day 0 or 20 µg/g body weight/day cytosine arabinoside (ARA-C; Upjohn Co., Kalamazoo, MI) for 6 consecutive days from 8 days of age (experimental day 0) through 13 days of age (experimental day 5). Neonatal rats were also injected ip with specified concentrations of native sequence rKGF in PBS or PBS vehicle control from experimental day −1 through 11 or experimental day 0 through 11. Rats were scored for percent hair coverage (0 to 100% by increments of 10) by four independent observers blinded to the treatment groups on experimental days 6, 7, 8, 11, 13, 15, and 18. Mean scores were calculated for each rat pup daily. For each experimental day, hair coverage was evaluated, cyclophosphamide- or ARA-C-treated rats receiving rKGF were analyzed *versus* cyclophosphamide or ARA-C-treated rats receiving PBS vehicle control by one-way analysis of variance (two-tailed) coupled with the Bonferroni/Dunn *post hoc* test at a 5% significance level (Statview 4.02; Abacus Concepts). Results are reported as mean ± SEM.

Assessment of Keratinocyte Proliferation with Anti-BrdU

At 1 hour before sacrifice, each nude mouse was injected ip with BrdU (50 mg/kg body weight; Aldrich Chemical Co., Milwaukee, WI). BrdU was detected in zinc formalin-fixed, paraffin-embedded 3-µm histological sections by avidin-biotin complex immunoperoxidase staining with an anti-BrdU monoclonal antibody (MAb; Dako Corp., Carpinteria, CA) as previously described.[16] Sections were lightly counterstained with hematoxylin. Assessment of follicular keratinocyte and sebaceous gland epithelial cell BrdU labeling was done by an observer blinded to the treatment groups using the ×20 objective. Only anagen follicles that were in full longitudinal section and that contained at least 10 BrdU-positive keratinocytes were counted. For each section examined, 10 anagen follicles and 10 sebaceous glands were counted and three sections from different individuals were examined per treatment group. The Kruskal-Wallis test of one-way nonparametric analysis of variance at a 5% significance level on Statview 4.02 was used to compare the number of BrdU-labeled keratinocytes per hair follicle in rKGF-treated *versus* control skin sections. Results are reported as the mean BrdU-labeled keratinocytes per hair follicle ± SEM and mean BrdU-labeled epithelial cells per sebaceous gland ± SEM.

Results

Localization of KGF and KGFR in Hair Follicles by in Situ Hybridization

In situ hybridization with a riboprobe specific for the KGFR splice variant revealed that KGFR mRNA was strongly expressed by keratinocytes in the basilar epidermis and all or nearly all keratinocytes in developing hair follicles of 17.5- and 20.5-day rat embryos (not shown), as well as 2-day-old neonatal rats (Figure 1). Adult mouse skin showed a similar, but less intense, pattern of KGFR mRNA expression (not shown). KGF mRNA was less intensely expressed

