Keratinocyte Growth Factor/Fibroblast Growth Factor 7, a Homeostatic Factor with Therapeutic Potential for Epithelial Protection and Repair

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Keratinocyte growth factor (KGF) is a paracrine-acting, epithelial mitogen produced by cells of mesenchymal origin. It is a member of the fibroblast growth factor (FGF) family, and acts exclusively through a subset of FGF receptor isoforms (FGFR2b) expressed predominantly by epithelial cells. The upregulation of KGF after epithelial injury suggested it had an important role in tissue repair. This hypothesis was reinforced by evidence that intestinal damage was worse and healing impaired in KGF null mice. Preclinical data from several animal models demonstrated that recombinant human KGF could enhance the regenerative capacity of epithelial tissues and protect them from a variety of toxic exposures. These beneficial effects are attributed to multiple mechanisms that collectively act to strengthen the integrity of the epithelial barrier, and include the stimulation of cell proliferation, migration, differentiation, survival, DNA repair, and induction of enzymes involved in the detoxification of reactive oxygen species. KGF is currently being evaluated in clinical trials to test its ability to ameliorate severe oral mucositis (OM) that results from cancer chemoradiotherapy. In a phase 3 trial involving patients who were treated with myeloablative chemoradiotherapy before autologous peripheral blood progenitor cell transplantation for hematologic malignancies, KGF significantly reduced both the incidence and duration of severe OM. Similar investigations are underway in patients being treated for solid tumors. On the basis of its success in ameliorating chemoradiotherapy-induced OM in humans and tissue damage in a variety of animal models, additional clinical applications of KGF are worthy of investigation.
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I. INTRODUCTION

Keratinocyte growth factor (KGF) was first isolated as an epithelial cell mitogen from the conditioned medium of the human embryonic lung fibroblast cell line, M426 (Rubin *et al.*, 1989). Initially, KGF was shown to stimulate DNA synthesis in BALB/MK mouse keratinocytes and subsequently exhibited mitogenic activity for a wide variety of epithelial cells (Rubin *et al.*, 1989, 1995). In contrast, no activity was seen on any nonepithelial cell types, such as fibroblasts, saphenous vein endothelial cells, melanocytes, or myoblasts (Halaban *et al.*, 1991; Ron *et al.*, 1993b; Rubin *et al.*, 1989). In addition to human embryonic lung fibroblasts, stromal cells from a variety of sources expressed KGF in culture. These included fibroblasts from human adult lung, skin, mammary gland, stomach, bladder, and prostate (Rubin *et al.*, 1995), as well as microvascular endothelial cells (Smola *et al.*, 1993) and smooth muscle cells (Koji *et al.*, 1994; Winkles *et al.*, 1997). It had long been postulated that epithelial cell proliferation during development as well as in adult organs was mediated by diffusible substances released from the underlying mesenchymal tissue (Cunha *et al.*, 1983; Sawyer and Fallow, 1983; Schor and Schor, 1987). The combination of KGF expression by stromal cells and activity specifically on epithelial cells supported the hypothesis that KGF functioned as just such a paracrine mediator of mesenchymal–epithelial communication.

Database analysis revealed that KGF was the seventh member of the fibroblast growth factor (FGF) family of structurally related signaling molecules

to be identified, and so it is also known as FGF7 (Finch *et al.*, 1989). In vertebrates, there are currently 22 identified FGFs that range in molecular mass from 17 to 34 kDa, and share 13–71% amino acid sequence identity. FGFs can be classified into several subfamilies, according to sequence homology within a conserved 120-amino acid core (Kim, 2001), as well as biochemical and/or developmental properties. On the basis of amino acid sequence comparison, KGF has been placed in an FGF subfamily that includes FGF10 and FGF22. Human KGF has 54% sequence identity with human FGF10 (Emoto *et al.*, 1997; Igarashi *et al.*, 1998) and 40% with human FGF22 (Nakatake *et al.*, 2001) within their conserved regions. Like KGF, FGF10 is synthesized predominantly by mesenchymal cells (Beer *et al.*, 1997), and appears to act primarily on epithelial cells (Emoto *et al.*, 1997; Igarashi *et al.*, 1998). Thus in addition to their sequence homology, KGF and FGF10 share a number of biological properties. An initial report indicated that FGF22 has a more limited pattern of expression than either KGF or FGF10, and in the skin was expressed by epidermal keratinocytes rather than mesenchymal cells (Beyer *et al.*, 2003; Nakatake *et al.*, 2001).

FGF activity on responsive cells is mediated by a family of high-affinity tyrosine kinase receptors (FGFRs) that are encoded by four structurally related genes (*FGFR1–4*) (Johnson and Williams, 1993; McKeehan *et al.*, 1998). Further heterogeneity among the FGFRs is generated by alternative splicing of transcripts, resulting in transmembrane protein tyrosine kinases with either two or three immunoglobulin (Ig)-like domains, with or without a highly acidic region also in the extracellular region (Johnson and Williams, 1993; Powers *et al.*, 2000). Specificity of FGF–FGFR binding is determined in part by alternative exons corresponding to the carboxy-terminal half of the third Ig domain and an adjacent ~20 residues of downstream sequence in FGFRs 1, 2, and 3. These alternative exons, designated IIIa, IIIb, and IIIc, generate receptor variants with different ligand-binding properties (Johnson and Williams, 1993; Powers *et al.*, 2000). Expression cloning of the KGF receptor revealed that it was encoded by IIIb variants of the *BEK/FGFR2* gene (FGFR2b) (Miki *et al.*, 1991). Binding studies demonstrated that KGF did not interact with any other FGFR variant (Miki *et al.*, 1991; Ornitz *et al.*, 1996). FGF10 bound preferentially to the FGFR2b receptor variant (Igarashi *et al.*, 1998), although it also associated with the IIIb splice variant of FGFR1, exhibiting approximately 10-fold lower affinity for it than for FGFR2b (Beer *et al.*, 2000; Lu *et al.*, 1999). At the time of writing, the target cell and receptor-binding specificity of FGF22 had not been reported. FGFR2b isoforms are found primarily in epithelial cells whereas FGFR2c isoforms have been observed in cells of mesenchymal origin. Thus, FGFR2b and FGFR2c proteins are expressed in a mutually exclusive manner in these cell lineages, through a positively regulated splicing mechanism that involves intron sequences adjacent to the isoform-specific

exons (Carstens *et al*., 1998, 2000; Del Gatto *et al*., 1997; Gilbert *et al*., 1993). The restricted pattern of FGFR2b distribution and remarkable specificity of KGF for FGFR2b isoforms account for the predominant epithelial activity of KGF.

One of the defining features of the FGF family is a strong affinity for heparin and heparan sulfate proteoglycans (HSPGs), the latter present on cell surfaces and in the extracellular matrix (Burgess and Maciag, 1989). FGF proteins contain HSPG-binding domains that are topologically defined by specific loop regions in the conserved FGF secondary structure (Ornitz and Itoh, 2001). The spatial arrangement of basic amino acid residues within these loops as well as distinctive structural features in the HSPG molecules have a critical role in determining individual FGF–HSPG interactions (Raman *et al*., 2003). Although heparin binding initially facilitated the purification of FGFs (Burgess and Maciag, 1989), including KGF (Rubin *et al*., 1989), subsequent studies established that HSPGs bind both FGFs and FGFRs and have a critical role in strengthening the affinity of their mutual interactions (Pellegrini *et al*., 2000; Schlessinger *et al*., 2000). However, the function of HSPGs in FGF signaling is complex, and dependent both on the specific FGF–FGFR interaction and the ability of FGFs to bind different HSPGs (Ostrovsky *et al*., 2002). Furthermore, in some situations, HSPGs inhibit FGF activity (Aviezer *et al*., 1994; Bonneh-Barkay *et al*., 1997).

Although KGF originally was suspected to have important activities during embryonic development, we now believe that its primary function is that of a homeostatic factor in the mature organism. In particular, KGF serves to maintain the barrier function of epithelial tissues. Several *in vitro* and *in vivo* studies have demonstrated that KGF has potent cytoprotective and regenerative activities in a variety of epithelial contexts. For this reason, efforts have been underway to identify clinical applications for KGF in which the integrity of epithelial surfaces is at risk, and preservation or rapid restoration of these tissues would be of benefit. Specifically, KGF is currently being evaluated in clinical trials sponsored by Amgen (Thousand Oaks, CA) to test its ability to ameliorate severe oral mucositis (OM) that results from cancer chemoradiotherapy.* In the following pages, we review the molecular properties of KGF, the cellular responses it elicits, and pertinent signaling mechanisms. After summarizing observations from transgenic and knockout models, we describe the salient findings from many *in vivo* injury models that provided the foundation for a series of ongoing clinical trials. The design and results of these clinical studies are presented, along with a concise view of future directions for KGF clinical development.

*Palifermin is the name for recombinant human KGF that will be used in the clinical setting. Note that a truncated derivative of FGF10 was given the name Repifermin.

