Expression of Growth Hormone Receptor, Insulin-Like Growth Factor 1 (IGF-1) and IGF-1 Receptor mRNA and Proteins in Human Skin

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A cDNA corresponding to the membrane receptor for growth hormone (GH) was amplified by polymerase chain reaction (PCR) directly from human skin. The cDNA was cloned and found to have complete sequence homology to the extracellular domain of human liver GH receptor (GH-R). Northern analysis, using the cloned GH-R as probe, revealed relatively higher levels of GH-R transcripts in cultured human dermal fibroblasts compared to cultured keratinocytes or keratome biopsies. Semi-quantitative PCR analysis indicated that the level of GH-R mRNA in cultured melanocytes was similar to that in fibroblasts. The receptor protein encoded by GH-R mRNA in fibroblasts was shown by affinity cross-linking to have an apparent M_r of 115–120 kDa, similar to that of 3T3-F442A fibroblasts used as a control. mRNA transcripts for the major mediator of GH actions, insulin-like growth factor 1 (IGF-1), were detected by PCR in fibroblasts, melanocytes, and keratome biopsies, but not in keratinocytes. In contrast, IGF-1 receptor mRNA were abundant in cultured keratinocytes and skin biopsies, as determined by Northern analysis. IGF-1 but not GH (5-50 ng/ml) promoted clonal proliferation of cultured keratinocytes. In contrast, GH (10 ng/ml) after 5 d markedly increased fibroblast cell numbers (70%, $p < 0.009$) over 0.2% serum control. These data indicate that human skin cells possess the molecular elements necessary to respond to GH and raise the possibility that GH may influence skin growth in vivo. *J Invest Dermatol 99:343–349, 1992*

Growth hormone (GH) actions on linear bone growth and body metabolism have been studied for decades [1]. As proposed by the "somatomedin hypothesis" [2], many GH-mediated effects are exerted systemically via stimulation of hepatic synthesis of insulin-like growth factor 1 (IGF-1) [3,4]. Additional evidence for association between pituitary GH and hepatic production of IGF-1 has been obtained by cloning of GH receptor (GH-R) from liver of rabbit [5], human [5], rat [6], and mouse [7]. GH administered to older men with low plasma levels of IGF-1 has been reported to cause increased skin thickness [8]. In addition, there are generalized effects of excess GH production in acromegaly on epidermal structure and function such as skin tags, coarsening of facial features, and acanthosis nigricans [9,10]. These effects are believed to be the result of indirect actions of circulating GH, presumably via increased levels of liver-derived IGF-1 [1,4,10].

It is now evident that GH can act not only via systemic IGF-1 synthesized and secreted by the liver but also directly via GH-R expressed on many target tissues [11]. GH-R transcripts have recently been demonstrated in cultured cells in vitro [12,13], and in a number of rat tissues in vivo [6], but not in human skin. Positive immunoreactivity for GH-R and GH binding proteins (GHBP) was recently localized to basal and spinous keratinocytes, dermal fibroblasts, and hair matrix cells of the dermal papillae in human skin [14]. However, because the antibody used did not distinguish between the membrane-anchored GH-R and circulating GHBP [14], immunoreactivity alone does not provide direct evidence for expression of membrane-bound GH-R in skin.

In this work, we have cloned a cDNA for the extracellular domain of GH-R directly from human skin and examined GH-R gene and protein expression in cultured human skin cells. In addition, we have confirmed and extended previous reports by directly demonstrating IGF-1 and IGF-1 receptor (IGF-1R) mRNA expression in human keratinocytes, fibroblasts, and melanocytes.

Our findings indicate that fibroblasts and melanocytes, but not keratinocytes, are the major sources of GH-R mRNA transcripts in human skin. Fibroblasts and melanocytes, but not keratinocytes, also express IGF-1 mRNA. Because in vitro IGF-1 stimulates kera-

Manuscript received September 17, 1991; accepted for publication March 18, 1992.

These studies were presented in part at the national meeting of the Society for Investigative Dermatology in Seattle, Washington, in May 1991 (abstract 192).

This work was supported in part by the Babcock Fund for Dermatological Research (AT, JTE), National Institutes of Health grants R29-AR40016 (JTE) and R29-AR39691 (GJF), and the R. W. Johnson Pharmaceutical Research Institute.

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Abbreviations:
- BPE: bovine pituitary extract
- BSA: bovine serum albumin
- DMEM: Dulbecco's modified Eagle's medium
- EGF: epidermal growth factor
- FB: fibroblasts
- FCS: fetal calf serum
- GH: growth hormone
- GH-R: growth hormone receptor
- HC: hydrocortisone
- IGF-1: insulin-like growth factor 1
- IGF-1R: insulin-like growth factor 1 receptor
- KC: keratinocytes
- MC: melanocytes
- PCR: polymerase chain reaction

tinocyte growth via their IGF-1 receptors, these results suggest a role for IGF-1 in keratinocyte growth regulation, and identify dermal fibroblasts and melanocytes as potential extrahepatic sources of GH-induced IGF-1 synthesis in human skin.

MATERIALS AND METHODS

Skin Biopsies Keratome biopsies were procured from the buttocks of normal healthy volunteers, after obtaining written informed consent, using 1% lidocaine anesthetic as previously described [15]. Protocols for human subjects were approved by the University of Michigan Institutional Review Board. For RNA studies, biopsies were immediately frozen in liquid nitrogen and stored at −70°C until use (see below). Biopsies used for initiation of primary cell cultures were incubated with trypsin-EDTA to achieve a single cell suspension [15].