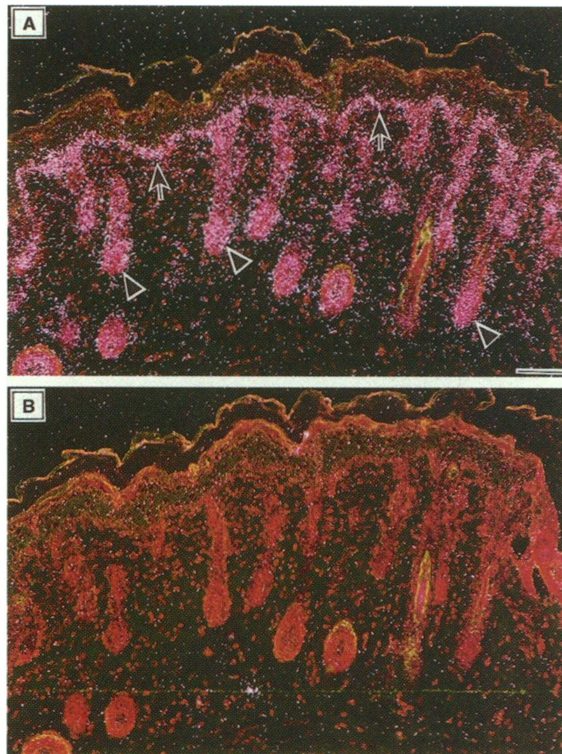

Figure 1. In situ *hybridization for KGFR in skin from a 2-day-old neonatal rat.* **A:** *Hybridized with an antisense riboprobe specific for the KGFR splice variant of FGFR-2, illustrating that KGFR mRNA is expressed by keratinocytes in the basilar epidermis (arrows) as well as keratinocytes in developing hair follicles, particularly within follicular bulbs (arrowheads).* **B:** *The sense strand control. Bar in* **A**, *100 μm.*

than was KGFR but was clearly localized within follicular dermal papillae, particularly in 2-day-old neonatal rats (Figure 2).

rKGF Stimulates Hair Growth in Athymic Nude Mice

When administered systemically (ip or sc) to athymic nude mice for 17 to 18 consecutive days, rKGF induced a significant dose-dependent increase in hair density ($P = 0.002$ by Kruskal-Wallis test) and a trend toward a dose-dependent increase in percent of body covered by hair ($P = 0.08$ by Kruskal-Wallis test; Figure 3). Hair growth was evident as early as 7 days after the onset of rKGF administration. When rKGF was administered at the same site sc, there was a marked increase in hair growth at the injection site in addition to the more generalized hair growth evident after ip administration (Figure 4). By 7 days after the termination of rKGF administration, all previously haired nude mice had lost their newly grown hair and resembled PBS-treated control nude mice (not shown).

The skin of rKGF-treated nude mice exhibited prominent follicular hypertrophy as well as slight to moderate sebaceous gland hypertrophy (Figure 5, A and B). In addition, while control nude mouse skin had numerous dystrophic follicles which were cystically dilated and contained fragmented hair shafts and keratinized debris, rKGF-treated nude mouse skin had relatively normal appearing follicles that contained well differentiated and unfragmented hair shafts which continued through the follicular ostia to the skin's surface (Figure 5, A and B). Examination of BrdU-labeled sections revealed that rKGF induced a moderate increase in BrdU-labeled follicular keratinocytes, particularly within follicular bulbs (Figure 5, C and D).

rKGF Simulates Proliferation of Follicular and Sebaceous Gland Keratinocytes

rKGF-treated nude mice exhibited a significant increase in the number of BrdU-labeled keratinocytes per anagen follicle at all tested doses *versus* PBS-treated control mice ($P = 0.0035$ by Kruskal-Wallis test; Table 1). In addition, rKGF-treated nude mice also exhibited a significant dose-dependent increase in the number of BrdU-labeled epithelial cells per sebaceous gland ($P = 0.0001$ by Kruskal-Wallis test; Table 1). This increase in follicular keratinocyte and sebaceous gland epithelial cell proliferation was very similar to the increased epithelial proliferation we had previously reported for follicles and sebaceous glands of rKGF-treated rabbit dermal excisional wounds.[17]

rKGF Partially Ameliorates Cytosine Arabinoside-Induced Alopecia in Neonatal Rats

When rKGF was administered 1 day before the onset of ARA-C chemotherapy, rKGF-treated neonatal rats exhibited a dose-dependent increase in the percentage of hair remaining after chemotherapy. Neonatal rats receiving the highest dose of rKGF tested, 5 μg/g body weight/day for 13 days (experimental days −1 to 11), exhibited a statistically significant increase in percentage of hair coverage after ARA-C chemotherapy *versus* PBS-treated controls at all time points through day 15, after which time all experimental groups began to exhibit similar hair regrowth regardless of specific treatment (Figures 6 and 7). Administration of rKGF concurrent with, but not before, ARA-C chemotherapy (experimental days 0 to 11)