II. MOLECULAR BIOLOGY OF KGF

A. Protein

1. STRUCTURE

KGF was purified as a monomeric polypeptide with an apparent molecular mass of 26–28 kDa (Rubin *et al.*, 1989). The minor heterogeneity observed in molecular weight presumably was due to differences in glycosylation or partial proteolysis. The KGF cDNA encoded a 194-amino acid protein, including a classic signal peptide for secretion and one potential N (asparagine)-linked glycosylation site (Fig. 1) (Finch *et al.*, 1989). When expressed in bacteria, a biologically active protein was obtained with an apparent molecular size of 21 kDa and a specific activity approximately 10-fold greater than that of the native protein isolated from fibroblast-conditioned medium (Ron *et al.*, 1993a). A likely explanation for the difference in size was the absence of glycosylation from the bacterially expressed recombinant protein, suggesting that this posttranslational modification may in some way hinder KGF stability or interactions with its receptor (Ron *et al.*, 1993a). Analysis of recombinant KGF amino (N)-terminal truncation mutants demonstrated that the first 23 amino acid residues of KGF downstream from the signal sequence could be removed without decreasing mitogenic activity, whereas sequential deletion of the next 6 residues dramatically reduced biological activity (Nybo *et al.*, 1997; Osslund *et al.*, 1998; Ron *et al.*, 1993a). Similarly, heparin-binding properties were preserved with deletion of up to 28 amino acid residues of the mature molecule, but lost with the removal of an additional 10 residues (Ron *et al.*, 1993a).

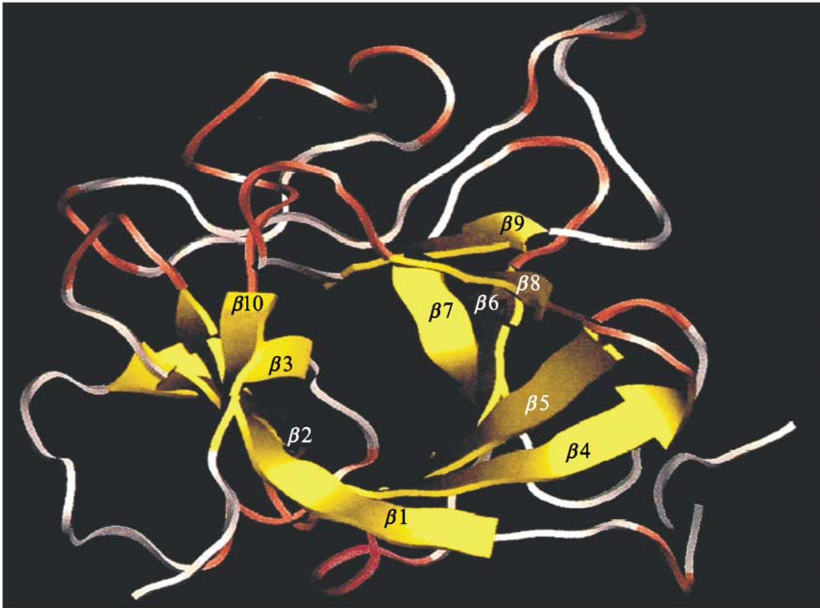
The crystal structure of KGF was determined at a resolution of 1.6 Å (Osslund *et al.*, 1998), and featured a β-trefoil motif similar to that of other FGF family members whose structures have been solved, as well as interleukin 1 (IL-1). This motif consists of 12 antiparallel β strands in which three pairs of the strands form a β-barrel structure, and the other three pairs cap the barrel with hairpin triplets, forming a triangular array (Ago *et al.*, 1991; Blaber *et al.*, 1996; Eriksson *et al.*, 1991; Graves *et al.*, 1990; Zhang *et al.*, 1991; Zhu *et al.*, 1991). The KGF structure has 10 well-defined β strands that form five double-stranded antiparallel β sheets (Fig. 2A and B), and a poorly defined sixth β-strand pair identified by a single β-sheet hydrogen bond between residues 168 and 172 (Osslund *et al.*, 1998).

A comparison of the three-dimensional structure of KGF with that of FGF10 revealed a high degree of homology (Osslund *et al.*, 1998; Yeh *et al.*, 2003). Furthermore, there are several distinctive traits unique to KGF subfamily members. For example, their $\beta 1$ strands are longer than those

Fig. 1 The amino acid sequence of recombinant human KGF. The N-terminal sequence of the native protein purified from fibroblast culture fluid began at residue C32; the presumptive signal sequence is shown to the right. Biological activity remained intact after removal of the first 23 residues, starting with C32, but further truncation resulted in a loss of activity. A derivative lacking the first 23 residues and containing an R175Q substitution was used to obtain the crystal structure of KGF. A single putative N-linked glycosylation site occurs at N45. Disulfide bonds linking C32 to C46 and C133 to C137 are indicated by solid lines. Site-directed mutagenesis demonstrated that the residues highlighted in red, blue, and yellow, which are present in the N terminus, β4/β5 loop, and β8 strand, respectively, are important for biological activity (Sher *et al.*, 2000, 2003). The shaded residues S153–M163 comprise a loop contributing to the putative secondary binding site for FGFR2b; alanine scanning indicated that W156 was particularly important for biological activity (Osslund *et al.*, 1998; Sher *et al.*, 1999). (Adapted from Osslund *et al.*, 1998, Fig. 1, p. 1682; numbering of residues has been changed from system used in that article to account for residues in the signal sequence.)

of FGF1 and FGF2, their β1/β2 and β9/β10 loops adopt different conformations because their lengths differ from the corresponding structures in FGF1 and FGF2 and, as mentioned previously, their β10/β11-strand pair was defined by a single hydrogen bond between the two strands. The N termini of FGF1 and FGF2 are considerably disordered, perhaps as a result of a *cis/trans* isomerization of prolines, such that the N-terminal segments were not defined in the electron density maps. In contrast, the KGF and

A



B

Starting residue	Ending residue	Beta strand designation	Double strand connection	Triple strand connections
Residue: 64	Residue: 71	β1	β4	β1, β4, β5
Residue: 76	Residue: 79	β2	β3	
Residue: 85	Residue: 88	β3	β2	
Residue: 98	Residue: 104	β4	β1, β5	β1, β4, β5
Residue: 107	Residue: 112	β5	β4	β1, β4, β5
Residue: 118	Residue: 121	β6	β7	
Residue: 127	Residue: 130	β7	β6	
Residue: 139	Residue: 143	β8	β9	
Residue: 149	Residue: 153	β9	β8	
Residue: 188	Residue: 192	β10	β1	

Fig. 2 KGF secondary structure. (A) The KGF structure has 10 well-defined β strands that form five double-stranded antiparallel β sheets. In addition, the β strand β4 is the central strand in a triple-stranded β sheet. This triple-stranded sheet is not present in FGF1 or FGF2, as the β strand β1 is significantly longer in KGF. A sixth poorly defined β strand pair is in the loop between residues 164 and 175, and is defined by only a single hydrogen bond between the two strands (this corresponds to the similarly defined β10/β11 pair in FGF10). (B) Pertinent information about the boundaries and connections of the β strands in KGF. (Adapted from Osslund *et al.*, 1998, Fig. 4, p. 1686, with numbering of residues modified as noted in the legend to Fig. 1.)

FGF10 N termini are substantially more ordered, in part because they do not exhibit this proline isomerization (note, however, that the crystal structure of KGF was determined with a truncated derivative that lacked a proline residue upstream of the conserved FGF core) (Osslund *et al.*, 1998). In addition, a four-amino acid N-terminal extension of the β1 strand, and a common glycine turn that causes the first five ordered amino acids to fold back toward the core structure, serve to provide additional stabilization (Osslund *et al.*, 1998; Yeh *et al.*, 2003). KGF also possesses a hydrogen bond between the backbone nitrogen of Y56 and the carbonyl of C133, which further contributes stability to the N terminus (Osslund *et al.*, 1998). Sequence comparison suggests that many of these features also may be present in FGF22 (Nakatake *et al.*, 2001). It has been hypothesized that these structural characteristics play a role in determining the receptor specificity of the KGF subfamily (Yeh *et al.*, 2003).

2. FGFR2B INTERACTIONS

By utilizing domain swapping and site-directed mutagenesis, the N-terminal portion and the β4/β5 loop of KGF were found to contribute to high-affinity binding to FGFR2b (Reich-Slotky *et al.*, 1995; Sher *et al.*, 2000). Point mutations in the loop connecting β9/β10 strands did not alter receptor-binding affinity (Sher *et al.*, 1999), but caused a decrease in mitogenic potency (Osslund *et al.*, 1998; Sher *et al.*, 1999) and receptor-mediated phosphorylation events (Sher *et al.*, 1999). W156 appeared to be particularly crucial, as its replacement by an alanine residue almost completely abolished mitogenic activity (Osslund *et al.*, 1998; Sher *et al.*, 1999). These results were similar to results previously reported for FGF2, in which the loop connecting β9/β10 strands was shown to form a secondary, low-affinity receptor-binding site that was required for receptor activation (Springer *et al.*, 1994).

Although a KGF–FGFR2b crystal structure has not been reported, the structure of a complex consisting of FGF10 and the ligand-binding portion of FGFR2b has been determined (Yeh *et al.*, 2003). This study identified unique contacts between FGF10 and two loops in the third Ig domain of FGFR2b (D3), designated βB′-βC and βC′-βE, that provide the basis for ligand/receptor-binding specificity. The majority of contacts occur between FGF10 and a wide cleft in D3. One side of the cleft consists of the βB′ strand of the βB′-βC loop; this strand is also present in the FGFR2c isoform. The other side of the cleft is composed of the βC′-βE loop, which is located in the second half of D3, and therefore is unique to FGFR2b. The N terminus, β1 strand, β4 strand, and β7/β8 loop of FGF10 made specific contacts with both sides of this cleft. These segments exhibit significant sequence diversity among the FGF family members. Mutations within the N terminus and β7/β8 loop of FGF10 resulted in molecules with reduced capacity to induce

DNA synthesis in BALB/MK mouse keratinocytes, underscoring the importance of these regions for receptor binding (Yeh *et al.*, 2003). The only FGF10–D3 contact outside of the D3 cleft occurred between β8 of FGF10 and the FGFR2b-specific βF-βG loop of D3.

