GCF2: expression and molecular analysis of repression

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Abstract

GC-binding factor 2 (GCF2) is a transcriptional repressor that decreases activity of the epidermal growth factor receptor (EGFR) and other genes. We have mapped the gene for GCF2 by fluorescence in situ hybridization (FISH) to chromosome 2q37. Sequence analysis of the GCF2 gene and cDNA showed that the gene consists of eight exons and introns and spans 73 kbp of DNA. Northern blot analysis showed that GCF2 mRNA was differentially expressed in many human tissues and cell lines. GCF2 mRNA was expressed as a 4.2 kb mRNA in most human tissues with the highest expression level in peripheral blood leukocytes and lowest expression in brain and testis. Additional transcripts of 6.6, 2.9 and 2.4 kb were found in some tissues but the only transcript detected in cancer cell lines was 4.2 kb with high levels found in seven Burkitts' lymphoma cell lines. Western blot analysis showed that GCF2 protein is present at high levels in Burkitts' lymphoma and several other cancer cell lines. GCF2 was found in both nuclear and cytoplasmic compartments in cells. Deletion mutants of GCF2 revealed that amino acids 429–528 are required for both DNA binding and repression of the EGFR promoter. Furthermore, GCF2 was able to substantially decrease activator protein 2 (AP2) enhancement of the EGFR promoter. Thus, GCF2 is a transcriptional repressor overexpressed in cancer cell lines with a role in regulating expression of the EGFR.
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1. Introduction

The epidermal growth factor receptor (EGFR) must be correctly regulated to ensure proper cell growth. Overexpression of the receptor is found in many tumors and cancer cell lines [1,2]. Overexpression occurs by gene amplification with or without gene rearrangement. Overexpression of EGFR in breast cancer correlates with failure on endocrine therapy [3,4]. The EGFR is considered a marker for cell transformation, and therefore it is an attractive target for clinical intervention [5,6]. High-level expression is also seen when there is no amplification in many cancer cell lines [7].

The regulatory region of the EGFR gene has been cloned and characterized [8–11]. Mechanisms have been proposed for regulation of the EGFR gene in breast cancer [12]. Many well-characterized transcription factors bind to the promoter region and influence transcription including specificity protein 1 (Sp1), AP2, p53, and Wilms' Tumor (WT1) [13–18]. The p53 homologue p63 has also been shown to regulate EGFR expression [19]. Less characterized transcription factors are also implicated in EGFR gene regulation [11,20–22]. Also, two repressors that bind to GC-rich sequences bind to the EGFR promoter [23,24]. More recently, a role for the early growth response gene (Egr-1) in upregulation of EGFR expression during hypoxia has been determined [25].

A 3.6 kbp cDNA that lacks a portion of the 3′ untranslated sequences was cloned and corresponds to a 4.2 kb mRNA. This cDNA was termed GC-binding factor 2 (GCF2) based on its homology to GC-binding factor (GCF) [26]. Characterization of this cDNA revealed a region of homology with GCF that includes the amino terminal (DNA binding) region and the 5′ untranslated region. The homology extends for 309 bp and has 98%

identity (305/309) in this region. The deduced protein sequence has a calculated molecular weight of 83 kDa but migrates as a 160 kDa protein when subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). GCF2 binds to a specific site in the EGFR promoter and represses transcription of the gene. When the GCF2 binding site in the promoter is mutated, the EGFR promoter is no longer repressed by GCF2, correlating GCF2 binding and repression [26]. In addition to the EGFR promoter, GCF2 is also able to repress the activity of the Rous sarcoma virus (RSV) and simian virus 40 (SV40) promoters. Cells treated with phorbol esters demonstrate an inverse correlation of GCF2 mRNA and EGFR mRNA expression.

In this study, we have initiated characterization of the GCF2 gene and its expression in human tissues and cancer cell lines. We further characterized GCF2 with respect to chromosomal and cellular localization and expression pattern in normal tissues and cancer cell lines. Using deletion mutants of GCF2, we have identified the region required for binding and repression. Furthermore, we determined that GCF2 is able to partially inhibit AP2 enhancement of EGFR promoter activity.

2. Materials and methods

2.1. Chromosomal localization

A genomic clone in a BAC vector was obtained from CHORI (Oakland, CA). The presence of the GCF2 gene was confirmed by PCR analysis with primers specific for the GCF2 cDNA. A 75-kbp-long GCF2 probe was labeled with biotin or digoxigenin (Random Primed DNA Labeling Kit, Boehringer-Mannheim, Indianapolis, IN), and used for in situ hybridization of human chromosomes derived from methotrexate-synchronized normal peripheral lymphocyte cultures. The conditions of hybridization, the detection of hybridization signals, digital-image acquisition, processing and analysis as well as the procedure for direct visualization of fluorescent signals on banded chromosomes were carried out as previously described [27].

2.2. Expression of GCF2 fusion proteins and antibody generation

Plasmid pGST/His was prepared by ligation of the BamHI and EcoRI fragment from pQE60, which contains several restriction sites and 6xHis, into pGEX-1λT (Amersham, Piscataway, NJ). The full-length GCF2 cDNA except stop codon was subcloned into the BamHI and XbaI sites of pGST/His to generate pGST/GCF2/His, all other pGST/GCF2 mutants/His were amplified by PCR and subcloned into pGST/His in the same way. Plasmid DNAs were sequenced using the Applied Biosystem model 373A sequencer to confirm that the fusion was in frame and that no mutations were present. Plasmid pGST/GCF2

or mutants/His were transformed to BL21 cells (Promega, Madison, WI) and correct clones were used to inoculate cultures of LB-ampicillin plus glucose and induced with IPTG (1 mM final concentration) at $A_{600} = 0.7$. The GST/ GCF2 and mutants/His fusion proteins were obtained by batch purification with GST-Sepharose according to the manufacturer's instructions and were subjected to a second purification using Ni-NTA resin. Protein concentrations were determined using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA). The purity and size of the mutant proteins were verified by SDS-PAGE and Coomassie staining.

