The Human Homologue of the Yeast CHL1 Gene Is a Novel Keratinocyte Growth Factor-regulated Gene*

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Keratinocyte growth factor (KGF)1 is a member of the fibroblast growth factor (FGF) family of mitogens that specifically acts on epithelial cells, including keratinocytes of the skin. To gain insight into the mechanisms of KGF action in this tissue, we attempted to identify genes that are regulated by KGF in keratinocytes. Using the differential display reverse transcription polymerase chain reaction technology, a gene was identified which was strongly induced in these cells by treatment with KGF but not with serum growth factors or pro-inflammatory cytokines. This gene seems to be part of a multigene family as assessed by Southern blot analysis. Molecular cloning and sequencing of the full-length cDNA revealed a strong homology with the yeast CHL1 gene. The latter encodes a putative helicase, which is involved in correct chromosome transmission and cell cycle progression. Furthermore, the CHL1 gene product and the protein encoded by the novel KGF-regulated gene were identical in size, indicating that we had cloned the human CHL1 homologue. This finding suggests a novel and specific role of KGF in correct chromosome segregation and/or cell cycle progression.

Materials and Methods

Keratinocyte growth factor (KGF)1 is a member of the fibroblast growth factor (FGF) family of mitogens that specifically acts on epithelial cells, including keratinocytes of the skin (1, 2). It is predominantly produced by mesenchymal cells and acts in a paracrine manner to regulate proliferation, differentiation, and other specific cell functions of epithelial cells (3). In vivo, KGF and its receptor, a splice variant of FGFR2 (4), have been implicated in morphogenetic processes of several organs such as the skin (5), the lung (6), the seminal vesicles (7), and the prostate (8). Furthermore, KGF can protect cells from oxidative damage (9) and from terminal differentiation (10). KGF differs in these respects from other epithelial cell mitogens such as epidermal growth factor (EGF), suggesting the existence of KGF-specific target genes, which are responsible for these effects. To gain insight into the molecular mechanisms of KGF action, we have used the differential display RT-PCR (DDRT-PCR) technology to identify and clone new genes that are specifically regulated by KGF. Using this strategy, we have recently identified a non-selenium glutathione peroxidase as a KGF-regulated gene, suggesting a role of KGF in the detoxification of reactive oxygen species.2 Here we report the identification and cDNA cloning of a novel KGF-regulated gene from cultured keratinocytes. Interestingly the novel cDNA revealed a striking homology to the yeast CHL1 gene that encodes a putative helicase involved in chromosome transmission and normal cell cycle progression. This finding suggests a novel role of KGF in these processes.

MATERIALS AND METHODS

Growth Factor Treatment of Keratinocytes—HaCaT keratinocytes (11) were grown to confluence in Dulbecco’s modified Eagle’s medium containing penicillin/streptomycin and 10% fetal calf serum (FCS), rendered quiescent by serum starvation for 24 h, and subsequently treated with 10 ng/ml recombinant human KGF, 10% FCS, 20 ng/ml EGF, 5 ng/ml transforming growth factor β1 (TGF-β1), 100 U/ml interleukin-1β (IL-1β), or 300 units/ml tumor necrosis factor-α (TNF-α) (Boehringer Mannheim), respectively. Cells were harvested at different time points after addition of these factors and used for RNA isolation. All experiments were repeated at least twice.

DDRT-PCR—DDRT-PCR was performed essentially as described (12). Briefly, 2 μg of total cellular RNA was reverse transcribed using 5'-TGCAGG-3' (primer A) as a primer. PCR was performed in a total reaction volume of 20 μl using 10 μM of 32P-dATP, 25 μM of primer A, 5 μM of primer B (5'-TTTCTTCC-3'), and 1 unit Taq polymerase (Perkin-Elmer). 40 cycles were performed (30 s at 94°C, 60 s at 42°C, 30 s at 72°C).