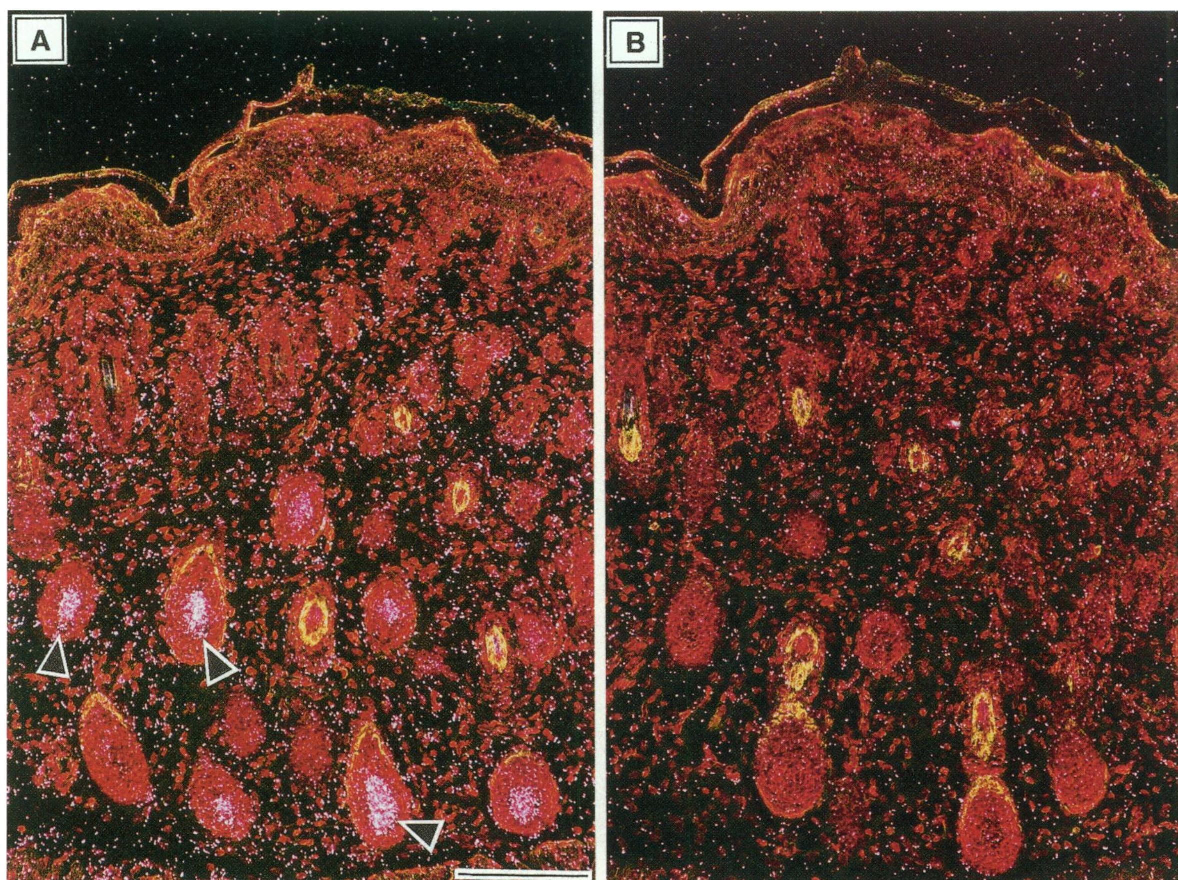

Figure 2. In situ *hybridization for KGF in skin from a 2-day-old neonatal rat.* **A**: *Hybridized with a KGF-specific antisense riboprobe, illustrating that KGF mRNA is clearly expressed within follicular dermal papillae (arrowheads).* **B**: *The sense strand control. Bar in* **A**, *50 μm.*

also increased the percentage of hair coverage *versus* PBS-treated controls, but not as markedly as with rKGF pretreatment (Figures 6 and 7).

In contrast to the partial protection rKGF administration demonstrated in ARA-C-induced alopecia, rKGF administration had no significant effect on the alopecia induced by cyclophosphamide chemotherapy (data not shown).