For antibody production, the GST–GCF2 fusion protein prepared from IPTG induced cells was dialyzed against saline and used to inoculate rabbits. Antiserum was raised in New Zealand rabbits and tested for its ability to immunoprecipitate GCF2 made in vitro. The crude antiserum was subjected to protein A chromatography using ImmunoPure immobilized Recomb Protein A according to the manufacturer's protocol (Pierce, Rockford, IL). The eluted IgG fraction was adjusted to 0.3 mg/ml with bovine serum albumin and dialyzed against phosphate buffered saline.

2.3. RNA isolation and blotting

From cultured cells, total RNA was isolated by guanidinium–thiocyanate–phenol–chloroform extraction [28]. Poly (A)$^+$ RNA was selected from the total RNA population by oligo (dT)-cellulose chromatography [29]. RNA was fractionated on a 1% formaldehyde–agarose gel and transferred to nitrocellulose [30]. RNA was UV crosslinked to the nitrocellulose filter, prehybridized, hybridized and washed as previously described [31]. Labeled cDNA probes were prepared by random primer extension of PCR generated fragments [32]. Tissue and cancer cell line blots were purchased (Clontech, Palo Alto, CA) and probed according to manufacturer's instructions.

2.4. In vitro transcription/translation and immunoprecipitation

The open reading frame of GCF2 was amplified by the polymerase chain reaction (PCR) and subcloned into pCI-TE2A (Novagen, Madison, WI). GCF2 was synthesized in vitro into the presence of ^{35}S-methionine with the coupled transcription translation system (TNT) from Promega. The in vitro prepared proteins were analyzed on SDS-polyacrylamide gels [33]. To test the GCF2 antiserum, in vitro translated products (10 μl) were incubated with GCF2 antiserum (20 μl) on ice in RIPA buffer (100 μl) of for 3 h. Fifty microliters of a 10% suspension of Staphylococcus aureus was added and incubation continued for 30 min. The samples were pelleted and washed four times with RIPA buffer. The samples were resuspended in 20 μl SDS gel loading buffer, boiled for 3 min, loaded onto a 6% SDS polyacrylamide gel and subjected to electrophoresis at 200 V

A.
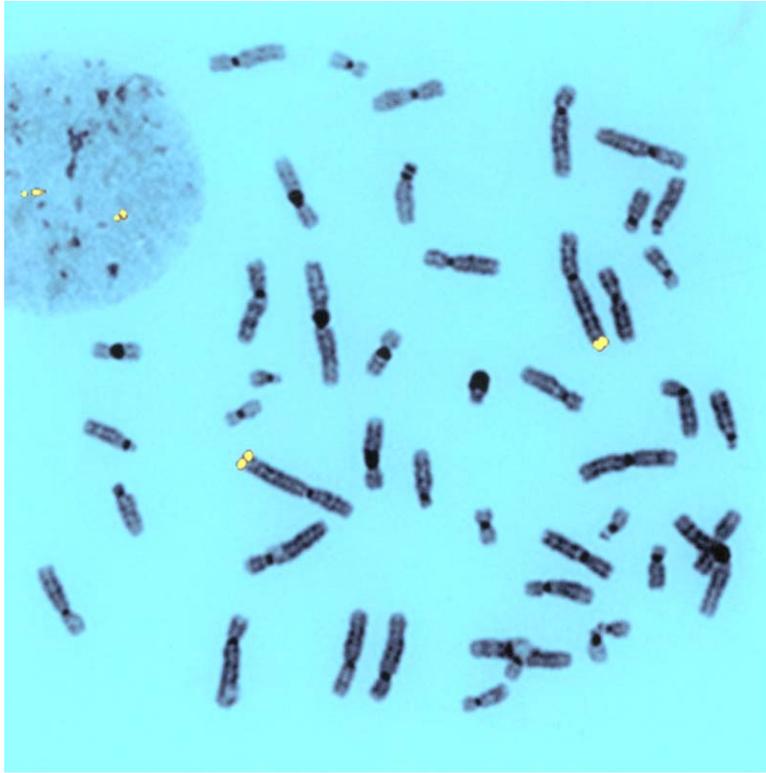
B.
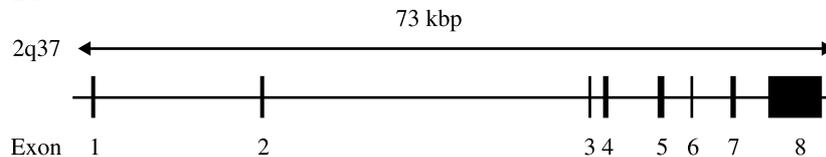
Exon	Amino acids
1	1 - 33
2	34 - 51
3	52 - 63
4	64 - 105
5	106 - 162
6	163 - 193
7	194 - 240
8	241 - 752

Fig. 1. Chromosomal localization and gene structure of GCF2. (A) Chromosome localization of the GCF2 gene by FISH. Digital image of a metaphase derived from methotrexate-synchronized normal human peripheral leukocytes after hybridization with GCF2 probe and DAPI counterstaining. Both chromosomes 2 have signals on of the long arm of chromosome 2 at band q36-37. (B) Exon/intron structure of GCF2 with amino acids encoded by each exon.

for 1 h. The gel was fixed in 20% methanol/10% acetic acid, immersed in Enlightning™ (Perkin-Elmer, Boston, MA) for 30 min, dried and exposed to film at − 80 °C.