RNA Isolation and RNA Protection Assays—Total cellular RNA and RNA protection assays were carried out as described (13, 14). Probe DNA: (i) 297-base pair cDNA fragment corresponding to the 3'-end of the KRG-2 cDNA. This fragment had been obtained by DDRT-PCR (ii); 275-base pair probe corresponding to amino acids 664–756 of the KRG-2 open reading frame.

Northern Blot Analysis—Isolation of polyadenylated RNA was performed by affinity chromatography on oligo(dT)-cellulose, using a Pharmacia RNA isolation kit. 3 μg of polyadenylated RNA from quiescent HaCaT keratinocytes and KGF-treated HaCaT cells were used for Northern blot analysis as described (14), using the 32P-labeled DDRT-PCR cDNA fragment as a probe.

Southern Blot Analysis—Chromosomal DNA from human fibroblasts was digested with HindIII, EcoRI, or BamHI restriction endonucleases, respectively, fractionated by agarose gel electrophoresis and transferred to nitrocellulose filters. Filters were hybridized under high stringency conditions with the 32P-labeled cDNA probe described above. Cloning of a Full-length KRG-2 cDNA—To obtain a full-length KRG-2 cDNA, a DNA library was generated from 6 μg of polyadenylated RNA of KGF-stimulated HaCaT cells using the Uni-Zap XR cDNA cloning kit (Stratagene) and the Gigapack Gold III packaging extract (Stratagene) as described by the manufacturer. Filters were hybridized and washed at high stringency using standard methods (15). The DDRT-PCR cDNA fragment was used as a probe.

In Vitro Transcription/Translation—For in vitro transcription/translation studies the complete KRG-2 cDNA was subcloned into the transcription/translation system (Promega) using T3 RNA polymerase and [35S]methionine (1000 Ci/mmol) (Amersham Corp.). Proteins were...
FCS-stimulated cells were analyzed by RNase protection assay using a 32P-labeled antisense probe corresponding to the cloned DDRT-PCR fragment. The time after KGF or FCS stimulation is indicated on top of the figure. 1000 cpm of the hybridization probe were loaded in the lane labeled “probe” and used as a size marker. 50 μg of tRNA were used as a negative control. B, Northern blot analysis. 3 μg of polyadenylated RNA from quiescent (control) and KGF-stimulated keratinocytes (5 h after addition of KGF) were analyzed by Northern blotting using a 32P-labeled cDNA corresponding to the DDRT-PCR fragment. C, quiescent HaCaT keratinocytes were incubated with 300 units/ml TNF-α, 5 ng/ml TGF-β1, 20 ng/ml EGF, or 100 units/ml IL-1β for different time periods. 20 μg of total cellular RNA from these cells were analyzed by RNase protection assay for KRG-2 mRNA expression.

RESULTS AND DISCUSSION

Identification of a KGF-regulated Gene by DDRT-PCR—To elucidate the mechanisms of KGF action in keratinocytes, we attempted to identify genes that are regulated by this growth factor in keratinocytes. For this purpose we used the DDRT-PCR technology, which allows the identification of differentially expressed genes within different cell populations. The HaCaT keratinocyte cell line, which is known to express functionally expressed genes within different cell populations. The HaCaT keratinocyte cell line, which is known to express functional KGF receptors,3 was used for these studies. Quiescent cells were treated for 1.5, 5, and 8 h with purified KGF. RNAs from three independent experiments were analyzed by DDRT-PCR for differentially expressed genes in KGF-treated and nontreated cells. One of the fragments was exclusively detected after amplification of cDNA from KGF-stimulated cells but not from quiescent or FCS-treated keratinocytes (data not shown). It was therefore isolated from the gel, reamplified, and cloned. Induction of the corresponding gene by KGF was confirmed by RNase protection assay (Fig. 1A) and Northern blot analysis (Fig. 1B). The gene was expressed at low levels in quiescent keratinocytes but was strongly induced within 5–8 h after addition of KGF. In contrast to KGF, induction of this gene was not seen with FCS (Fig. 1A). Since this is the second KGF-regulated gene that we identified, it was designated KRG-2. Northern blot analysis demonstrated the presence of a single 4.3-kilobase mRNA (Fig. 1B).