Discussion

In this study we have demonstrated that KGFR is strongly expressed in developing hair follicles and that KGF is expressed in follicular dermal papillae. We have also shown that exogenously administered rKGF stimulates hair growth in *nu/nu* athymic nude mice by stimulating follicular proliferation and inducing normalization of the *nu/nu* follicular keratin differentiation defect. Lastly, we have shown that exogenously administered rKGF partially protects against the alopecia induced by ARA-C chemotherapy in neonatal rats when administered before the onset of chemotherapy.

Although there are a significant number of growth factors and cytokines known to influence hair growth and development, most, including all members of the FGF family reported to have effects on hair follicles other than KGF (aFGF, bFGF, and FGF-5), inhibit hair growth and/or development.[3,7,8,11,12] There are currently only three growth factors or cytokines and one growth factor antagonist that have been reported to stimulate hair follicles, hepatocyte growth factor, IL-1, KGF, and a PTHrP antagonist. Hepatocyte growth factor has stimulated hair follicles in organ culture,[6] but its potential *in vivo* effects on hair follicles have not yet been described. IL-1 has been reported to both stimulate[9] and inhibit[10] hair follicle growth in organ culture, but its potential *in vivo* effects on hair follicles have also not yet been described. Lastly, a PTHrP antagonist has been reported to stimulate

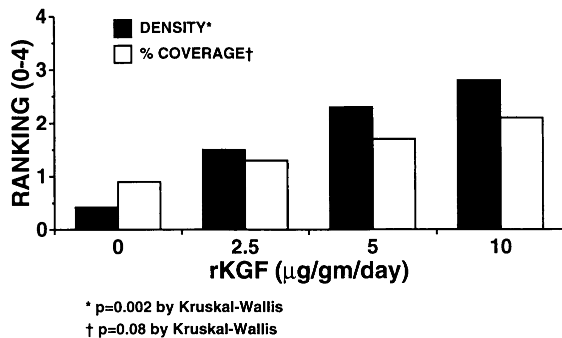

Figure 3. *Hair density and percentage of hair coverage in athymic nude mice treated sc for 17 consecutive days with rKGF* versus *vehicle control. Mice were evaluated for hair density and hair coverage by three independent observers blinded to the treatment groups who scored each mouse on a scale of 0 to 4 for hair density and percent of body covered by hair (0, complete alopecia, to 4, normal hair density and 100% hair coverage). The four treatment groups were statistically analyzed for differences in both hair density and percentage of hair coverage by the Kruskal-Wallis test of one-way nonparametric analysis of variance at a 5% significance level. rKGF induces a statistically significant dose-dependent increase in hair density* (P = *0.002) and a trend toward a dose-dependent increase in percent of body covered by hair* (P = *0.08).*

hair follicles both *in vitro* and *in vivo*,[31] although PTHrP itself inhibits hair growth.[7] Therefore, KGF is the only endogenous growth factor or cytokine that has been described to date to have a stimulatory effect on hair follicle growth and differentiation *in vivo*.

KGF is also the only growth factor or cytokine that has stimulated hair growth in athymic nude mice. The only other substance that has been reported to stimulate hair growth in nude mice is cyclosporin A (CyA),[32,33] an immunosuppressive fungal metabolite that is inhibitory to T lymphocytes.[34] The mechanism of CyA's action on hair follicles is unknown, but CyA, like KGF, stimulated follicular proliferation and normalized the *nu/nu* follicular keratinization defect,[31] raising the possibility that some of CyA's effects may have been mediated via up-regulation of endogenous KGF. In contrast to KGF and CyA, minoxidil, a potent vasodilator[35] that can stimulate hair regrowth in human androgenetic alopecia (male pattern baldness),[36] had no effect on the alopecia of nude mice.[33]

It has recently been reported that IL-1 up-regulates KGF mRNA expression,[37] suggesting that some or all of IL-1's reported stimulatory effects on hair follicles[9] may be mediated via up-regulation of endogenous KGF, although a contradictory report of IL-1's inhibitory effects on hair follicles[10] casts some doubt on this hypothesis. However, as IL-1 is one of the growth factors/cytokines that have a cytoprotective effect in ARA-C-induced alopecia,[27,28] it is possible that KGF may play a role in the mechanism of IL-1 induced cytoprotection in this model. In addition to IL-1, the only other growth factors or cytokines reported to have cytoprotective effects in the ARA-C-induced alopecia model are EGF and aFGF,[29] both of which inhibit hair growth and development when administered to normal animals,[2,11] suggesting that their mechanism of action in the chemotherapy-induced alopecia model must differ from that of KGF.