2.5. Preparation of cell lysates and cell fractionation

Cells were harvested by scraping or centrifugation and resuspended in TD buffer at 10^6 cells per 100 μl. The cells were subjected to three cycles of freeze/thaw followed by sonication for 30 s. The samples were incubated at 37 °C for 10 min and samples centrifuged at $12,000 \times g$ for 5 min. The supernatant was collected and protein determination made using the Bio-Rad reagent. Cell fractionation was performed as described by Dyer and Herzog [34]. Briefly, cells were pelleted and resuspended in sucrose buffer containing NP-40. The lysate was centrifuged at $500 \times g$ to pellet nuclei. The supernatant was taken and processed to isolate cytoplasmic proteins. The pellet was resuspended in sucrose buffer, washed with a low salt buffer and lysed in high salt
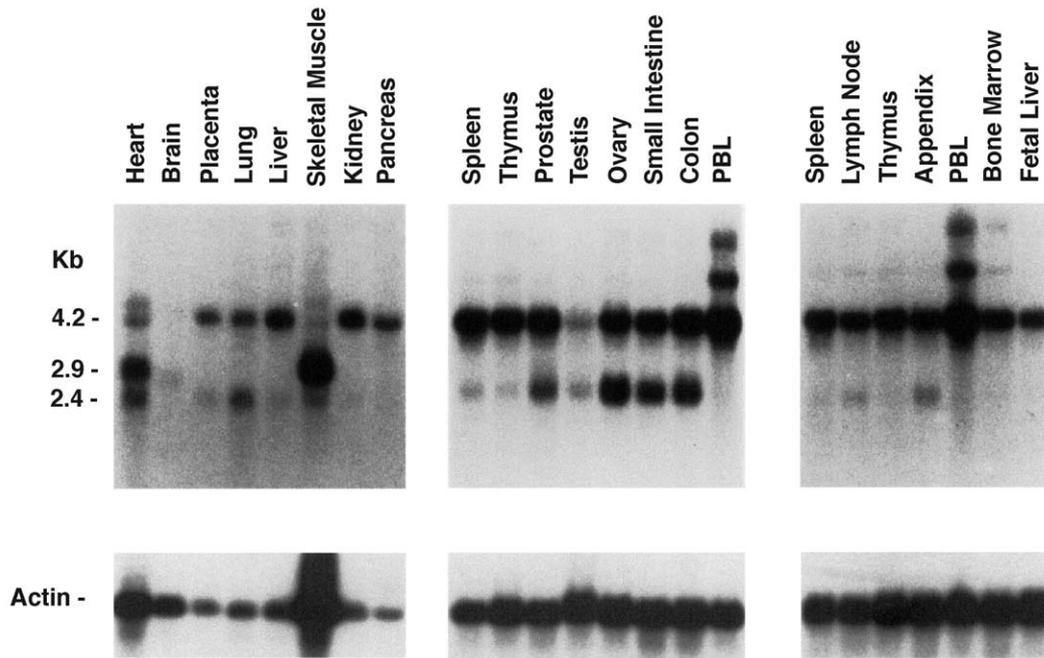
Fig. 2. GCF2 mRNA expression in normal human tissues. Northern hybridization analysis was performed using tissue RNA blots purchased from Clontech. A radiolabeled 1.1 kbp GCF2 cDNA fragment was hybridized according to Clontech's protocol. The tissues from which the RNAs were obtained are indicated above the figure. Sizes of hybridizing GCF2 RNAs are on the left of the figure. Results from reprobing the blot with actin are shown below the figure.

buffer. A nuclear extract was produced and stored at -80 °C. For phorbol ester experiments, nuclear extracts were prepared from cells treated with DMSO (control) or PMA (40 ng/ml for 2 h) by the method of Dignam et al. [35].

2.6. Western blotting

Protein extracts were subjected to gel electrophoresis on a 6% SDS-PAGE. The proteins were transferred to nitrocellulose and the nitrocellulose incubated in TPBS for 30 min. The GCF2 antiserum was added and incubation continued for 1 h at 37 °C. The nitrocellulose was washed three times with TPBS and the blot developed using the VectaStain ABC method according to the manufacturer's protocol or the ECL plus detection kit (Amersham).

2.7. Electrophoretic mobility shift assay

Mobility shift assays were performed as described previously [13]. A double-stranded oligonucleotide containing the GCF2 binding site was prepared by annealing two complementary oligonucleotides containing nucleotides -249 to -229 of the EGFR promoter in a buffer containing 10 mM Tris, pH 8.0, 500 mM NaCl and 1 mM EDTA. Equimolar amounts of the complementary oligonucleotides were mixed in a 1.5 ml Eppendorf centrifuge tube and placed in a heat block at 95 °C. The heat block was allowed to cool to room temperature and the sample was desalted on a G-25 Sephadex column. The double-stranded oligonucle-

otide was end-labeled with P-32 using T4 polynucleotide kinase and gamma ATP. For the gel shift analysis, the end-labeled double-stranded oligonucleotide was incubated with GCF2-His and/or AP2 at room temperature (23 °C) for 15 min in the presence of 10 mM Tris pH 7.5, 1 mM $MgCl_2$, 0.5 mM EDTA, 0.5 mM DTT, 50 mM NaCl, 50 µg/ml poly (dI–dC)·(poly dI–dC) and 4% glycerol. Samples (10 µl) were loaded onto a 5% polyacrylamide gel and subjected to electrophoresis at 150 V for 2 h using $0.5 \times$ TBE ($1 \times$ TBE = 89 mM Tris, 8 mM boric acid and 2 mM EDTA, pH 8.3) as running buffer. After electrophoresis, gels were transferred to Whatman 3 MM paper and exposed to Kodak XAR film with intensifying screens at -70 °C.