Fibroblast Cultures and Growth Assays Papillary dermal fibroblast (FB) cultures were initiated from keratome biopsies and propagated for up to 10 passages. Celles were seeded at $1-2 \times 10^4$ cells/cm^2 in Dulbecco's modified Eagle's medium media (DMEM) supplemented with 10% fetal calf serum (FCS, GIBCO), re-fed every 48 h, and passaged once they had reached $6-8 \times 10^4$ cells/cm^2. For cell-proliferation experiments, FB growing in DMEM plus 10% FCS were harvested and 5×10^3 cells were seeded in 1 ml of DMEM plus 0.2% FCS in 35-mm dishes and allowed to attach and grow for 48 h. At this time the medium was replaced and cells were treated in DMEM plus 0.2% FCS to which had been added protein carrier-free pituitary GH (CALBIOCHEM, San Diego, CA) at indicated concentrations. FB were allowed to grow for 3 or 5 d at which time they were trypsinized and counted in a hemocytometer.

Keratinocyte Cultures and Growth Assays Primary cultures of human KC were derived from keratome biopsies and subcultured in MCDB 153 medium modified for high-density growth [16] supplemented with 0.15-mM Ca^{++}, hydrocortisone (HC, 0.5 μg/ml), bovine pituitary extract (BPE, 0.4%), insulin from bovine pancreas (5 μg/ml), and epidermal growth factor (EGF, 10 ng/ml) from murine salivary gland. KC cultures were passaged when 60% confluent, approximately 7×10^4 cells/cm^2, and used between passages 2 and 5. Cell replication studies were conducted using the clonal growth assay [17]. Cells growing in the above medium were trypsinized and seeded at 2×10^3 cells per 35-mm dish in the modified keratinocyte basal medium (KBM) to which had been added HC and BPE, as above, and EGF (1 ng/ml) and either 500 ng/ml bovine insulin (Sigma), or IGF-1 and GH at varying concentrations. Cells were incubated for 12 d without a further change of medium, fixed in formaldehyde, stained with crystal violet [18], and photographed.

Melanocyte and 3T3-F442A Fibroblast Cultures Epidermal melanocytes were obtained from neonatal foreskin and cultured essentially as described [19] in melanocyte growth medium (MGM; Clonetics, San Diego, CA). 3T3-F442A fibroblasts (preadipocytes) were kindly provided by Dr. Christy Carter-Su, University of Michigan, and cultured as described previously [20].

RNA Preparation and Northern Blotting Total RNA preparation from tissues as well as cultured cells was carried out essentially as described [21,22]. Ploy $(A)^+$-enriched RNA was prepared using a commercial kit (Pharmacia, Uppsala, Sweden). After agarose gel electrophoresis, total or poly $(A)^+$ RNA was transferred in 10 × SSC (1XSSC is 150 mM NaCl, 15 mM sodium citrate, pH 7.0) onto Zeta probe membranes (Bio Rad, Richmond, CA). Prehybridization, hybridization, and washes of blots were performed as described in detail elsewhere [22].

Polymerase Chain Reaction (PCR) and Semi-Quantitative PCR PCR was carried out using 1 μg of total RNA extracted from cultured cells or epidermal biopsies. RNA was reverse transcribed into a complementary DNA [23], using M-MLV H^- reverse transcriptase (200 units, Gibco BRL) and random hexamers as primers (25 pmol), at 37°C for 30 min, in a total volume of 20 μl buffer A [50 mM Tris (pH 8.3), 0.3 mM $MgCl_2$, 20 mM DTT, 500 mM dXTP, 20 units RNAse inhibitor]. Forty cycles of PCR amplification (94°C, 60 seconds, 50°C, 30 seconds, 72°C, 90 seconds) were performed in 80 μl using *Taq* DNA polymerase, buffers, and nucleotides as described below for semi-quantitative PCR. After amplification, 20 μl aliquots were analyzed by agarose gel electrophoresis as described [23]. These PCR conditions produced results that correlated well with Northern blotting.

The relative amounts of GH-R mRNA levels in KC, FB, and MC were determined by semi-quantitative PCR. To 1 μg total RNA from each cell type was added 1.2 pg β-globin mRNA (Gibco BRL) as an internal control, and the mixtures were reverse transcribed as described above. The cDNA thus synthesized was three-fold serially diluted three times (i.e., the original reaction was diluted 3, 9, and 27 times) in buffer A, and to each dilution (20 μl) GH-R and β-globin specific primers (100 pmol each) were added. GH-R and β-globin target sequences were co-amplified by PCR in a total volume of 80 μl containing 40 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 125 mM dXTP, and 2 units *Taq* DNA polymerase (Perkin Elmer Cetus). PCR amplification was carried out for 21 cycles because we have determined that under these conditions the amount of DNA formed is proportional to the amount of input RNA. Following PCR amplification the reaction products were separated by agarose gel electrophoresis and transferred under alkaline conditions to nylon membrane (Nytran, Schleicher and Schuell). Membranes were simultaneously hybridized to random primed ^{32}P-labeled cDNA probes for GH-R and β-globin, as described above for Northern analysis. Radioactivity in each band was quantified using a phosphorimager (Molecular Dynamics). Results are expressed as GH-R band intensity relative to that of β-globin for each sample.