Because key determinants of FGF10–FGFR2b binding involve structural features that typify the KGF subfamily, it is likely that these properties also are relevant to KGF–FGFR2b interactions. For example, the primary role of D3 in FGF10–FGFR2b binding was consistent with an earlier observation that a D3 derivative bound KGF with high affinity (Cheon *et al.*, 1994). Furthermore, the portion of the FGF10 N terminus involved in receptor binding corresponded to KGF residues previously reported to be required for potent biological activity (Osslund *et al.*, 1998; Ron *et al.*, 1993a). As described previously, the β4 strand and N terminus of KGF also have been implicated in FGFR2b binding (Osslund *et al.*, 1998; Reich-Slotky *et al.*, 1995; Ron *et al.*, 1993a; Sher *et al.*, 2000). A mutation in the β8 loop of KGF reduced both receptor-binding affinity and mitogenic activity (Sher *et al.*, 2003), like similar mutations introduced into FGF10 (Yeh *et al.*, 2003). Mutagenesis and peptide-binding studies indicated that the FGFR2b-specific βC′-βE loop, which comprised part of the D3 cleft involved in FGF10 binding, participated directly in KGF binding (Bottaro *et al.*, 1993; Gray *et al.*, 1995; Wang *et al.*, 1999a). Finally, mutations within the FGFR2b-specific, βF-βG loop of D3, a region that makes contact with FGF10 outside of the D3 cleft (Yeh *et al.*, 2003), have been shown to reduce KGF binding (Gray *et al.*, 1995).

Besides its interactions with D3, FGF10 induced a rotation of the second Ig loop of FGFR2 (D2), resulting in specific contacts with FGF10 (Yeh *et al.*, 2003). Thus, although FGF10 and presumably KGF interactions with D3 are of primary importance, binding to D2 occurred as well. Interactions with both D2 and D3 also have been documented for FGF2 binding to FGFR1 (Plotnikov *et al.*, 1999; Venkataraman *et al.*, 1999) and for FGF1 binding to FGFR2 (Stauber *et al.*, 2000).

The FGF10–FGFR2b crystal structure did not identify receptor contacts with the β9/β10 loop of FGF10. As noted previously, this loop is thought to function in KGF as a secondary receptor-binding site. Because the FGF10–FGFR2b complex was generated as a binary unit in the absence of heparin, it is possible that such interactions would be detected in a higher order complex consisting of FGF10 or KGF, FGFR2b, and heparin oligosaccharide. On the basis of prior work with other FGF–FGFR combinations, two ligand molecules would be expected to associate with two FGFR2b molecules. In such complexes involving FGF1 or FGF2 and FGFRs, individual FGF molecules form contacts with two receptor molecules via primary and secondary binding sites (Pellegrini *et al.*, 2000; Plotnikov *et al.*, 1999; Schlessinger *et al.*, 2000; Stauber *et al.*, 2000; Venkataraman *et al.*, 1999).

We surmise that in similar complexes of KGF or FGF10 and FGFR2b, the $\beta9/\beta10$ loop would be in contact with a second FGFR2b molecule.

3. HSPG Binding and Activity

Heparin/HSPG binding has long been recognized to have a major impact on FGF activity. Many early studies indicated that HSPGs stimulated the activity of FGF1 and FGF2, presumably by increasing their local concentration at the cell surface, or by inducing conformational changes in the FGFs that enhanced their stability and/or FGFR interactions (Aviezer *et al.*, 1994; Klagsbrun and Baird, 1991; Mansukhani *et al.*, 1992; Rapraeger *et al.*, 1991). More recent crystallographic work showed that HSPG molecules stabilize the formation of FGF–FGFR complexes by binding both to ligand and receptor molecules (Pellegrini *et al.*, 2000; Schlessinger *et al.*, 2000). HSPG enhanced the binding of individual FGF and FGFR molecules to each other, and facilitated the association of higher order FGF–FGFR complexes.

In contrast to FGF1 and FGF2, initial reports demonstrated that heparin inhibited KGF activity (Reich-Slotky *et al.*, 1994; Ron *et al.*, 1993a; Strain *et al.*, 1994). Moreover, a reduction in the amount of endogenous proteoglycan on the surface of BALB/MK mouse keratinocytes or rat myoblast–FGFR2b transfectants decreased FGF1 binding and mitogenicity, whereas corresponding KGF activities increased (Reich-Slotky *et al.*, 1994). Subsequently, glypican-1 was identified as an HSPG that stimulated FGF1 signaling, but inhibited KGF activity in various assays (Berman *et al.*, 1999; Bonneh-Barkay *et al.*, 1997). In cells lacking HSPG, heparin exhibited a biphasic effect on KGF activity: at physiological levels KGF binding to FGFR2b was enhanced, whereas higher heparin concentrations were inhibitory (Berman *et al.*, 1999; Hsu *et al.*, 1999; LaRochelle *et al.*, 1999). Presumably, when heparin is added to cells that express ample quantities of HSPG, the overall amount of heparin/HSPG at the cell surface may be sufficiently high to inhibit KGF activity.

Cell-free analysis revealed that HSPG was required for KGF binding to soluble monomeric FGFR2b (Hsu *et al.*, 1999), but not necessary for binding to preformed FGFR2b dimers (LaRochelle *et al.*, 1999). Moreover, the KGF–FGFR2b complex formed when KGF and monomeric FGFR2b were incubated with heparin contained two FGFR2b molecules. These results implied that HSPG stimulated KGF binding to FGFR2b by promoting receptor dimerization and, consequently, the simultaneous binding of a single KGF molecule with two FGFR2b molecules. Other studies demonstrated that HSPG facilitated monomeric interactions between KGF and FGFR2b (Berman *et al.*, 1999; Ostrovsky *et al.*, 2002). Combined with the evidence that HSPG binds both to KGF and FGFR2b (Hsu *et al.*, 1999; LaRochelle *et al.*, 1999), the general pattern of HSPG effects on KGF–FGFR2b

interactions appears to resemble that documented for other FGF–FGFR combinations.

Nonetheless, as noted at the outset of this section, there are differences in the way HSPGs regulate KGF compared with other FGFs. Differences even exist between KGF and FGF10: heparin stimulated FGF10 mitogenic activity on BALB/MK cells, whereas KGF activity was reduced (Igarashi *et al.*, 1998). Contrasting effects of HSPG on FGF function can be attributed in large measure to the distinct activities, tissue distributions, and FGF–FGFR specificities of differentially modified HSPG molecules (Allen and Rapraeger, 2003; Chang *et al.*, 2000; Friedl *et al.*, 1997; Kreuger *et al.*, 2001; Ostrovsky *et al.*, 2002; Powell *et al.*, 2002; Pye *et al.*, 2000; Ye *et al.*, 2001). For instance, 2-O- and 6-O-desulfated heparin activated FGF1 signaling via FGFR2b but had no effect on KGF signaling (Ostrovsky *et al.*, 2002). Alternatively, heparin oligosaccharides rich in 3-O-sulfate were effective at protecting KGF from protease digestion, implying that this sulfated HSPG interacted with KGF (Ye *et al.*, 2001). Interestingly, experiments with selectively sulfated heparin molecules indicated that O-sulfated groups were critical for FGF10 activity during lung bud formation. Furthermore, the effect of FGF10 in branching morphogenesis was in part determined by regional distribution of O-sulfated HSPGs (Izvolsky *et al.*, 2003a,b). Taken together, these data indicate that the presence of specific patterns of HSPG modification represents a critical determinant for FGF binding, and may provide an important mechanism whereby ligands with similar FGFR-binding properties, such as KGF and FGF10, can elicit different biological responses *in vivo*.

A number of studies have provided valuable insights about the HSPG-binding domain of KGF. An analysis with peptides spanning structural motifs in the KGF protein identified regions in the N terminus (residues 64–103) and C terminus (residues 148–194) that contributed to heparin binding (Kim *et al.*, 1998). Superimposition of the Cα trace of the KGF β-trefoil scaffold with that of FGF2 revealed that there was significant structural homology between the putative KGF HSPG-binding domain and that of FGF2 (Faham *et al.*, 1996; Raman *et al.*, 2003). However, the spatial arrangement of the basic amino acid residues and their side-chain conformations within the pocket were significantly different (Osslund *et al.*, 1998; Raman *et al.*, 2003; Ye *et al.*, 2001). In particular, the nonpolar residue V143 of KGF, which superimposed with K126 of FGF2, could not participate in HSPG binding, although T154 may replace some of the binding energy lost due to the valine substitution (Osslund *et al.*, 1998). Heparin oligosaccharides of six to eight residues were sufficient for FGF1- and FGF2-induced FGFR dimerization and activation (Aviezer *et al.*, 1994; Guimond *et al.*, 1993; Ornitz *et al.*, 1992). In contrast, only relatively long oligosaccharides stimulated receptor binding and activation by KGF (Hsu *et al.*, 1999; Ostrovsky *et al.*, 2002).

Furthermore, protection of KGF from protease digestion required longer heparin oligosaccharides compared with those required to protect FGF1 and FGF2 (Ye *et al*., 2001). These data indicated that the positive charge of the HSPG-binding site of KGF was weaker and more dispersed than that of FGF2. Such differences may account for the contrasting HSPG-binding specificities of individual FGFs (Raman *et al*., 2003). Understanding the HSPG-binding properties of KGF not only enhances our knowledge about the molecular basis of its activities, but also may provide insight about mechanisms that govern the bioavailability and pharmacokinetics of recombinant KGF used in a clinical setting (see Section VII).