2.8. Transfections and luciferase assays

NIH3T3 (2×10^5) cells grown in triplicate in 35-mm-diameter wells were transfected with the expression vectors by lipofectamine (Invitrogen). The EGFR luciferase reporter construct (pER1-Luc) containing the EGFR promoter was prepared by ligation of the 1.1 kbp *Hin*dIII promoter fragment from pEGFR-CAT1 into pGL3-Basic (Promega). The reporter plasmid (0.1 µg) was cotransfected with the indicated amount of expression vectors. DNA concentration was kept constant by addition of herring sperm DNA. Cells were harvested 24 h after transfection and cell extracts prepared according to the protocol from Analytical Luminescence Laboratory (San Diego, CA). All luciferase activities were normalized for protein concentration and
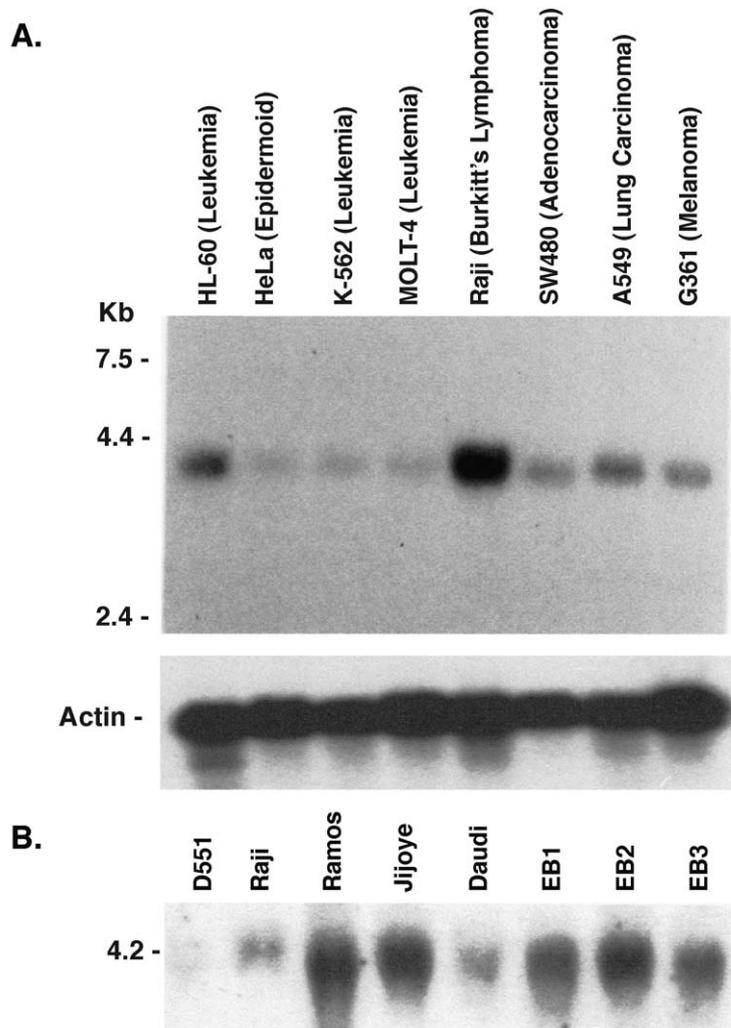
Fig. 3. GCF2 mRNA expression in cancer cell lines. (A) Northern hybridization analysis was performed using the cancer cell line RNA blot from Clontech. A radiolabeled 1.1 kbp GCF2 cDNA fragment was hybridized according to Clontech's protocol. The names of the cell lines and type of cancers from which they were obtained are indicated. RNA marker sizes are indicated on the left. (B) GCF2 mRNA expression in Burkitts' lymphoma cell lines. Northern blot analysis was performed as in A with RNA isolated from seven Burkitts' lymphoma cell lines (lanes 2–7). RNA from D551, a normal human fibroblast cell line, was present in lane 1. Actin levels did not significantly change for any of the samples (data not shown). (C) GCF2 mRNA expression in breast cancer cell lines. Northern blot analysis was performed as in A with RNA isolated from eight breast cancer cell lines. Actin levels did not significantly change for any of the samples (data not shown).

transfection efficiency using B-galactosidase. All experiments were performed in triplicate.

3. Results

Chromosome preparations obtained from cultured peripheral lymphocytes derived from two healthy donors were hybridized with a GCF2 probe. The majority, if not all of the metaphases with informative signal and a minimal nonspecific background, had symmetrical fluorescent signal on the terminal band of the long arm of chromosome 2, the largest submetacentric chromosome readily identifiable by size. Thirty labeled chromosome spreads recorded from both samples exhibited signal at the same site on both chromo-

somes 2. This signal was localized on DAPI counterstained metaphases at the region 2q36-37, where we assign the location of the GCF2 gene (Fig. 1A). The cDNA sequence for GCF2 was compared to the human genome sequence using Blast (National Center for Biotechnology Information, Bethesda, MD). The GCF2 cDNA matched sequences located at chromosome 2q37. The comparative analysis revealed the presence of eight exons and seven introns (Fig. 1B). Exon 8 is the largest and encodes for 511 of the 752 amino acids.