The oligonucleotide primers for PCR were based on published mRNA sequences and were as follows. Three GH-R mRNA primers were to the extracellular domain of the receptor [5], primer 1 (5′-CATCGGATCCTCTGGAAGTGAGGCCACAGCAG-3′), primer 2 (5′-CATCGGATCCCACTCAAGAATGGACTCAAGA-3′), and primer 3 (5′-CATCGGATCCGTAAATTGGCTCATCTGAGG-3′); human IGF-1R α-chain [24], primer 1 (5′-AATAACATTGCTTCAGAGCTG-3′) and primer 2 (5′-GATGGTGCCGTCGGCATACTT-3′); human IGF-1 [25], primer 1 (5′-ACGGCTGGACCGGAGACGGTC-3′), primer 2 (5′-CTACTTGCGTTCTTCAAATGT-3′); and human β-globin, primer 1 (5′-GAAGAAGTTGGTGGTGAGGCC-3′) and primer 2 (5′-ATTGGCCACACCAGCCACCAC-3′). GH mRNA expression in skin was examined by two degenerate oligonucleotide primers designed to the coding sequence of human pituitary GH [26] and its corresponding region in bovine [27], primer 1 (5′-A[G/A]CCAT[G/T][T/C]CCTT [G/A]TCC[G/A]G[C/G]C-3′) and primer 2 (5′-GTCCTTCC[T/G]GAAGCAG[T/G]AGAGCA-3′). These regions were selected for their high frequency of conserved nucleotides, thus allowing one set of primers to be used for detection of GH mRNA from both human skin and bovine pituitary (positive control). All primers were synthesized by the University of Michigan DNA core facility. Identity of PCR products was confirmed (see below) by enzyme restriction mapping, DNA blot hybridization with ^{32}P-labeled specific cDNA probes, or DNA sequencing [21,22]. Unless otherwise stated, all chemicals were of reagent grade and purchased from commercial sources.

DNA Probes, cDNA Cloning, and Sequencing A plasmid clone (number 59294) encoding the 30 amino acid signal peptide and 193 amino acids of the α-subunit of human IGF-1R was obtained from American Type Culture Collection (ATCC) Rockville, MD). Plasmids for the α- and β-chains of insulin receptor were gifts from Dr. Ora Rosen, Sloan-Kettering Cancer Institute. Given the structural similarities and ligand overlap of insulin receptor and IGF-1R, Southern blots of human genomic DNA digested with several restriction enzymes were hybridized with insulin receptor and IGF-1R cDNA probes to monitor their specificity and sensitivity. The porcine IGF-1 cDNA clone (designated sigf.3) [22], has

been shown to hybridize to human IGF-1 sequence [21]. The GH-R cDNA probe was a PCR fragment amplified in this work directly from epidermal biopsies. This fragment was gel purified using the Geneclean II kit (BIO 101, La Jolla, CA), cleaved by *Bam*HI and ligated using T4 DNA ligase [22] into the *Bam*HI-digested pGEM3Z plasmid (Promega, Madison, WI). The β-globin probe used for DNA hybridization was also a PCR product. DNA sequence analysis and comparison of cloned genes were carried out as previously described [22].

Affinity Cross-Linking Studies in Fibroblasts and Keratinocytes FB were cultured as above whereas 3T3-F442A cells were grown in 100-mm dishes as described previously [20]. Cells were washed, switched, and maintained in DMEM containing 0.1% BSA for 12–18 h prior to cross-linking experiments. KC were grown as above to 60–80% confluency and then switched to modified MCDB medium lacking HC, BPE, insulin, and EGF for 48 h. Cross-linking experiments were performed as previously described [20]. Briefly, after rinsing cells in KRP buffer (0.12 M NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1.2 mM $MgSO_4$, 10 mM $NaPO_4$, pH 7.4), binding was carried out at 4°C for 6 h in the same buffer containing 1% BSA and 30 ng/ml [^{125}I]-human GH (NEN-DuPont, Wilmington, DE) (for FB and KC) and 10 ng/ml (3T3-F442A) cells. These conditions were chosen because lower concentrations of iodinated GH did not produce reproducible results in FB. After washing with cold KRP plus 1% BSA, cross-linking was performed for 15 min at 4°C in KRP buffer to which was added 1 mM disuccinimidyl suberate (DSS, Pierce Chemical Co., Rockford, IL), freshly prepared in DMSO. Cell lysates were prepared by detaching monolayers in the presence of a solution containing protease inhibitors, 25 mM HEPES buffer, 0.1% Triton X-100, and 0.5 mM DTT, and electrophoresed in 8% polyacrylamide gels with a tris-glycine discontinuous buffer system [20].

RESULTS

Expression of GH-R mRNA and Protein in Human Skin A 720-base pair (bp) DNA fragment was obtained from human skin RNA by reverse transcription using GH-R primers 1 and 3. This DNA fragment was re-amplified with a nested (internal) 5′-primer (GH-R primer 2, nucleotides 270–289) and primer 3 yielding the expected fragment of 523 bp, which also included the 20 bp *Bam*HI-adaptor sequence (results not shown). The PCR fragment was cloned into pGEM3Z and digested with *Bam*HI resulting in the release from the plasmid vector of a 503-bp insert whereas *Bam*HI/*Eco*RV and *Bam*HI/*Ava*II digests each yielded the predicted subfragments (data not shown). The skin-derived PCR product was partially sequenced and exhibited 100% homology to the nucleotide sequence of human liver GH-R [5] (results not shown).