In addition to HSPG, KGF also binds dermatan sulfate, the predominant glycosaminoglycan in skin, and its activity is potentiated by this interaction (Trowbridge *et al*., 2002). Furthermore, besides associating with the heparan moieties of HSPG, KGF interacts with specific domains in the protein core of perlecan, an HSPG expressed primarily at cell surfaces and in basement membranes (Mongiat *et al*., 2000), and this interaction might contribute to the stimulatory effect of perlecan on KGF activity (Ghiselli *et al*., 2001). KGF also binds to other protein components of the extracellular matrix, in particular, collagens I, III, and IV, using the consensus sequence glycine-proline-hydroxyproline (Gly-Pro-Hyp) as the binding motif (Ruehl *et al*., 2002). Association with these collagens may further determine the spatial distribution of KGF.

B. Expression

1. MESENCHYMAL–EPITHELIAL PARADIGM

KGF and FGFR2b expression in cell culture, as well as KGF target cell specificity, suggested a role for this pathway in mediating mesenchymal–epithelial interactions known to be important both during organogenesis and in the adult. To investigate this possibility, *in situ* hybridization (ISH) expression studies were performed with mouse embryos. FGFR2b transcripts were detected during gastrulation, but their spatial distribution was diffuse and overlapped with that of the FGFR2c transcript (Orr-Urtreger *et al*., 1993). However, as development progressed, FGFR2b transcripts became prominent in the surface ectoderm and its derivative structures, including the mammary gland, as well as in epithelial cells of the respiratory, gastrointestinal (GI), and urogenital systems (Finch *et al*., 1995a; Orr-Urtreger *et al*., 1993).

KGF transcripts were detected in mesenchymal cells adjacent to epithelia that expressed FGFR2b (Finch *et al*., 1995a; Mason *et al*., 1994). Although KGF and FGFR2b typically were coexpressed in developing organs by

midgestation (Finch *et al.*, 1995a), expression of FGFR2b often preceded that of KGF during organogenesis (Mason *et al.*, 1994; Orr-Urtreger *et al.*, 1993). For instance, FGFR2b transcripts were evident in the surface epithelium as early as 10 days postcoitus (dpc), whereas KGF expression was first detected in the dermis between 15.5 and 16.5 dpc, corresponding to the time when the epidermis changes from a simple to a stratified epithelium (Mason *et al.*, 1994). In contrast, the temporal expression of FGF10 and FGFR2b was similar in developing organs. In lung, KGF transcripts were not present until 14.5 dpc (Bellusci *et al.*, 1997), and were distributed in a diffuse manner throughout the entire lung mesenchyme (Finch *et al.*, 1995a; Mason *et al.*, 1994). However, FGF10 transcripts were expressed in the mesenchyme adjacent to distal buds from the earliest stages of lung development (11.5 dpc) (Bellusci *et al.*, 1997; Park *et al.*, 1998). A similar distinction in the temporal and spatial distribution of KGF and FGF10 transcripts was observed in the ventral prostate (Thomson and Cunha, 1999). These results suggested that KGF contributed to mesenchymal–epithelial communication, particularly in the later stages of organogenesis, but that other factors such as FGF10 probably had a more critical role in organ development. This interpretation was reinforced by the phenotypes of pertinent knockout mouse models (see Section V).

KGF and FGFR2b transcripts were also widely expressed in adult tissues, including skin (Werner, 1998), lung (Ulich *et al.*, 1994), breast (Pedchenko and Imagawa, 2000b), and organs of the GI system (Housley *et al.*, 1994) and reproductive tracts (Koji *et al.*, 1994; Thomson *et al.*, 1997). ISH indicated that the spatial distribution of transcripts closely followed the paradigm established during development, with KGF expressed in the stroma and FGFR2b in the epithelium. These data suggested that KGF was a potential homeostatic factor for epithelia in the adult.

2. OTHER PATTERNS

ISH analysis revealed that KGF was expressed during development in other locations not associated with mesenchymal–epithelial communication. Expression was first seen in the heart at 8.5 dpc and persisted until 11 dpc (Mason *et al.*, 1994). Transcripts were detected throughout the myocardium in both trabeculated and compact regions, although signal was stronger in the atrium than the ventricle. Both KGF and FGFR2b transcripts were detected in the perichondrium and cartilage of limbs, ribs, pelvic bones, larynx, and trachea (Finch *et al.*, 1995a). Furthermore, KGF and FGFR2b transcripts were jointly expressed in developing skeletal muscle (Finch *et al.*, 1995a; Mason *et al.*, 1994), as well as in smooth muscle of the small intestine (Finch *et al.*, 1995a). KGF mRNA was also transiently expressed in the developing forebrain around 14.5 dpc in three separate regions of the

ventricular zone, where the progenitor cells of neurons and glia proliferate (Mason *et al*., 1994). These data suggested that KGF may have diverse roles during development in tissues not governed by mesenchymal–epithelial interactions.

a. Epithelial Cells

Although numerous studies of KGF expression *in vitro* and *in vivo* indicated that KGF was primarily expressed by stromal cells from a variety of organs, there also have been reports of KGF expression by normal epithelial cells. KGF expression was observed by ISH in the endometrial epithelium of porcine uterus, and was especially prominent during days 12 and 15 of the estrous cycle and pregnancy (Ka *et al*., 2000). KGF expression was upregulated by 17β-estradiol, and postulated to stimulate the proliferation and differentiation of the conceptus trophectoderm (Ka *et al*., 2000). KGF transcripts were detected in cultured ovarian surface epithelial cells derived from bovine ovaries, and KGF stimulated the growth of these cells *in vitro* (Parrott *et al*., 2000). Ovarian epithelial cells are of mesodermal origin, arising from the mesothelial lining of the abdomen, and express a mixture of classically epithelial and mesenchymal markers (Parrott *et al*., 2000). Conceivably, this mixed lineage may account for the coexpression of KGF and receptor. In addition, KGF transcript was detected in primary lens epithelial cells (Weng *et al*., 1997). Taken together, these findings suggest that certain normal epithelial cells express KGF and may be able to respond to it, although further investigation is required to establish the physiologic significance of these observations.

b. Vascular Cells

KGF expression has been detected in a variety of vascular smooth muscle cells. For instance, ISH analysis identified KGF mRNA in smooth muscle cells from the spiral arteries of monkey endometrium (Koji *et al*., 1994). Cultured smooth muscle cells from human saphenous vein and iliac artery expressed and secreted mitogenically active KGF. KGF transcripts were detected in normal and atherosclerotic human arteries, although FGFR2b transcripts were not observed (Winkles *et al*., 1997). However, others described FGFR2b expression by cultured rat vascular smooth muscle cells. Furthermore, KGF was shown to stimulate the growth of these cells by approximately 10% (Onda *et al*., 2003). Even though this represented a small increase, it appeared to be statistically significant.

Although KGF lacks activity on cultured endothelial cells derived from large vessels, there is one report that it stimulated neovascularization in the cornea and proliferation of endothelial cells derived from small vessels (Gillis *et al*., 1999). Because FGFR2b was not detected in these cells, it was hypothesized that KGF may act on microvascular endothelial cells

through an as yet undiscovered high-affinity receptor. The significance of this observation awaits further confirmatory studies.

c. Lymphocytes

Epithelial tissues contain T cells that express the $\gamma\delta$ T cell receptor (TCR). $\gamma\delta$ T cells have evolved to recognize antigen in a different manner and perform a broader set of functions than T cells expressing $\alpha\beta$ TCRs. Activated $\gamma\delta$ T cells from the skin, intestine, and vagina express KGF, whereas intraepithelial $\alpha\beta$ T cells, as well as all lymphoid $\alpha\beta$ and $\gamma\delta$ T cells, did not produce measurable quantities of KGF (Boismenu and Havran, 1994; Rakasz *et al*., 1996). It was postulated that $\gamma\delta$ T cells recognized antigens expressed by injured epithelial cells, and this triggered the synthesis of KGF to minimize damage to epithelial surfaces, and hasten wound repair.

$\alpha\beta$ T cells isolated from the thymus also have been reported to express KGF in a developmentally regulated manner (perhaps negative results mentioned previously were due to use of a less sensitive assay). KGF expression was undetectable in $CD3^{-}4^{-}8^{-}$ thymocytes, but was readily observed in mature $CD4^{+}$ and $CD8^{+}$ thymocytes (Erickson *et al*., 2002). Exposure of thymocyte-depleted fetal thymic lobes to KGF resulted in decreased thymic epithelial expression of class II major histocompatibility complex, and stimulated expression of IL-6. In intact fetal thymic organ cultures, KGF inhibited the generation of $CD4^{+}$ thymocytes (Erickson *et al*., 2002). These findings implied that KGF–FGFR2b signaling participated in the development and function of thymic epithelium. As lymphocytes are derived from mesoderm, it is reasonable to consider KGF release by these cells as a special example of its paracrine mode of action on epithelial cells.