Since GCF2 acts to repress EGFR promoter activity, we were prompted to examine the expression of this gene in normal tissues and cancer cell lines. Northern blot hybridization was used to detect GCF2 mRNA. As shown in Fig. 2, GCF2 mRNA expression varied in normal tissues. In
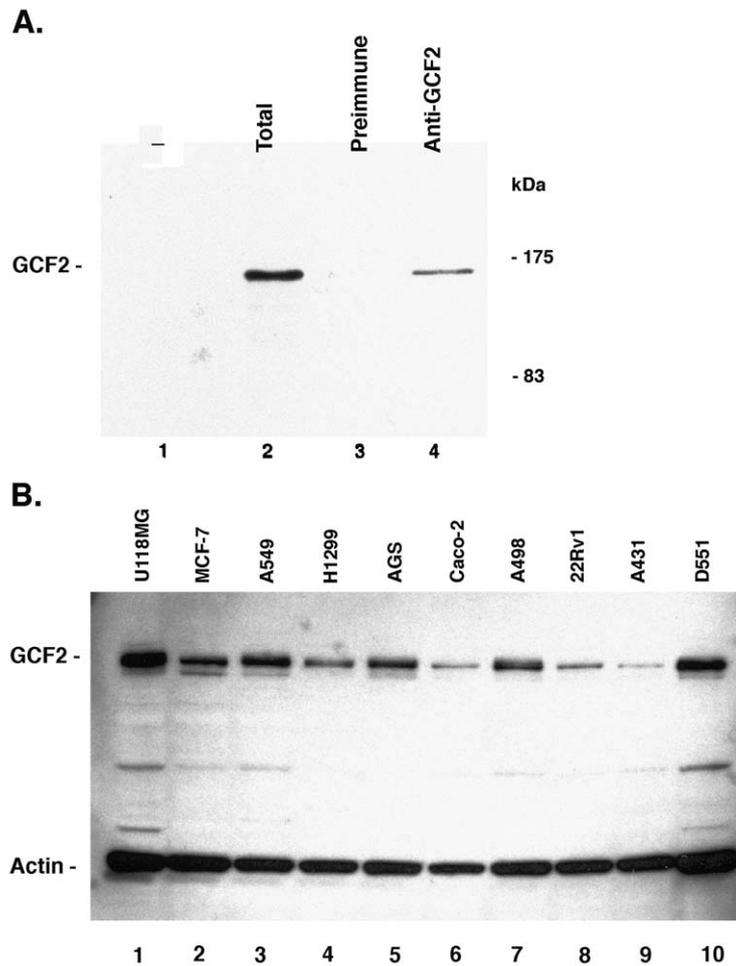
Fig. 4. Characterization of GCF2 antiserum and Western blot analysis. (A) In vitro translated GCF2, prepared by coupled transcription/translation, was immunoprecipitated with antiserum from rabbits immunized with a GST-GCF2 fusion protein or with preimmune serum from the same rabbit. The immunoprecipitated products were analyzed on a 6% SDS-polyacrylamide gel. Lanes: (1) Translation products without DNA; (2) total translation products using GCF2 DNA; (3) immunoprecipitation with preimmune serum; and (4) immunoprecipitation with GCF2 antiserum. Molecular weight markers are shown. (B) Total protein extracts were prepared from cell lines indicated above the blot and subjected to electrophoresis on a 6% SDS-polyacrylamide gel. Proteins were transferred to nitrocellulose and GCF2 detected using the IgG fraction from the antiserum.

addition to the 4.2 kb mRNA, some tissues expressed a 2.4 and/or a 2.9 kb mRNAs. Brain and testis showed very little expression of the 4.2 kb mRNA although other tissues except heart and skeletal muscle had an abundant amount. Heart and skeletal muscle both predominantly expressed a 2.9 kb mRNA. Peripheral blood leukocytes expressed very high levels of the 4.2 kb mRNA but also two larger RNAs of approximately 6.6 and 9 kb. The 2.4 kb mRNA was expressed highest in ovary, colon and small intestine. We next examined expression of GCF2 mRNA in various cancer cell lines (Fig. 3A). Unlike normal tissues, only the 4.2 kb mRNA was detected. The highest level of GCF2 mRNA was found in Raji cells derived from a Burkitts' lymphoma. Leukemia and epidermoid cancer cell lines expressed relative lower levels of GCF 2 mRNA. We examined additional Burkitts' lymphoma cell lines for GCF2 mRNA. High-level expression was detected in

RNA from all seven lines we examined (Fig. 3B). Again, only the 4.2 kb mRNA was detected.

To examine protein expression of GCF2, an antiserum was raised against a GST–GCF2 fusion protein. The antiserum was tested by immunoprecipitation of in vitro translated GCF2 (Fig. 4A). Antiserum but not preimmune serum was able to specifically immunoprecipitate GCF2. The IgG fraction of the antiserum was purified by protein A Sepharose chromatography and used in Western blotting experiments to detect GCF2 expression in cells (Fig. 4B). GCF2 was detected as a 160 kDa protein and was found expressed at high levels in A498 cells (derived from a kidney carcinoma), A549 cells (derived from a lung carcinoma), AGS cells (derived from a gastric adenocarcinoma) and U118MG cells (derived from a glioblastoma).