PCR analysis of RNA isolated from cultured cells identified FB, melanocytes (MC), and KC as three sources of GH-R RNA transcripts in skin (Fig 1*A*, liver is the positive control). Cultures of KC, however, were often found to contain 2–4% MC that might have contributed to the GH-R mRNA signal detected in these cells by PCR. Therefore, Northern analysis and semi-quantitative PCR were performed to determine the relative abundance of GH-R mRNA in the cultured cells and keratome biopsies. A single GH-R mRNA of 5.3–5.5 kb was detected in poly (A)$^+$ RNA that was most abundant in FB, but present only at very low levels in KC or skin biopsies (Fig 1*B*). No mRNA for GHBP, which in rat and mouse [28] is expressed from the same transcription unit as GH-R but has a smaller size of approximately 1.2–1.4 kb, was seen in Northern blots. Hybridization of the same RNA blot to the control gene, cyclophilin [29], revealed that equal amounts of poly (A)$^+$ RNA were loaded onto the gel (Fig 1*B*). These data indicated that GH-R mRNA levels in FB were significantly greater than in KC or skin biopsies. Cell type–specific expression of GH-R mRNA in epidermal cells was further defined by semi-quantitative PCR analysis. This revealed that GH-R mRNA transcript levels normalized to the β-globin mRNA internal control were approximately equal in cultured MC and FB (Fig 1*C*). In contrast, very low levels of GH-R

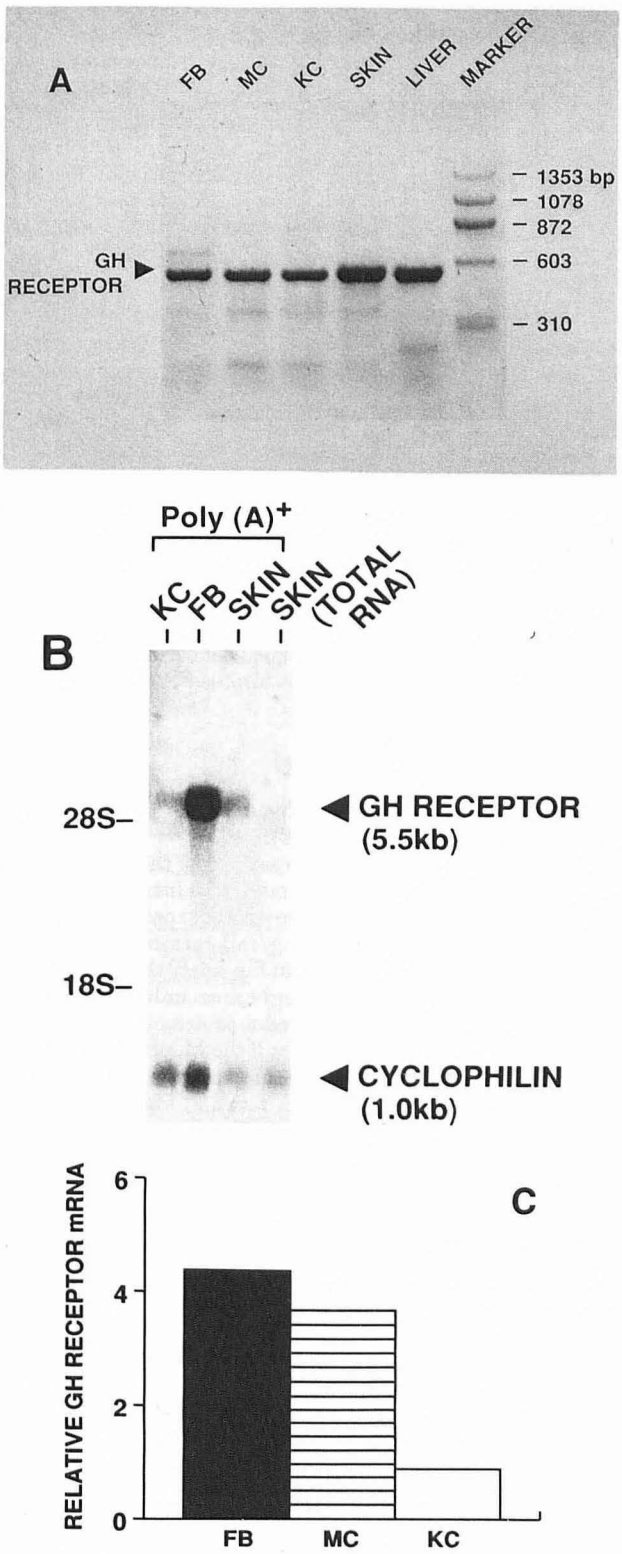


Figure 1. GH-R gene expression in human skin. *A)* GH-R mRNA was detected by PCR in cultured fibroblasts (FB), melanocytes (MC), keratinocytes (KC), skin, and liver (control). *B)* Northern hybridization of ^{32}P-labeled GH-R probe to poly (A)$^+$ RNA (5 μg) from KC, FB, and skin, or total RNA (40 μg) from skin. The absence of a signal in the lane containing total RNA indicates that the signal was not due to cross-hybridization of GH-R cDNA to 28S ribosomal RNA *(B)*. The same RNA blot was hybridized against cyclophilin cDNA probe, as an internal control for gel loading. Positions of 28S and 18S ribosomal RNA markers are shown. *C)* Semi-quantitative PCR determiniation (average of three measurements) of GH-R mRNA in FB, MC, and KC. Results are normalized to exogenous input β-globin mRNA as described in *Materials and Methods*.