3. UPREGULATION AFTER INJURY

KGF expression was dramatically upregulated after cutaneous injury in mouse and human full-thickness excisional wounds (Marchese *et al*., 1995; Werner *et al*., 1992). Increased KGF expression was confined to the dermal compartment. KGF transcripts also were elevated after tissue damage in models of surgical bladder injury (Baskin *et al*., 1997), chemically induced kidney injury (Ichimura *et al*., 1996), exposure of neonatal rabbit lungs to hyperoxia (Charafeddine *et al*., 1999), and acute lung injury resulting from bleomycin injection (Adamson and Bakowska, 1999). In an experimental model of fasting-induced gut atrophy, KGF was increased in the ileum, suggesting this may be an adaptive response to limit the extent of mucosal wasting (Estivariz *et al*., 2000). Furthermore, upregulation of KGF was documented in some human inflammatory diseases, including psoriasis (Finch *et al*., 1997) and inflammatory bowel disease (IBD) (Bajaj-Elliott *et al*., 1997; Brauchle *et al*., 1996; Finch *et al*., 1996). In both these

disorders, FGFR2b expression was observed in the epithelial cells, while KGF was synthesized in the underlying stroma. These studies suggested that KGF participates in a wide variety of epithelial preservation and/or repair processes.

4. CYTOKINE AND HORMONAL REGULATION

The many examples of KGF induction during acute or chronic epithelial injury and repair prompted studies to determine how KGF expression is regulated in these situations. IL-1 and IL-6, proinflammatory cytokines expressed by macrophages and polymorphonuclear leukocytes, stimulated either a large (IL-1) or moderate (IL-6) increase in KGF mRNA and protein synthesis in fibroblasts from multiple sources (Brauchle *et al*., 1994; Chedid *et al*., 1994; Tang and Gilchrest, 1996). KGF expression also was induced by serum, or various purified serum growth factors, including platelet-derived growth factor BB (PDGF BB) and transforming growth factor α (TGF-α) (Brauchle *et al*., 1994; Chedid *et al*., 1994). These results support the hypothesis that KGF upregulation after tissue injury is initiated by growth factors, such as PDGF BB and TGF-α, released from platelets. However, subsequent induction of KGF mRNA presumably is due to the release of proinflammatory cytokines, such as IL-1 and IL-6, from macrophages and polymorphonuclear leukocytes, which infiltrate the wound within 24 h of injury (Werner, 1998). The fact that KGF expression also was increased by phorbol esters and nonhydrolyzable cAMP analogs implied that induction was mediated by at least two different pathways, involving protein kinase C and cAMP-dependent protein kinases (Brauchle *et al*., 1994).

Sex steroid hormones are thought to affect epithelial cells via an indirect mechanism involving an initial interaction with nearby stromal cells. KGF has been implicated as a potential paracrine mediator of steroid hormone action on epithelia in organs of the male and female reproductive tracts, including seminal vesicle, prostate, and endometrium (Alarid *et al*., 1994; Koji *et al*., 1994; Sugimura *et al*., 1996). Androgen stimulated KGF expression in adult rat prostate stromal cells (Yan *et al*., 1992), as well as human fetal prostate stromal cells *in vitro* (Levine *et al*., 1998). FGF10 also was upregulated by androgen in normal prostate stromal cells (Lu *et al*., 1999). However, the significance of these findings is unclear as subsequent studies indicated that KGF and FGF10 might not be direct targets of androgen activity *in vivo* (Nemeth *et al*., 1998; Thomson *et al*., 1997). Progesterone treatment of rhesus monkeys resulted in a marked elevation in KGF transcript levels in stromal cells of the endometrium (Koji *et al*., 1994), whereas estrogen was reported to induce KGF expression in mammary stromal cells (Pedchenko and Imagawa, 2000a,b).

KGF expression was negatively regulated by glucocorticoids (Brauchle *et al*., 1995; Chedid *et al*., 1996; Tang and Gilchrest, 1996). Dexamethasone decreased the transcriptional rate and destabilized the KGF transcript (Chedid *et al*., 1996). In contrast, nonsteroidal antiinflammatory agents had no effect on KGF synthesis.

C. Gene Structure and Promoter Analysis

The *FGF* genes are highly conserved in their overall structure, consisting of three exons that encode the primary translation product and intervening introns situated in the same locations relative to the protein-coding sequences (Ornitz and Itoh, 2001). The *KGF* gene conforms to this structure, with introns between the three coding exons positioned after nucleotides 731 and 835 in the cDNA sequence (Kelley *et al*., 1992). Both the human and rat *KGF* genes contain an intron in the 5′ untranslated region. The intron in the human gene is approximately 650 bp long and located between nucleotides 179 and 180 (Finch *et al*., 1995b), whereas the intron in the rat gene is 585 bp long and located in a similar position (Fasciana *et al*., 1996). The locus of the human *KGF* gene is chromosome 15q13-q22 (Zimonjic *et al*., 1997). However, a portion of the gene consisting of exon 2, exon 3, the intron between them, and an element encoding 3′ noncoding sequence of the KGF transcript was amplified during evolution to approximately 16 copies and dispersed to multiple chromosomes. These KGF-like sequences are transcriptionally active, differentially regulated in various tissues, and comprise three distinct classes of coding sequence that are 5% divergent from each other and from the true KGF gene product (Kelley *et al*., 1992). However, the functional significance of these sequences is not clear, as it is not known whether they direct protein synthesis. Interestingly, such dispersion of *KGF*-like genes was evident to a varying extent in the genomic DNAs of chimpanzee, gorilla, and orangutan, but not in gibbon, old world monkeys, mouse, or chicken (Kelley *et al*., 1992; Zimonjic *et al*., 1997), reflecting stepwise changes that have occurred in the genomes of the great apes and humans during evolution (Zimonjic *et al*., 1997).

The 5′ flanking regions of the human and rat *KGF* genes have been cloned and the transcription initiation sites determined (Fasciana *et al*., 1996; Finch *et al*., 1995b). The human promoter contains two transcription start sites, whereas only one was identified in the rat promoter region. The location of the first (and major) start site in the human promoter is identical to that present in the rat. Putative promoter sequences, TATTTA and CCAAT, were identified 31 and 50 bp upstream from the first mRNA start site. For the human 5′-flanking region, transient transfection analysis identified a basal promoter region located between bases −225 and +190 (Finch

et al., 1995b). Within this region, the human and rat sequences are 78% homologous, but upstream of this point the sequences diverge.

The basal promoter from the human gene directed transcription of a chloramphenicol acetyltransferase (*CAT*) reporter gene in fibroblasts and myoblasts, but no activity was observed when it was transfected into epithelial or lymphoid cells or macrophages. Consistent with cytokine regulation of KGF expression, transcription from the basal *KGF* promoter was induced by IL-1, IL-6, and forskolin. These results indicated the presence of *cis*-acting elements that are responsible for selective activation of the *KGF* promoter only in cells that normally express KGF mRNA (Finch *et al*., 1995b). Experiments performed with the rat *KGF* promoter region showed that the segment between bp −1200 and −1900 was involved in upregulation by the synthetic androgen R1881 after transient transfection into LNCaP prostatic cells (Fasciana *et al*., 1996). Furthermore, a longer construct containing the region up to bp −4700 had significantly higher activity than did the −1900 bp construct, indicating the presence of additional activating sequences. The inducibility of the human *KGF* promoter region by androgens has not been examined.

An *in vitro* study of the human *KGF* promoter region focused on determining the role of a novel regulatory element, TGAGGTCAG, in mediating the induction of *KGF* transcription (Zhou and Finch, 1999). This element is homologous to binding sites for both the ATF/CREB (TGACGTCA) and C/EBP (TGNNGNAAG) families of transcription factors, both of which have been implicated in mediating induction of gene expression in response to extracellular stimuli. This element conferred sensitivity to induction by forskolin when cloned in front of a heterologous simian virus 40 (SV40) promoter. Gel mobility supershift assays indicated that two members of the ATF family, ATF1 and ATF2, were present in the nuclear protein complex bound to this region. Furthermore, purified ATF2 protein bound to the TGAGGTCAG sequence. There was no evidence of C/EBP transcription factors in the complex.

Further information concerning the regulation of *KGF* gene expression came from an *in vitro* skin model consisting of human keratinocytes cocultured with immortalized fibroblasts derived from either wild-type, c-$jun^{-/-}$, or $junB^{-/-}$ mouse embryos (Szabowski *et al*., 2000). The epithelium resulting from coculture with c-$jun^{-/-}$ fibroblasts contained a smaller number of cell layers and the number of proliferating cells in the basal layer was markedly reduced compared with skin in which wild-type fibroblasts had been used. Moreover, KGF expression was not detected in the c-$jun^{-/-}$ fibroblasts. In contrast, $junB^{-/-}$ fibroblasts contained high basal levels of KGF, and they stimulated hyperproliferation of the keratinocytes with an increased number of epithelial cell layers (Szabowski *et al*., 2000). Previous work had shown that keratinocytes secrete IL-1, which induces KGF

expression in fibroblasts, and KGF in turn stimulates keratinocytes (Maas-Szabowski *et al*., 1999, 2000; Smola *et al*., 1993). Because c-*jun*$^{-/-}$ fibroblasts did not express KGF, even after treatment with IL-1, it appears that c-Jun is not only critical for basal KGF expression, but also for IL-1 induction of *KGF* transcription (Szabowski *et al*., 2000). In contrast, the presence of high levels of KGF in *junB*$^{-/-}$ fibroblasts indicated that JunB was a negative regulator of *KGF* transcription. Two potential AP-1-binding sites have been identified in the *KGF* promoter region (Finch *et al*., 1995b), raising the possibility that c-Jun directly activates *KGF* transcription by binding to these sites.

III. CELLULAR AND MOLECULAR RESPONSES TO KGF

Expression studies indicated that KGF might play a role in the regulation of epithelial homeostasis in adult organs, particularly during epithelial repair. The following account summarizes responses to KGF that contribute to homeostatic and repair processes.