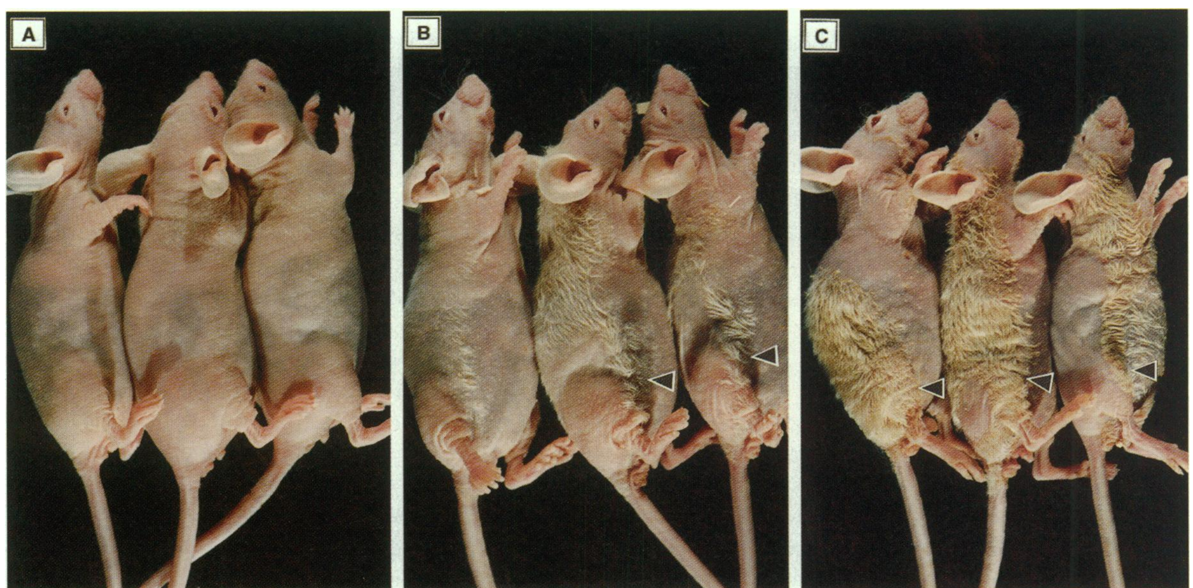

Figure 4. *Athymic nude mice injected sc in the right flank (arrowheads in* B *and* C *illustrate injection sites) with rKGF or vehicle control for 17 consecutive days.* A: *Vehicle control mice.* B: *Mice injected with 5 µg of rKGF per gram of body weight per day.* C: *Mice injected with 10 µg of rKGF per gram of body weight per day. A rKGF dose response is evident with a particular increase in hair growth at and near the injection sites (arrowheads).*