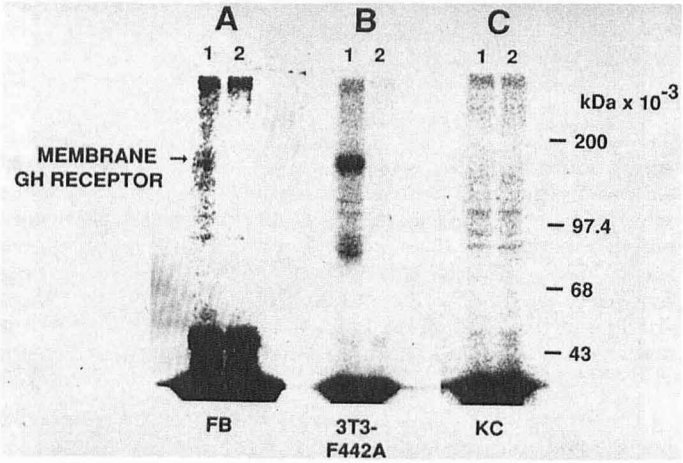


Figure 2. Affinity cross-linking of [^{125}I]-GH to its receptor in skin cells. GH-R was cross-linked on dermal FB *(A, lane 1)* or control 3T3-F442A cells *(B, lane 1)*. Specificity of cross-linked receptor was determined by incubation of cell monolayers with iodinated GH in the presence of a hundredfold excess; 1 and 3 μg unlabeled GH for 3T3-F442A and FB, respectively (*A* and *B, lane 2*). *C)* Monolayers of KC were incubated with iodinated GH in the absence *(lane 1)* or presence *(lane 2)* of a hundredfold excess (3 μg) unlabeled GH and cell lysates were run on SDS gels. Abbreviations as in Fig 1.

mRNA were detected in KC by this method (Fig 1*C*), consistent with the Northern analysis (Fig 1*B*).

We next examined by affinity cross-linking the presence of GH-R protein in cultured human KC and FB. In intact FB monolayers and control 3T3-F442A cells, known to express the membrane GH-R [20], [^{125}I]-GH (10 or 30 ng/ml) formed a complex with GH-R of M_r 135–140 kDa (*lane 1* in Fig 2*A,B*) that was specifically reduced by a hundredfold (1 or 3 μg) excess unlabeled human GH (*lane 2* in Fig 2*A,B*). This indicated a protein of about M_r 115–120 kDa for GH-R in both cell types if the M_r of iodinated GH was subtracted. In contrast, no evidence for expression of GH-R protein was observed in cultured KC (*lanes 1* and *2*, Fig 2*C*), even under conditions where IGF-1R was readily cross-linked (data not shown). The cross-linking results were compatible with measurements of GH-R mRNA levels, indicating that FB and MC, but not KC, expressed GH-R. This also suggests that FB and MC could potentially respond to GH by IGF-1 production.

Pattern of IGF-1 mRNA Expression in Skin Expression of transcripts for IGF-1, the major mediator of GH effects, was next examined in skin cells. Using exon-specific primers, IGF-1 mRNA was detected by PCR in human dermal FB, skin (n = 3) and liver but not in KC cultures from three individuals (Fig 3*A*). Cultured KC were also negative for IGF-1 mRNA expression by Northern blotting (data not shown), which further substantiated the PCR data. In a second PCR experiment depicted in Fig 3*B*, IGF-1 mRNA expression was demonstrated in FB from a different individual, MC, skin, and liver. The integrity of KC RNA samples shown in Fig 3*A,B* was confirmed by PCR amplification of cyclophilin [29], as shown. The presence of GH mRNA transcripts in skin was investigated by two degenerate PCR primers. However, no mRNA for GH was found in normal human skin, although it was detected in bovine pituitary RNA (Fig 3*C*). Because the results indicated that IGF-1 was produced in skin cells, we next examined expression of receptors for IGF-1 in FB, KC, and skin biopsies.

IGF-1 Receptor mRNA Expression IGF-1R mRNA was detected by PCR in FB, KC, and skin (Fig 4*A*). Liver expressed very low levels of IGF-1R transcripts, consistent with a previous report [4]. Because the physiologic role of IGF-1 is in part determined by the distribution and concentration of its receptors on target cells [4],