A. Mitogenicity

Initially, KGF was shown to stimulate DNA synthesis in BALB/MK mouse keratinocytes, B5/589 human mammary epithelial cells, and CCL208 rhesus monkey bronchial epithelial cells (Rubin *et al*., 1989). Subsequently, additional responsive cells were identified: human keratinocytes, rat and human prostatic epithelial cells, rat hepatocytes, type II alveolar cells, corneal epithelial cells, and bovine granulosa cells (Rubin *et al*., 1995). No activity was seen on fibroblasts, saphenous vein endothelial cells, melanocytes, or myoblasts (Halaban *et al*., 1991; Ron *et al*., 1993b; Rubin *et al*., 1989). At least in the case of BALB/MK cells, proliferation in serum-free medium required a combination of KGF and insulin or insulin-like growth factors (IGFs) (Rubin *et al*., 1989). Besides stimulating mitogenic activity in a wide variety of epithelial cell types *in vitro*, several studies have confirmed that KGF is a powerful mitogen for epithelial cells in many different organ systems *in vivo*. These latter results are described in detail in Sections V and VI.

B. Motility

During wound healing, keratinocyte migration is evident immediately after injury and is initially confined to cells at the wound edge. KGF enhanced the migration of normal human keratinocytes in a dose-dependent

manner that was inhibited by incubation with KGF-neutralizing monoclonal antibody (Tsuboi *et al*., 1993). KGF-treated keratinocytes displayed increased attachment to collagen and fibronectin, and KGF preferentially induced keratinocyte migration on these substrates (Putnins *et al*., 1999). KGF was subsequently shown to promote type II alveolar motility in a variety of *in vitro* models (Atabai *et al*., 2002; Galiacy *et al*., 2003; Isakson *et al*., 2001; Waters and Savla, 1999). Keratinocyte migration during re-epithelialization is preceded by detachment from the underlying basement membrane and accompanied by successive degradation of the wound clot, dermal material, and granulation tissue. These remodeling processes involve matrix metalloproteinases (MMPs), which degrade essentially all extracellular matrix components. KGF has been reported to enhance the heparin-dependent synthesis of MMP-1 (collagenase) by keratinocytes (Putnins *et al*., 1996), and to directly induce the synthesis of MMP-9 (gelatinase) (Putnins *et al*., 1995), MMP-10 (stromelysin-2) (Madlener *et al*., 1996), MMP-13 (collagenase-3) (Uitto *et al*., 1998), and urokinase-type plasminogen activator activity (Putnins *et al*., 1995; Tsuboi *et al*., 1993; Zheng *et al*., 1996). These data suggest that KGF regulates the production of proteases that are active in tissue remodeling during wound healing.

C. Differentiation

In a skin equivalent model, KGF induced expression of integrin $\alpha_5\beta_1$, and delayed expression of keratin 10 and transglutaminase, markers of terminal keratinocyte differentiation (Andreadis *et al*., 2001). KGF stimulated the expression of differentiation-specific markers in human keratinocytes in response to an increase in extracellular calcium, consistent with the idea that KGF was involved in the initiation of the early stages of differentiation (Marchese *et al*., 1990, 1997). KGF regulates the expression of the gene encoding the estrogen-responsive B box protein (EBBP) in keratinocytes (Beer *et al*., 2002). *In vivo*, EBBP is expressed at high levels in basal keratinocytes. Stable overexpression of EBBP in HaCaT keratinocytes enhanced the early differentiation process, and may mediate the differentiation-stimulating activity of KGF under permissive conditions (Marchese *et al*., 1990).

KGF also influences epithelial differentiation in the lung. Type I alveolar cells are flat, relatively metabolically inactive cells that are primarily involved in gas exchange. Type II cells are cuboidal, metabolically active cells that synthesize and secrete surfactant protein. After lung injury the type II cells proliferate, line the alveolar septae, and then differentiate into type I cells to facilitate the reconstitution of normal alveolar parenchymal

architecture. Type II cells are also prominent in the fetal lung, where they are thought to play a role in lung morphogenesis. In a cell culture system in which type II alveolar cells develop characteristics of type I cells over time, incubating cells in the presence of KGF maintained the type II phenotype (Borok *et al.*, 1998b; Isakson *et al.*, 2001; Rice *et al.*, 2002). Addition of KGF-neutralizing antibody to isolated lung epithelium decreased fibroblast conditioned medium-stimulated surfactant synthesis by 50% (Chelly *et al.*, 2001). Furthermore, administration of KGF to fetal rat type II cells in various mesenchyme-free culture systems induced distal epithelial differentiation and expression of a number of genes encoding surfactant proteins (Cardoso *et al.*, 1997; Chelly *et al.*, 1999; Deterding *et al.*, 1997; Mason *et al.*, 2002; Sugahara *et al.*, 1995; Xu *et al.*, 1998b). Expression of the surfactant-A (SP-A) and surfactant-D (SP-D) genes is regulated in part by the C/EBPα transcription factor (He and Crouch, 2002; Li *et al.*, 1995). KGF increased the expression of C/EBPα in type II alveolar cells (Mason *et al.*, 2003). In addition to the surfactant proteins, surfactant also contains phospholipid. KGF stimulated lipogenesis in type II cells and the subsequent conversion of the newly synthesized fatty acids into phospholipids (Mason *et al.*, 2003). The induction of the genes for fatty acid synthase, stearoyl-CoA desaturase-1, and epidermal fatty acid-binding protein, mediated in part by increased expression of C/EBPα, as well as the transcription factors C/EBPδ and SREBP-1, likely contributed to the increased fatty acid synthesis in KGF-treated type II cells (Mason *et al.*, 2003).

Administration of recombinant KGF to adult rats caused a marked and selective induction of mucin-producing goblet cells throughout the GI tract (Housley *et al.*, 1994). Members of the intestinal trefoil factor (ITF) family of proteins are selectively expressed in intestinal goblet cells and their expression correlates with intestinal goblet differentiation. In a study of the H2 subclone of the human HT29 colonic epithelial cell line, KGF promoted H2 differentiation into goblet cells as reflected by increased ITF expression (Iwakiri and Podolsky, 2001). Furthermore, KGF regulated mouse ITF transcription through the goblet cell silencer inhibiter, which is essential for goblet cell-specific expression of ITF (Iwakiri and Podolsky, 2001). *In vivo*, KGF enhanced expression of ITF2 and ITF3 throughout the intestine (Fernandez-Estivariz *et al.*, 2003).

KGF stimulated the proliferation of pancreatic exocrine cells and cyto-differentiation to a ductal epithelial phenotype (Miralles *et al.*, 1999; Yi *et al.*, 1994b), while repressing the formation of endocrine cells (Elghazi *et al.*, 2002; Yi *et al.*, 1994b). After KGF was removed from the culture medium, pancreatic precursor cells differentiated into endocrine cells (Elghazi *et al.*, 2002). Thus, KGF may be useful in expanding the population of precursor cells that subsequently can be induced to form insulin-expressing cells.

D. Branching Morphogenesis

Epithelial branching morphogenesis is a highly coordinated process involving cell proliferation, cell–cell and cell–matrix interactions, and remodeling of the basement membrane. A number of studies have demonstrated that KGF can stimulate growth and branching morphogenesis in various fetal explants including seminal vesicle (Alarid *et al.*, 1994), prostate (Sugimura *et al.*, 1996), and pancreas (Miralles *et al.*, 1999). However, as discussed elsewhere in this review, FGF10 rather than KGF is the predominant mesenchymal mediator of epithelial branching morphogenesis during organogenesis. Nonetheless, KGF may provide a proliferative signal that contributes to the formation of branches during development (Park *et al.*, 1998; Post *et al.*, 1996).

E. Antiapoptotic Effects

In postconfluent cultures of normal human keratinocytes, KGF promoted tight packing of cells characterized by a small basal cell morphology, suggesting that it could prevent terminal differentiation and/or apoptosis (Hines and Allen-Hoffmann, 1996). In support of this hypothesis, these cultures produced fewer cross-linked cell envelopes, and exhibited less membrane-associated transglutaminase activity and nucleosomal fragmentation compared with untreated cultures (Hines and Allen-Hoffmann, 1996). KGF-treated hepatocytes exhibited decreased apoptosis in response to actinomycin D and tumor necrosis factor (TNF) than did control cells, or cells treated with hepatocyte growth factor or epidermal growth factor (Senaldi *et al.*, 1998). In a mouse model of total parenteral nutrition, which is associated with high levels of intestinal epithelial cell apoptosis, administration of KGF decreased apoptosis and increased expression of antiapoptotic Bcl-2 proteins (Wildhaber *et al.*, 2003). In the lung, KGF administration induced type II alveolar cell proliferation. On withdrawal of KGF the hyperplastic type II cells underwent apoptosis, and a normal alveolar epithelium consisting primarily of type I cells was restored (Fehrenbach *et al.*, 1999). Therefore, KGF not only stimulated the proliferation of type II cells, but maintained their phenotype by preventing both apoptosis and terminal differentiation into type I cells. KGF also inhibited hyperoxia-induced apoptosis of alveolar epithelial cells, probably by repressing the expression of apoptotic mediators such as p53, p21, Bax, and Bcl-x (Barazzone *et al.*, 1999; Buckley *et al.*, 1998).

In a mouse model of oxidant-induced lung injury, expression of KGF from a tetracycline-inducible, lung-specific transgenic promoter protected the lung epithelium from the hyperoxic insult. Furthermore, KGF induced the

antiapoptotic Akt pathway, and inhibition of this activation by expression of a dominant-negative Akt mutant blocked KGF-mediated protection of the epithelium (Ray *et al.*, 2003). In another study, FGFR2b was shown to interact with p21-activated protein kinase 4 (PAK4), a newly identified member of the PAK family of proteins that are regulated by the Rho family GTPases Rac and Cdc42. A dominant-negative PAK4 mutant blocked KGF inhibition of caspase-3-dependent apoptosis in epithelial cells subjected to oxidant stress (Lu *et al.*, 2003). Thus, Akt and PAK4 appear to be important mediators of the antiapoptotic activity of KGF.