To determine the location of GCF2 within the cell, Western blotting was performed with nuclear and cytosolic
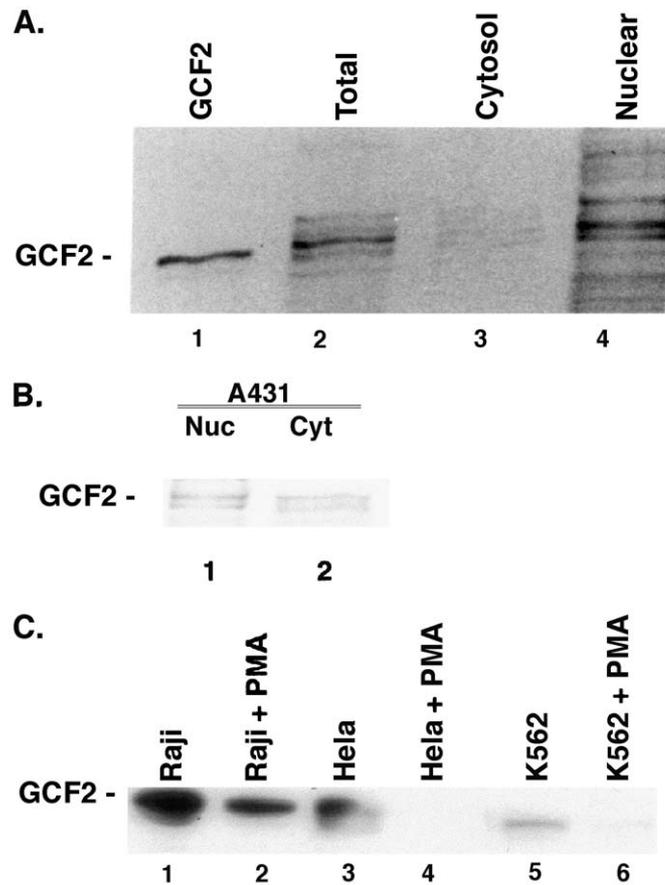
Fig. 5. Cellular localization of GCF2. (A) HUT 102 cells were fractionated into nuclear and cytosolic components as presented in Materials and methods. Protein extracts were subjected to Western blot analysis with GCF2 antiserum. Lanes: (1) GCF2 His fusion protein as a control; (2) total protein extract; (3) cytosolic fraction; and (4) nuclear fraction. (B) A431 cells were fractionated into nuclear and cytosolic components as presented in Materials and methods. Protein extracts were subjected to Western blot analysis with GCF2 antiserum. Nuc = nuclear, Cyt = cytosolic. (C) Nuclear extract were prepared from the cell lines listed above the figure after treatment with or without PMA. Western blot analysis was performed using GCF2 antibody.

fractions prepared from HUT-102 cells that express high levels of GCF2. The majority of GCF2 was found in the nuclear fraction but some GCF2 was detected in the cytosol (Fig. 5A). In A431 cell, GCF2 is distributed almost equally in nuclear and cytosolic fractions (Fig. 5B). We had previously shown that GCF2 levels decreased when EGFR expression increased due to phorbol ester treatment of cells. We examined the consequence of phorbol ester treatment on GCF2 cellular location. Nuclear extracts from Raji, Hela and K562 cells were examined for GCF2 nuclear expression. All nuclear extracts showed decreased GCF2 nuclear expression after phorbol ester treatment (40 ng/ml for 2 h) (Fig. 5C). GCF2 represses EGFR transcriptional activity by binding to a specific site in the promoter. To determine the region of GCF2 required for binding and repression of promoter activity, we performed electrophoretic mobility shift assays and cotransfection experiments with deletion mutants of GCF2. GCF2 deletion mutants that contained amino acids 428–752 (lanes 2–5 and 12) were able to bind the GCF2 binding site located in the EGFR promoter whereas deletion mutants that contained amino acids 529–

752 (lane 6) could not (Fig. 6). A GCF2 deletion mutant expressing amino acids 429–528 was also able to bind the GCF2 binding site oligonucleotide (lane 18). Consistent with the hypothesis that GCF2 binding is required for repression, cotransfection assays showed that amino acids 429–528 were necessary for full repression of EGFR promoter activity (Fig. 7). Whereas full-length GCF2 reduced EGFR promoter greater than 80%, GCF2 429–528 reduced activity by greater than 70%, indicating the requirement of this region for DNA binding and repression.