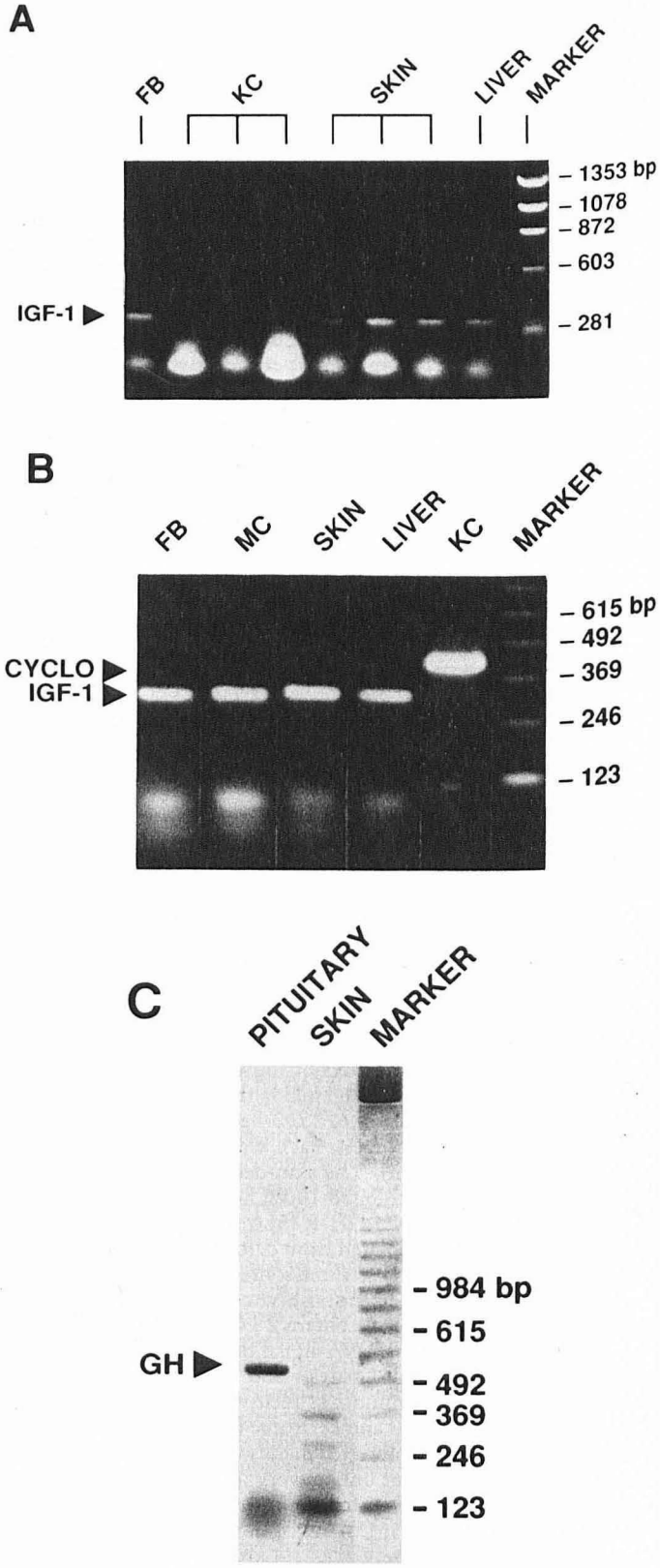


Figure 3. IGF-1 mRNA expression in human epidermal cells. *A)* IGF-1 mRNA was detected by PCR in cultured FB, normal human skin (n = 3), and liver, but not in KC (n = 3). *B)* IGF-1 transcripts were shown by PCR to be expressed in FB (derived from a different individual than one used in *A*), in MC, skin, and liver. Integrity of KC RNA was verified by PCR amplification of cyclophilin mRNA indicated by an *arrow* as a fragment of 450 bp above the IGF-1 band. *C)* GH mRNA was undetectable by PCR in human epidermal biopsies although it was present in bovine pituitary (positive control). Abbreviations as in Fig 1.