As discussed previously, KGF was upregulated by progesterone in primate endometrium during the menstrual cycle (Koji *et al.*, 1994). At the end of the cycle, withdrawal of progesterone induces the luteal–follicular transition (LFT), which is marked by menstrual sloughing, apoptotic regression of the basalis zone, and a 9-fold decrease in the abundance of KGF transcripts. Addition of exogenous KGF during the LFT inhibited apoptosis in the basalis zone, and had a marked trophic effect on the spiral arteries (Slayden *et al.*, 2000). Therefore, the progesterone-dependent increase in KGF expression may serve to inhibit glandular apoptosis during the nonfertile menstrual cycle.

F. Cytoprotection

The marked induction of KGF after injury raised the possibility that in addition to participating in the physical repair of wounded tissue, KGF might also have cytoprotective activities that would limit cellular damage from external insults and the associated inflammatory response. For example, hyperoxic injury to the lung results in alveolar hemorrhages, exudates, and inflammatory infiltrates (Panos *et al.*, 1995). However, administration of exogenous KGF prevented or attenuated much of the injury caused by this toxic exposure (see Section VI.B). DNA strand break is one of the earliest abnormalities that occurs in cells exposed to oxidative stress and can lead to cell death by apoptotic and necrotic pathways. *In vitro*, KGF diminished the formation of strand breaks in A549 alveolar epithelial cells exposed to radiation (Takeoka *et al.*, 1997) or hydrogen peroxide (Wu *et al.*, 1998). The protective effect of KGF on these cells was blocked by inhibitors of DNA polymerases α, δ, and ε (Takeoka *et al.*, 1997; Wu *et al.*, 1998), and inhibitors of protein kinase C and tyrosine kinases (Wu *et al.*, 1998). Addition of KGF to cultures of type II cells isolated from hyperoxic rats resulted in a significantly decreased number of oxygen-induced DNA strand breaks compared with control cells (Buckley *et al.*, 1998). KGF also had cytoprotective effects on epithelial cells in the GI tract of whole animals, as discussed in Section VI.D.

The molecular mechanisms of the cytoprotective effects of KGF include the induction of genes encoding enzymes that limit oxidative stress. For instance, in HaCaT keratinocytes KGF upregulated the expression of a nonselenium glutathione peroxidase, known as peroxiredoxin VI, which plays a role in the detoxification of hydrogen peroxide and organic peroxides (Frank *et al*., 1997). Furthermore, in mice treated with KGF, increased expression of peroxiredoxin VI and glutathione-*S*-transferase (GST) was observed in the oral squamous epithelium and the intestine, indicating that KGF can regulate expression of these genes in different tissues *in vivo* (Farrell *et al*., 2002; Jonas *et al*., 2000). GST also inactivates reactive oxygen species and is a target gene for Nrf2, a transcription factor that interacts with leucine zipper proteins to bind *cis*-acting, antioxidant response elements in the promoters of genes that encode cytoprotective enzymes (Mulcahy *et al*., 1997; Rushmore *et al*., 1990; Venugopal and Jaiswal, 1996, 1998). Nrf2, itself, is a target of KGF action (Braun *et al*., 2002). Whereas Nrf2 was expressed at high levels in basal keratinocytes of hyperproliferative wound epithelium, Nrf2 knockout mice exhibited no delays in wound healing (Braun *et al*., 2002). This may be explained by upregulation of the related Nrf3 transcription factor, which also appeared to be induced by KGF (Braun *et al*., 2002).

IV. SIGNAL TRANSDUCTION

Whereas significant progress has been made in delineating FGF-mediated signal transduction pathways, there have been relatively few studies that have specifically examined KGF signaling. However, the data currently available suggest that KGF signaling involves pathways common to other FGFs. In this section we provide a brief overview of FGF signaling pathways, and then review data that specifically pertain to KGF.

FGF signaling is initiated when high-affinity binding of an FGF ligand and its cognate receptor induces receptor dimerization, activation of its intrinsic tyrosine kinase activity, and autophosphorylation of the cytoplasmic domains of the receptor (Powers *et al*., 2000). Autophosphorylation sites located within the catalytic core are involved in regulation of kinase activity, whereas autophosphorylation in other regions of the cytoplasmic domain increases the affinity of binding sites for Src homology 2 (SH2) or phosphotyrosine-binding (PTB) domains of effector proteins. On activation, tyrosine-phosphorylated FGFRs function as platforms for the assembly of a variety of signaling proteins, including phospholipase C_{γ} (Mohammadi *et al*., 1991) and Crk, an SH2/SH3-containing adaptor protein that may link FGFR to the downstream signaling molecules Shc, C3G, and Cas (Larsson *et al*., 1999).

Recruitment of signaling proteins in response to receptor stimulation is also facilitated by indirect mechanisms involving membrane-linked docking proteins. FGF stimulation leads to tyrosine stimulation of two highly homologous 90-kDa proteins identified as SNT-1 or FRS2α (Kouhara *et al*., 1997; Wang *et al*., 1996) and FRS2β (Ong *et al*., 2000). The FRS2/SNT proteins are targeted to the plasma membrane by myristoylation at the N terminus and contain a PTB domain that mediates interaction with FGF or nerve growth factor receptors (Dhalluin *et al*., 2000; Meakin *et al*., 1999; Ong *et al*., 2000; Xu *et al*., 1998a). The C-terminal region of FRS2 contains multiple tyrosine residues that are phosphorylated by active FGFRs, and serve as recognition motifs for SH2 domains of Grb2 and Shp2 (Hadari *et al*., 1998; Kouhara *et al*., 1997). The formation of the FRS2–Grb2 complex results in the recruitment of Ras to the plasma membrane, and its activation by Sos-dependent exchange of GDP for GTP. This in turn activates a signaling cascade consisting of the serine/threonine kinase Raf, the dual-specificity mitogen-activated protein kinase (MAPK) kinase MEK, and the MAPK isoforms Erk1 and Erk2. Alternatively, the assembly of a complex containing FRS2α, Grb2, and Gab1 induced by FGF stimulation results in activation of phosphatidylinositol 3-kinase (PI-3K) and downstream effector proteins such as the serine/threonine kinase Akt, whose cellular localization and activation are regulated by products of PI-3K enzymatic activity (Ong *et al*., 2000).

A group of four mammalian orthologs of the *Drosophila Sprouty* (*Spry*) gene have been identified as ligand-induced, feedback inhibitors of FGFR-mediated, MAPK signaling (Casci *et al*., 1999; Gross *et al*., 2001; Hacohen *et al*., 1998; Kramer *et al*., 1999; Reich *et al*., 1999; Yusoff *et al*., 2002). Sprys translocate to the plasma membrane in response to FGF signaling, and have many different binding partners, including various effectors of the MAPK pathway (Impagnatiello *et al*., 2001; Leeksma *et al*., 2002; Lim *et al*., 2000; Sasaki *et al*., 2003; Wong *et al*., 2001, 2002). Sprys require tyrosine phosphorylation to interact with their binding partners (Fong *et al*., 2003; Hanafusa *et al*., 2002). Mouse Spry2 inhibited FGF10-activated MAPK signaling by differentially binding to upstream target proteins (Tefft *et al*., 2002). Sprys are abundantly expressed in the epithelium during the early stages of branching morphogenesis (Hashimoto *et al*., 2002), and mSpry2 functions as a negative regulator of embryonic lung branching morphogenesis and growth (Mailleux *et al*., 2001).

KGF treatment of BALB/MK cells elicited the rapid tyrosine phosphorylation of a p90 protein that in all likelihood corresponded to the SNT/FRS2 proteins (Bottaro *et al*., 1990). Consistent with other FGFs, KGF administration caused a transient activation of MAPK in corneal epithelial cells (Liang *et al*., 1998), human epidermal keratinocytes (Zeigler *et al*., 1999), prostate DU145 cells (Mehta *et al*., 2001), and human endometrial

carcinoma cells (Taniguchi *et al.*, 2003). In keratinocytes the transient nature of the MAPK activation did not correlate with the sustained activation required for cell movement and MMP9 production, cellular responses that are integral to the process of invasion (Zeigler *et al.*, 1999). However, in human endometrial carcinoma cells, blocking the MAPK pathway completely neutralized the proliferative effect of KGF (Taniguchi *et al.*, 2003). KGF also stimulated a 2- to 3-fold increase in PI-3K activity in corneal epithelial cells, and phosphorylation of its downstream target, p70 S6K, with a corresponding increase in its activity (Chandrasekher *et al.*, 2001). As mentioned previously, Akt and PAK4 were required for KGF inhibition of apoptosis in different models of oxidant-induced cell injury (Lu *et al.*, 2003; Ray *et al.*, 2003).