Induction of the Novel Gene in Keratinocytes Is a Specific Effect of KGF—Since expression of KRG-2 was only detected in KGF-stimulated cells but not in serum-treated keratinocytes, we further determined the specificity of this effect for KGF. For this purpose, quiescent HaCaT cells were treated with different growth factors and cytokines and analyzed by RNase protection assay for KRG-2 expression. As shown in Fig. 1, A and B, KGF strongly induced expression of the novel gene, whereas EGF, another potent keratinocyte mitogen, as well as the proinflammatory cytokine IL-1β, had no significant effect (Fig. 1C). By contrast, inhibitors of keratinocyte proliferation, such as TGF-β1 and TNF-α, caused a slight reduction of KRG-2 expression (Fig. 1C). Taken together, these findings suggest that induction of KRG-2 is a novel and specific effect of KGF.

Multiple KRG-2-like Genes Exist in the Human Genome—To determine if KRG-2 is a unique gene or a member of a multigene family, human chromosomal DNA was digested with different restriction enzymes and used for Southern blot analysis. The fragment that had been obtained by DDRT-PCR was used as a probe. As shown in Fig. 2, multiple bands of variable intensities were obtained after a high stringency wash of the filter, suggesting the existence of several closely related genes in the human genome.

KRG-2 Is Highly Homologous to the Yeast CHL-1 Gene—To further characterize the KRG-2 gene and its product, we isolated the full-length cDNA from a cDNA library of KGF-treated keratinocytes. The longest insert (3.8 kilobase) was sequenced from both strands. As shown in Fig. 3A, the cDNA consists of a 212-nucleotide 5’-noncoding region, a 2568-nucleotide open reading frame, and a 975-nucleotide 3’-noncoding region, whereby the last 297 nucleotides are identical to the fragment obtained by DDRT-PCR. The cDNA encodes a protein of approximately 100 kDa as determined by in vitro transcription/translation studies (Fig. 4). Using a fragment from the open reading frame as a template for RNase protection assays, we confirmed the results obtained with the probe from the 3’-end, which are shown in Fig. 1. Sequence comparison of the nucleotide sequence with known sequences from the EMBL data base demonstrated a significant homology with the yeast CHL1 gene. The latter had been identified in a screen for yeast mutants with decreased chromosome transmission fidelity (16). Mutants lacking the CHL1 gene exhibit a 200-fold increase in the rate of chromosome III missegregation per cell division due to sister chromatid loss and sister chromatid nondisjunction (17). Furthermore, these mutants display a signif-

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3 S. Werner, unpublished data.
somes. These results might provide an explanation for the portion of these genes has been duplicated as part of a larger genes was almost identical to our cloned cDNA (21). A large published sequence of the carboxyl-terminal region of one of these identified that might encode the KRG-2 protein. Thus, the pub-

significant delay in cell cycle progression in G2/M (17). The CHL1 gene encodes an 861-amino acid protein, which is similar in size to the KRG-2 protein. Furthermore, both proteins revealed a 32% identity and a 55% similarity at the amino acid level (Fig. 3B), suggesting that KRG-2 is the human homologue of CHL1. The most striking homologies between the yeast and human proteins were observed in regions which represent functional elements. These include the A and B motifs of ATP binding proteins, whereby the latter resembles the modified B motif present in proteins with helicase activity. These enzymes are involved in many biological processes that require unwinding of double-stranded DNA and RNA, such as DNA replication and repair, transcription, splicing, translation, and also the segregation of chromosomes at mitosis (for review, see Refs. 18 and 19). In addition, the CHL1 product and the KRG-2 proteins contain a highly conserved helix-turn-helix motif (for review, see Ref. 20), suggesting that they bind to DNA.

Recently, two closely related human genes have been identified that might encode the KRG-2 protein. Thus, the published sequence of the carboxyl-terminal region of one of these genes was almost identical to our cloned cDNA (21). A large portion of these genes has been duplicated as part of a larger human telomeric repeat sequence found on many chromosomes. These results might provide an explanation for the
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