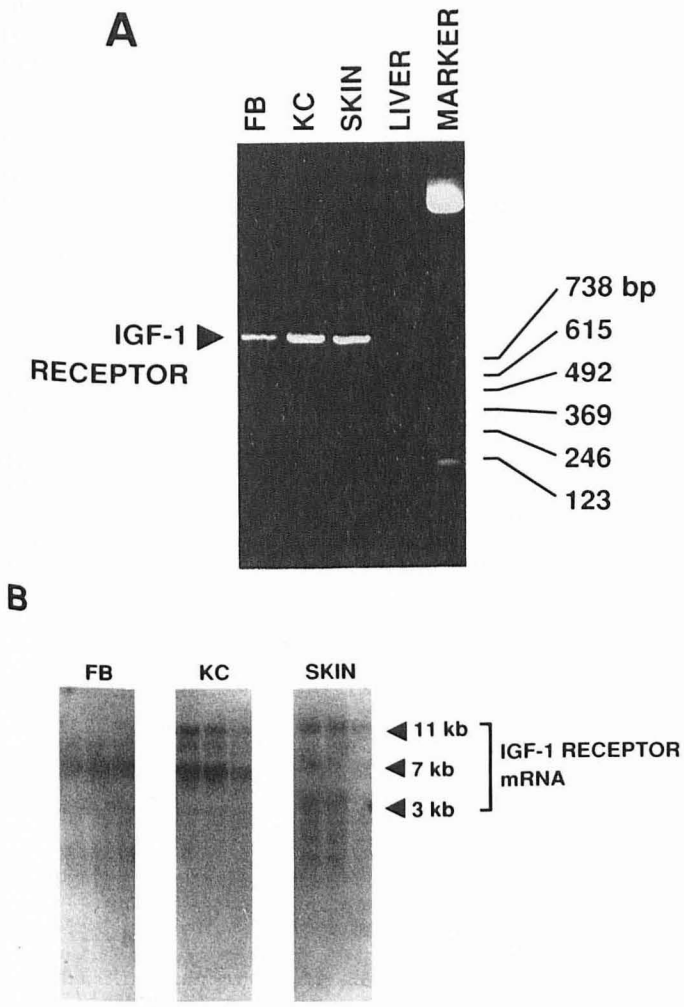


Figure 4. IGF-1R gene expression in human epidermal cells. *A)* IGF-1R mRNA was identified by PCR in FB, KC, and skin, and in lesser amounts in liver RNA. *B)* Relative abundance and size of IGF-1R transcripts were determined by Northern analysis of 50 μg of total RNA per *lane.* Multiple transcripts of approximately 3 kb, 7 kb, and 11 kb were detected in FB, KC, and skin (n = 3). Abbreviations as in Fig 1.

the relative abundance and size of IGF-1R mRNA transcripts in FB, KC, and skin were examined by Northern hybridization. Northern analysis revealed that the steady-state levels of IGF-1R mRNA (three major species of approximately 3.0, 7.0, and 11 kb) in cultured KC were at least as great as that in FB (Fig 4*B*). IGF-1 receptor transcripts displayed a differential pattern of expression in each cell types. Hybridization of the IGF-1R probe to Southern blots of genomic DNA digests produced a profile distinct from that generated by hybridization with insulin receptor cDNA, indicating the specificity of IGF-1R transcripts detected here (results not shown).

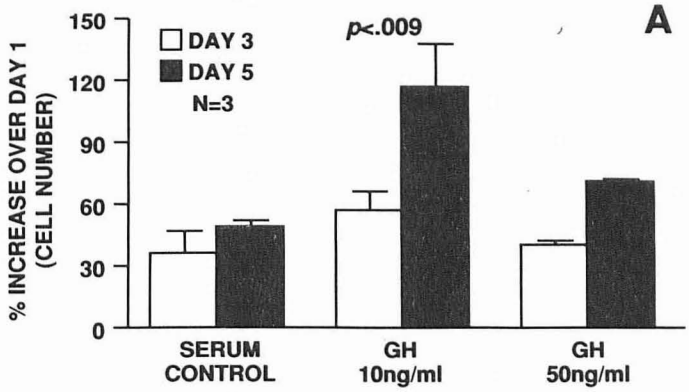
Effects of GH and IGF-1 on Epidermal Cell Growth The ability of GH to stimulate growth of dermal FB shown to express GH-R was next examined in vitro. In the absence of serum, GH at several concentrations had no effects on cell numbers or thymidine incorporation into DNA (data not shown), in agreement with previous reports [30]. However, addition of GH at 10 ng/ml in the presence of 0.2% serum stimulated cell replication after 3 d by about 21% over serum control (Fig 5*A*, *open bars*). After 5 d, FB cell numbers increased by about 70% ($p < 0.009$) over serum control (Fig. 5*A*, solid bars). GH at higher doses (50 ng/ml) had little or no effects on FB growth beyond that achieved by 0.2% serum alone (Fig 5*A*).

Recombinant GH at 5–50 ng/ml (data for 20 ng/ml shown) had no effect in stimulating clonal growth of epidermal KC. In contrast, either insulin at 50–5000 ng/ml or IGF-1 at 0.5–50 ng/ml, used as control (Fig 5*B*, data for 5 ng/ml IGF-1 and 500 ng/ml insulin shown), promoted clonal growth of KC cultures, consistent with previously published data [31]. GH in combination with either insulin or IGF-1 also did not produce any significant effect beyond that seen with either insulin or IGF-1 alone as shown (Fig 5*B*), or at several other concentrations used (data not shown).

DISCUSSION

GH receptor gene expression has been previously demonstrated in epiphyseal chondrocytes and osteoblast-like cells in vitro [12,13] and various rat tissues in vivo [6], but not in skin. Thus this report constitutes the first direct evidence for expression of GH-R in human skin in vivo and cultured human MC and dermal FB in vitro.

FB and MC cultures used in these studies were essentially free of other cell types as the few KC initially present in the primary cultures differentiate on subculturing in the media used to propagate FB and MC and thus are subsequently eliminated. KC cultures,