We observed that the GCF2 binding site from -249 to -233 in the EGFR promoter overlapped with an AP2 binding site located -253 to -232. We used cotransfection experiments to determine if GCF2 could inhibit AP2 enhancement of the EGFR promoter. Transfections of NIH3T3 cells with increasing concentrations of an AP2 expression vector increased EGFR promoter activity in a dose-dependent manner. Conversely, transfection with a GCF2 expression vector decreased EGFR promoter activity in a dose-dependent manner. Cotransfection with AP2 and GCF-2 expression vectors resulted in decreased EGFR
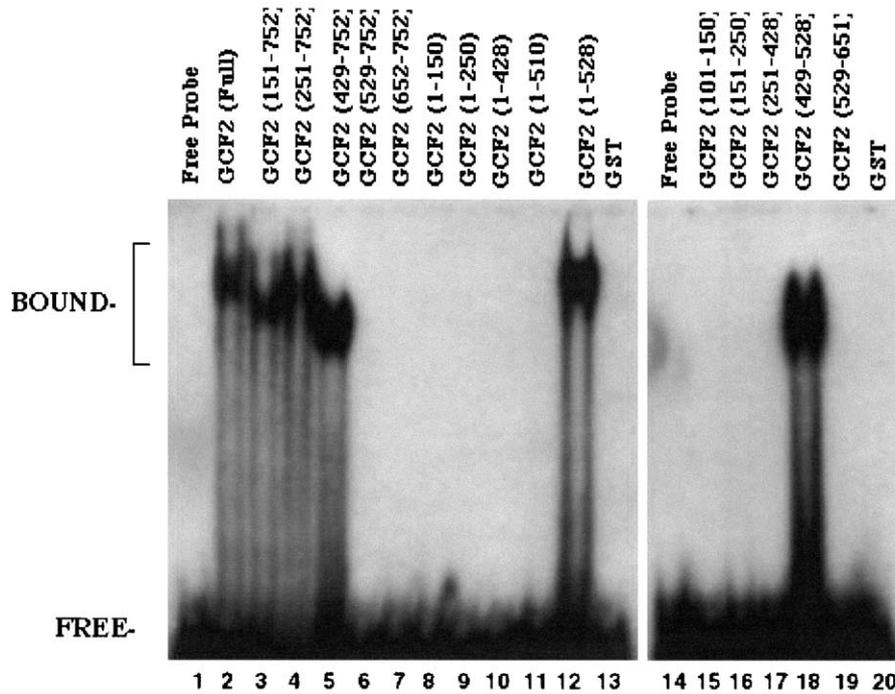
Fig. 6. Electrophoretic mobility shift with GCF2 deletion mutants and EGFR GCF2 binding site. The GCF2 binding site oligonucleotide was incubated with equivalent amounts of GCF2 His proteins (indicated above each lane) and assays performed as described in Materials and methods. The bound area indicates the region of the shifted bands.
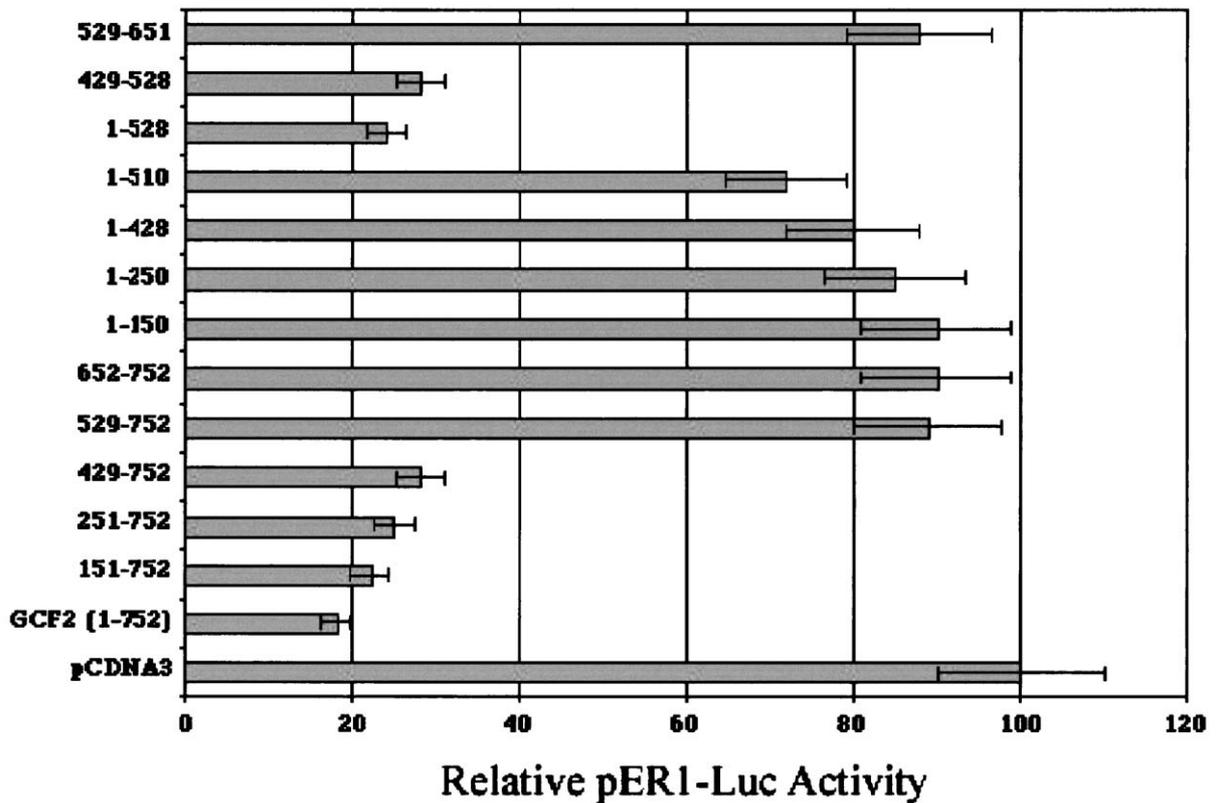
Fig. 7. Repression activity of GCF2 deletion mutants. Eukaryotic expression constructs (2 μg) containing the open reading frame for GCF2 amino acids regions indicated to the left of the figure were cotransfected into NIH3T3 cells with pER1 Luc as described in Materials and methods. Luciferase activity was measured in lysates prepared 24 h after transfection and is expressed relative to the activity of pCDNA3. Activity is corrected for protein concentration and transfection efficiency.
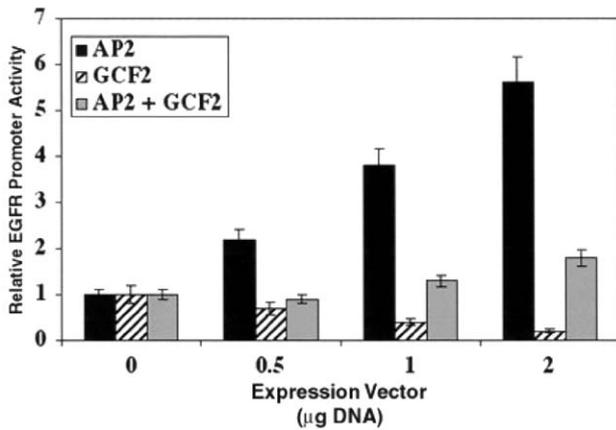
Fig. 8. GCF2 inhibits AP2 enhancement of the EGFR promoter. NIH3T3 cells were transfected with pER1 Luc (0.1 µg) and indicated concentrations of pRSV AP2 and pCMV GCF2. Activity is relative to the sample containing no GCF2 or AP2 plasmid. Varying amounts of control vector was used to keep the amount of DNA constant.