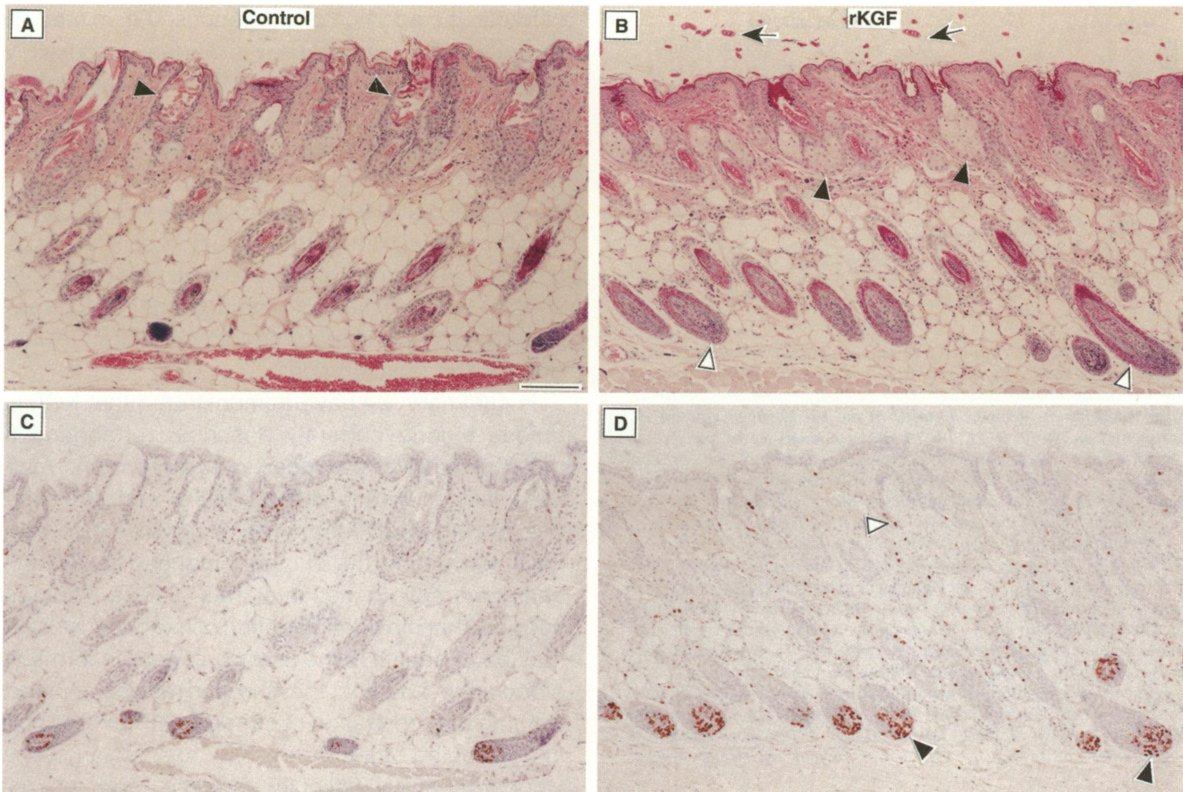

Figure 5. *H&E- and BrdU-stained sections of skin from control (A and C) and rKGF-treated (B and D) athymic nude mice. A: Cystically dilated hair follicles filled with fragmented hair shafts and keratinized debris (arrowheads) that are normally present in nude mouse skin. B: rKGF-treated skin exhibits prominent follicular hypertrophy (open arrowheads) as well as moderate sebaceous gland hypertrophy (filled arrowheads) with a normalization of follicular morphology and the presence of hair shafts above the skin's surface (arrows). D: A moderate increase in BrdU-labeled follicular keratinocytes, particularly within follicular bulbs (filled arrowheads), as well as a slight increase in BrdU-labeled peripheral epithelial cells in sebaceous glands (open arrowhead) in rKGF-treated nude mouse skin* versus *control nude mouse skin (C). Bar in A, 100 μm.*

Table 1. *Proliferating Follicular Keratinocytes and Sebaceous Gland Epithelial Cells in rKGF-Treated Athymic Nude Mice*

rKGF (μg/g/day)	BrdU-positive keratinocytes per anagen follicle	BrdU-positive epithelial cells per sebaceous gland
0	22.2 ± 1.8 *	1.1 ± 0.2 †
2.5	34.5 ± 2.4 *	1.7 ± 0.4 †
5	35.7 ± 2.9 *	2.2 ± 0.4 †
10	32.8 ± 2.0 *	3.2 ± 0.4 †

Mice were administered rKGF or PBS vehicle control ip for 18 consecutive days, then administered BrdU ip 1 hour before harvest. The number of BrdU-positive keratinocytes per follicle and epithelial cells per sebaceous gland were counted by an observer blinded to the treatment groups. Only anagen follicles that were in full longitudinal section and that contained at least 10 BrdU-positive keratinocytes were counted. Ten anagen follicles and ten sebaceous glands were counted for each section examined, and three sections from different individuals were examined per treatment group. Results are presented as mean BrdU-positive cells ± SEM.