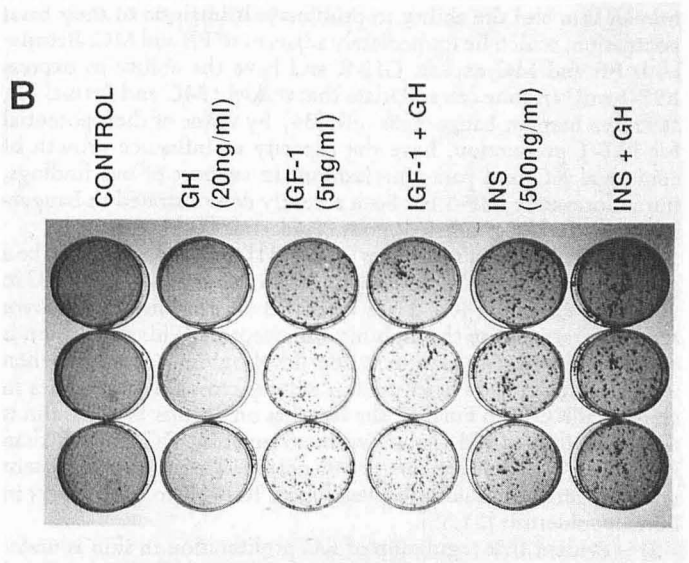


Figure 5. Effects of GH on proliferation of cultured human FB and KC. *A)* FB were maintained in DMEM plus 0.2% serum for 48 h prior to the indicated treatments on day 1. Cells were counted on day 3 *(open bars)* and day 5 *(solid bars)*. Cell numbers are expressed as percentage increase over the number of cells on day 1. The *bar graphs* designated "serum control" indicate increases in FB cell numbers in the presence of 0.2% serum alone. Data are mean ± SEM of three independent experiments. *B)* 2×10^3 KC were grown in 35-mm dishes in 5 ml modified MCDB medium in the presence of the indicated concentrations of either insulin, IGF-1, or GH. Cells were incubated for 12 d without a further change of medium and clonal growth was measured by colony size [17].

however, may contain 2–4% MC, whose RNA may have significantly contributed to the GH-R signal detected by PCR and Northern analysis in cultured KC. Judged by the low GH-R mRNA levels in KC cultures and epidermal biopsies, which are themselves composed of 95% KC, we conclude that human KC express probably little if any GH-R transcripts. The size of the receptor mRNA in FB and skin samples was similar to that reported for the membrane-bound GH-R mRNA species (4.8–5.1 kb), which mediates GH action in liver [5]. Other investigators have also observed a 5.2-kb GH-R transcript in human skin (Dr. M.J. Waters, University of Queensland, Queensland, Australia, personal communication). We have shown that both GH-R mRNA and protein are expressed in FB consistent with the expression of membrane-anchored GH-R previously reported in other cell types [5,20]. In addition, we have directly demonstrated for the first time that the size of membrane-bound GH receptor is approximately M_r 120 kDa in human dermal FB.

GH-R/GHBP was previously immunolocalized in mammalian skin [14]. However, the antibodies utilized in that study recognized both the binding protein and membrane-bound receptor [32], making it impossible to determine the exact nature of the GH-R in skin [14]. On the other hand, based on the specificity of the PCR primers, the size of the single GH-R mRNA in Northern blots and the size of cross-linked receptor on SDS gels, we conclude that human skin expresses the membrane-bound GH-R, which presumably is involved in mediating direct and indirect effects of GH. Previous work [30] has shown that GH in vitro is mitogenic within its physiologic plasma concentration (10–30 ng/ml) [1,8]. We also found that GH at 50 ng/ml did not stimulate FB proliferation in vitro. This refractoriness to higher doses of GH has been attributed to the increased rate of GH-R internalization, which is believed to attenuate GH responsiveness in target cells [28].

GH effects are mediated primarily by IGF-1 production [30,33] and the ability of GH to induce IGF-1 production in human FB has been previously demonstrated [30], although this issue was not directly addressed in the present study. In addition to FB, we found IGF-1 mRNA transcripts in MC, distinct pigment forming dendritic cells in human skin. KC comprise close to 95% of the cells in human skin and the ability to proliferate is intrinsic to their basal population, which lie immediately adjacent to FB and MC. Because both FB and MC express GH-R and have the ability to express IGF-1 mRNA, one can speculate that resident MC and dermal FB, as shown here, or Langerhans cells [34], by virtue of their potential for IGF-1 production, have the capacity to influence growth of epidermal KC in a paracrine fashion. In support of our findings, immunoreactive IGF-1 has been recently demonstrated in Langerhans cells in UV-irradiated mouse epidermis [34].

In agreement with a previous report [31], we found IGF-1 to be a potent stimulator of KC growth. Our data also suggest that in KC in vitro, the effects of IGF-1 are mediated mainly through its own receptors rather than through insulin receptors. This conclusion is compatible with the extremely low-level binding obtained when iodinated insulin has been used in affinity cross-linking studies in epidermal KC [31]. Further, the fact that on a molar basis insulin is nearly a hundredfold less active in stimulating KC growth than IGF-1 [21,31] lends support to our notion. This is also consistent with the recent immuno-localization of IGF-1R to basal layers in human epidermis [21,35].

It is evident that regulation of KC proliferation in skin is under the control of a vast array of intrinsic and extrinsic factors produced locally with short-distance paracrine or autocrine effects [36]. Given the pivotal location of FB and MC in skin, their ability to produce IGF-1 may therefore have important implications in regulation of epidermal growth, inflammatory conditions, and cutaneous wound healing. Although systemic IGF-1 may play a critical role in the proposed scheme, our results provide circumstantial evidence that GH may regulate skin growth through local production of IGF-1. The fact that GH is produced and secreted by immuno-regulatory cells [37] emphasizes a potential role for GH/IGF-1 axis in tissues other than classical endocrine organs. The extent to which the molecular elements identified here actually contribute to normal and abnormal cutaneous growth in vivo is currently under investigation.

We acknowledge the valuable technical assistance of Diane Boman, Soheila Benrazavi, and Y-J. H. Chow. We are also grateful to Mr. Ted Hamilton for the statistical analyses. We thank Dr. Christy Carter-Su for providing the 3T3-F442A fibroblasts, and for many valuable discussions during this project.

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