promoter activation, indicating that GCF2 repressed AP2-mediated promoter activation (Fig. 8). Therefore, these results support a competition for binding model for GCF2 repression.

4. Discussion

We report here that GCF2 is differentially expressed in human cell lines and tissues. At least three RNA species from human tissues hybridized to GCF2 cDNA. The 4.2 kb mRNA reported previously was found in all tissues examined with very low levels detected in brain and testis. Fetal liver, bone marrow, thymus, spleen, lymph node, pancreas, liver, placenta and kidney expressed the 4.2 kb mRNA as the predominant species of RNA. Heart and skeletal muscle had very low levels of the 4.2 kb mRNA but possessed an abundant level of a 2.9 kb mRNA. In addition, a high level of a 2.4 kb mRNA was found in RNA from ovary, small intestine and colon. Peripheral blood leukocytes expressed not only a high level of the 4.2 kb mRNA but also significant levels of a 6.6 and a 9 kb mRNA. The relationship between these species of RNA remains to be determined. In cancer cell lines, the only mRNA that hybridized to GCF2 cDNA was 4.2 kb.

The position of the GCF2 gene on chromosome 2 overlaps with an aphidicholine-inducible common fragile site (FS). Over the last several years, compelling evidences linking FS to the process of tumor development have accumulated. Chromosome translocations, amplification of proto-oncogenes, deletion of tumor suppressor genes, and integration of oncogenic viruses all result from the specific breakage of genomic DNA at FSs, suggesting a causative role for FSs in cancer [36]. Chromosome abnormalities involving region 2q36-37 have been described in Buschke–Ollendorff syn-

drome (2q31-ter), cerebrotendinous xanthomatosis (CTX) (2q33-ter) and Klein–Waardenburg syndrome (2q35-37) as well as in alveolar rhabdomyosarcoma with a specific translocation t(2;13) [37,38]. Molecular analyses might reveal a role of GCF2 gene in these diseases. Significantly, in B and T cell lymphoma cell lines, we detected higher levels of GCF2 expression than in other tumor cell lines. High-level expression may be associated with chromosomal alterations and suggests that GCF2 gene is important in pathogenesis of malignant lymphomas. The level of GCF2 was also high in kidney, lung, gastric and glioblastomas cancer cell lines with variable in a panel of breast cancer cell lines. The expression level of GCF2 may reflect differences in the regulatory proteins that control GCF2 expression in these cells.

GCF2 was found primarily in the nuclear compartment within the cell but a measurable amount was also cytoplasmic. The percentage of GCF2 found in the nuclear or cytoplasmic fractions was variable within different cell lines. For example, GCF2 was predominantly in the nuclear fraction of extracts from prepared from HUT-102 cells but was equally distributed in A431 cells (derived from an epidermoid carcinoma) (Fig. 5). Several protein bands of approximately 160 kDa were reactive with the GCF2 antibody. We suspect that posttranslational modifications may be involved in the determination of cellular location of GCF2. We have shown that phorbol esters reduce nuclear GCF2 levels in several cell lines. Apparently, GCF2 levels are reduced prior to PMA-enhanced EGFR expression. This is consistent with a previous kinetic analysis of GCF2 and EGFR expression after phorbol ester treatment of cells [26].

In terms of regulation, GCF2 expression decreases upon phorbol ester treatment of cells [26]. It is increased, however, by treatment of PC12 cells with nerve growth factor [39]. In both of these instances, EGFR expression is inversely correlated. In cells, the nuclear level of GCF2 is decreased by factors that activate EGFR expression. This allows for increased EGFR expression that is returned to steady state levels as GCF2 levels are increased. We speculate that posttranslational modifications such as phosphorylation may be the trigger for translocation of GCF2 between nuclear and cytoplasmic compartments.

GCF2 has been shown to repress transcription of the EGFR and PDGF-A chain promoters [26,40]. GCF2 binds to the PDGF-A chain promoter and competes with several zinc-finger transcription factors. We examined the effect of expressing both GCF2 and AP2, a known activator of EGFR expression, in cells. GCF2 partially inhibited AP2 enhancement of EGFR expression. However, the combination of GCF2 and AP2 did not alter the DNase I footprint in size or intensity (data not shown). These results suggest that GCF2 represses EGFR promoter activity by competition with positive activators such as AP2. In this report, we have shown that GCF2 requires amino acids 429–528, which contains a lysine-rich motif, for repression and DNA binding.

GCF2 contains 752 amino acids of which 100 appear involved in DNA binding and repression. The amino terminal domain contain coil–coil motif with homology to those found in flightless [41–43]. These domains allow for protein–protein interaction and thus may provide an additional activity for GCF2. Further analysis is necessary to more thoroughly define the mechanism(s) by which GCF2 is able to effect changes in gene expression.

Acknowledgements

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