*$P = 0.0035$ by Kruskal-Wallis test.

†$P = 0.0001$ by Kruskal-Wallis test.

Although IL-1, EGF, aFGF, and KGF all protected neonatal rats from alopecia induced by ARA-C, all factors failed to protect neonatal rats from cyclophosphamide-induced alopecia. ARA-C is a cell cycle-specific chemotherapeutic agent that selectively damages cells which are in S phase synthesizing DNA.[38] Cyclophosphamide, on the other hand, is a non-cell-cycle-specific chemotherapeutic agent that alkylates nucleic acids, thereby inducing cell death in any cell that has entered the cell cycle.[39] The finding that all growth factors/cytokines tested to date protect only against ARA-C-induced alopecia suggests that the underlying mechanism of alopecia induction by ARA-C and cyclophosphamide must differ. In addition, as ARA-C selectively inhibits cells that are in S phase, KGF must be cytoprotective in the ARA-C-induced alopecia model by a mechanism other than increasing the number of follicular keratinocytes that are in S phase.

Although administration of exogenous rKGF to athymic nude mice stimulated hair follicles to proliferate and differentiate into normal, mature hairs, and administration of exogenous rKGF to rabbit ear dermal wounds stimulated follicular proliferation,[17] targeted overexpression of KGF to epidermal and follicular basal keratinocytes of transgenic mice inhibited hair follicle growth and development.[13] Although the results from these studies appear to be

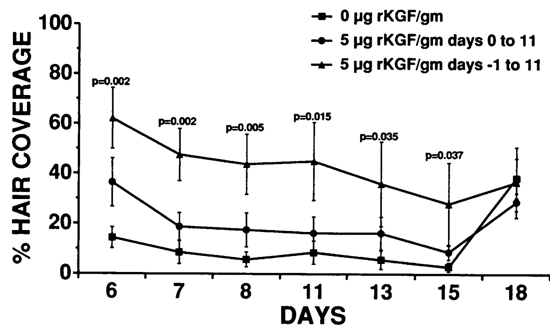

Figure 6. *Percentage of hair coverage in neonatal rats after 6 days (experimental days 0 to 5) of ARA-C chemotherapy. Rats were scored for percentage of hair coverage (0 to 100% by increments of 10) by four independent observers blinded to the treatment groups on experimental days 6, 7, 8, 11, 13, 15, and 18. Mean scores were calculated for each rat pup daily. For each experimental day that hair coverage was evaluated, ARA-C-treated rats receiving 5 µg/g body weight/day rKGF were analyzed* versus *ARA-C-treated rats receiving PBS vehicle control by one-way analysis of variance (two-tailed) coupled with the Bonferroni/Dunn* post hoc *test at a 5% significance level. Results are reported as mean ± SEM. When administered 1 day before the onset of ARA-C chemotherapy (experimental day −1), rKGF at 5 µg/g body weight/day induces a statistically significant increase in percentage of hair coverage after ARA-C chemotherapy* versus *PBS-treated controls at all time points through day 15, after which time all experimental groups exhibit similar hair regrowth regardless of specific treatment. Administration of 5 µg/g body weight/day rKGF concurrent with, but not before, ARA-C chemotherapy (experimental days 0 to 11) also increases the percentage of hair coverage* versus *PBS-treated controls but not as markedly as with rKGF pretreatment.*

contradictory, it is possible that constant unregulated overexpression of KGF in the transgenic mice may have overridden normal follicular growth control mechanisms and inhibited follicular development, whereas the addition of exogenous KGF in models in which KGF was present in normal or possibly suboptimal levels stimulated follicles. A stimulatory role for KGF in normal follicular growth and development is further supported by a recent study that showed follicular morphological abnormalities and a marked decrease in follicle numbers in transgenic mice with targeted inhibition of KGFR signaling in epidermal basal and follicular outer root sheath keratinocytes,[18] findings that also suggested that KGF is required for normal hair follicle growth and development.

In summary, we have demonstrated that KGF is unique among currently described endogenous growth factors or cytokines in its ability to normalize follicular keratin differentiation and stimulate hair growth in athymic nude mice while also partially abrogating the alopecia induced by ARA-C chemotherapy in neonatal rats. These findings suggest that the administration of exogenous rKGF may be of benefit in the therapy of certain forms of alopecia. In addition, these *in vivo* results, coupled with the demonstration of strong KGFR expression in developing hair follicles, KGF expression in developing dermal follicular papillae, and the recently reported follicular abnormalities in transgenic mice with targeted inhibition of KGFR signaling in follicular outer root sheath keratinocytes,[18] strongly suggest that KGF is an important

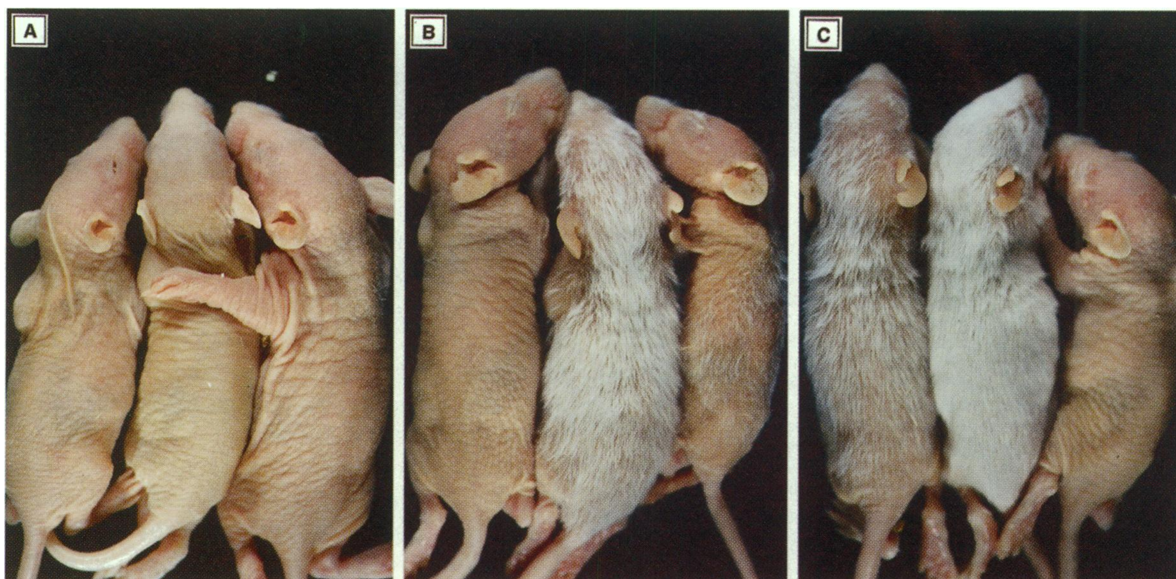

Figure 7. *Sixteen-day-old neonatal rat littermates on experimental day 8 after treatment with ARA-C for 6 consecutive days (experimental days 0 to 5).* A: *Three rat pups treated with ARA-C plus PBS (vehicle control).* B: *Three rat pups that received 5 µg/g body weight/day rKGF beginning concurrently with ARA-C (from experimental day 0 through day 11).* C: *Three rat pups that received 5 µg/g body weight/day rKGF beginning 1 day before receiving ARA-C (from experimental day −1 through day 11). A rKGF-induced cytoprotective effect is evident, with rats receiving rKGF beginning 1 day before the onset of ARA-C chemotherapy having the most hair coverage, rats receiving rKGF beginning concurrently with onset of ARA-C chemotherapy having an intermediate degree of hair coverage, and all three rats receiving only ARA-C and no rKGF exhibiting nearly complete alopecia.*

endogenous paracrine mediator of normal hair follicle growth, development, and differentiation.

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