V. TRANSGENIC AND KNOCKOUT MODELS

A number of studies have examined the consequences of targeting KGF expression to various epithelial cell populations, using tissue-specific promoters in transgenic models. When KGF was targeted to the epidermis, using the keratin-14 promoter, the mice were weak and frail, and exhibited grossly wrinkled skin. There was an increase in epidermal thickness accompanied by alterations in epidermal growth and differentiation (Guo *et al.*, 1993). Furthermore, hair follicle morphogenesis was suppressed in these animals, as was adipogenesis. With age, gross transformations in the epidermis and tongue epithelium developed, and animals exhibited increased salivation and altered differentiation of salivary glands. Similarly, when KGF was overexpressed in the embryonic liver of transgenic mice, using an apolipoprotein E promoter, there was marked epidermal papillomatous acanthosis and hyperkeratosis in the skin, with a notable decrease in the number of developing hair follicles. These animals also were characterized by marked hyperplasia and cystic dilation of the cortical and medullary kidney collecting duct system, a phenotype resembling infantile polycystic kidney disease in humans (Nguyen *et al.*, 1996). Overexpression of KGF in the mouse lung epithelium either constitutively (Simonet *et al.*, 1995) or conditionally (Tichelaar *et al.*, 2000) caused a pulmonary malformation that resembled pulmonary cystadenoma in humans. The embryonic lungs had dilated saccules lined with columnar epithelial cells and normal alveolar structure. Embryos constitutively expressing KGF in the lung epithelium died before reaching term (Simonet *et al.*, 1995). Ectopic expression of KGF in pancreatic beta cells resulted in significant intraislet duct cell proliferation, and the appearance of hepatocytes within the islets of

Langerhans (Krakowski *et al*., 1999). In transgenic mice with KGF overexpressed in the eye, hyperproliferation was observed in embryonic corneal epithelial cells that subsequently differentiated to form functional lacrimal gland-like structures (Lovicu *et al*., 1999).

The interpretation of these transgenic studies with regard to the normal function of KGF is unclear, because endogenous KGF expression is under regulatory control and usually does not occur in responsive epithelial cells. Perhaps the major conclusion from these investigations is that KGF has strong activity in a variety of epithelial cells *in vivo*. Persistent production of KGF in transgenic animals caused permanent histological abnormalities in targeted tissues. An extreme example was observed in mice that expressed KGF as a transgene under the control of the mouse mammary tumor virus long terminal repeat (MMTV LTR). These animals were characterized by mammary epithelial cell hyperplasia and, after multiple pregnancies that presumably induced high levels of transgenic KGF expression, they developed mammary adenocarcinomas (Kitsberg and Leder, 1996). In contrast, when recombinant KGF was given to animals, the histological changes seen in multiple epithelial cell lineages were rapidly reversed after cessation of KGF treatment (Housley *et al*., 1994; Ulich *et al*., 1994; Yi *et al*., 1994a,b, 1995). This was consistent with the idea that physiologic mechanisms exist to mediate KGF responses and to resolve them in accordance with homeostasis.

KGF knockout mice were viable and appeared to be essentially normal (Guo *et al*., 1996). However, further examination revealed subtle phenotypes involving the hair, kidneys, and bladder. Over time, the fur of mice lacking KGF developed a matted and greasy appearance, similar to the rough mouse, whose recessive mutation maps at or near the *KGF* locus on mouse chromosome 2. This defect appeared to be restricted to the cells giving rise to the hair shaft (Guo *et al*., 1996). The developing ureteric bud and mature renal collecting system of KGF knockout mice were markedly smaller than in wild-type kidneys (Qiao *et al*., 1999), consistent with an earlier study showing the responsiveness of urothelium to recombinant KGF (Yi *et al*., 1995). Furthermore, mature kidneys in the knockout mice had 30% fewer nephrons than wild-type kidneys (Qiao *et al*., 1999). *In vitro* experiments demonstrated that KGF augmented ureteric bud growth and, through associated inductive mechanisms, increased the number of nephrons that formed in rodent metanephric kidney organ cultures (Qiao *et al*., 1999). These results indicated that KGF modulated the extent of ureteric bud growth during development and the number of nephrons that eventually formed in the kidney. In KGF knockout mice the bladder urothelium was markedly thinner than that of wild-type mice and lacked the intermediate cell layers present in wild-type animals (Tash *et al*., 2001). Primary

urothelial cell cultures maintained without KGF stopped dividing, and expressed markers associated with terminally differentiated umbrella cells (Tash *et al*., 2001). Thus, KGF may be required for normal bladder urothelial stratification, and delay the differentiation of urothelial cells into post-mitotic umbrella cells.

The importance of FGFR signaling for epidermal wound healing was demonstrated in mice expressing a dominant-negative FGFR2b derivative in basal keratinocytes of the epidermis. These animals displayed a severely reduced rate of keratinocyte proliferation at wound edges, resulting in delayed reepithelialization (Werner *et al*., 1994). In contrast, KGF knockout mice exhibited no abnormalities in epidermal wound healing (Guo *et al*., 1996), suggesting that despite its strong induction after injury, other FGFR2b ligands could compensate for its absence. In this regard, FGF10 was detected in the wound epidermis of KGF knockout mice, suggesting that it might substitute for KGF in the healing of skin wounds (Jameson *et al*., 2002). However, it should be noted that only full-thickness incisional wound healing was examined in KGF null mice (Guo *et al*., 1996). Excisional wound repair, which requires a far greater extent of reepithelialization than incisional wounds, was not studied in these mice (Werner and Grose, 2003). Interestingly, FGF22 was strongly expressed in the wound epidermis of wild-type mice during the final stages of healing (Beyer *et al*., 2003). This raises the intriguing possibility that FGF22 may be an FGFR2b antagonist whose function is to inhibit the proliferative effects of KGF and FGF10 once tissue repair is near completion. However, verification of this hypothesis will require more information about the receptor-binding and biological activity of FGF22. In contrast to results in the skin injury model, KGF knockout mice exhibited more severe inflammation in the colon and a delay in tissue repair compared with wild-type mice after treatment with dextran sodium sulfate, a model for IBD (Chen *et al*., 2002). This implied that KGF had a specific, nonredundant role in limiting damage to the intestine.

The absence of gross developmental abnormalities in the KGF knockout mouse demonstrated that KGF did not have an important role in organogenesis. However, expression of a soluble dominant-negative FGFR2b receptor under the control of a metallothionein promoter resulted in markedly impaired development of many epithelial organs including the kidney, lung, various cutaneous structures, and exocrine and endocrine glands (Celli *et al*., 1998). The mouse embryos also displayed severe defects in craniofacial and limb formation. This implied that FGF ligands that could bind FGFR2b had a critical role in these developmental processes. Strikingly, the phenotype of FGF10 knockout mice exhibited many of these traits (Ohuchi *et al*., 2000; Sekine *et al*., 1999). FGF10 knockout mice died at birth as a result of a lack of lung development. Although the trachea was normal, subsequent

pulmonary branching was minimal (Sekine *et al.*, 1999). Limb bud development was markedly impaired because of the failure to form an apical ectodermal ridge. When expression of FGFR2b isoforms was specifically disrupted in mice, defects were seen in many organs (De Moerlooze *et al.*, 2000; Petiot *et al.*, 2003; Revest *et al.*, 2001). These studies established that FGF10–FGFR2b signaling has an essential role in organogenesis and limb formation, whereas KGF appears to have a more significant role in epithelial homeostasis later in life.

VI. REGENERATIVE AND PROTECTIVE EFFECTS

As reviewed in the previous sections, much information has been obtained about KGF activity from a combination of *in vitro* and *in vivo* investigations. Taken together, these studies validated the concept that epithelial cells comprise the major targets of KGF action. Moreover, the multiplicity of KGF effects, including proliferation, migration, differentiation, cytoprotection, and inhibition of apoptosis, provides a mechanistic basis for its putative role as a homeostatic factor whose primary function is to maintain the integrity of epithelial tissues. The fact that KGF was upregulated after epithelial injury further suggested that it participates in normal tissue repair. This role distinguishes KGF from most other cytokines and provided a focus for efforts to identify clinical applications. It was hypothesized that exogenous KGF would augment the effects of the endogenous protein when epithelial tissues were at risk, and preservation or rapid restoration would be of clinical benefit. It was further surmised that, given its specificity for epithelial cells, this could be achieved without stimulating adverse inflammatory responses, fibrosis, or angiogenesis. This section summarizes the major findings from several animal models that have been used to examine the protective and regenerative properties of KGF.

A. Skin

The first experiments performed with KGF in animals involved its topical application to skin with the aim of determining its efficacy in stimulating epidermal wound repair. KGF increased the rate of reepithelialization in partial-thickness wounds of the porcine epidermis (Staiano-Coico *et al.*, 1993). In both partial- and full-thickness wounds there was a marked increase in the thickness of the epidermis after KGF treatment. In full-thickness wounds, this was associated with a deep rete ridge pattern, an increase in the number of serrated basal cells and increased deposition of

collagen fibers in the superficial dermis. Electron microscopy indicated that the serrated cells had better developed hemidesmosomes and thicker bundles of tonofilaments. These structural features suggested that KGF would enhance the strength and durability of healed wounds (Staiano-Coico *et al.*, 1993). A similar stimulation of reepithelialization and epidermal thickness was observed in wounds extending through the cartilage of rabbit ear (Pierce *et al.*, 1994). In addition, KGF increased the proliferation and differentiation of progenitor cells of hair follicles and sebaceous glands in the wound site. A subsequent study documented the expression of FGFR2b in basal keratinocytes and throughout the developing hair follicles, while KGF was detected in follicular dermal papillae of rat embryos and neonates (Danilenko *et al.*, 1995b). Moreover, subcutaneous or intraperitoneal administration of KGF in *nu/nu* athymic nude mice stimulated hair growth and sebaceous gland hypertrophy. In addition, KGF treatment 1 day before cytosine arabinoside reduced the extent of chemotherapy-induced alopecia in neonatal rats by ~50% (Danilenko *et al.*, 1995b). When KGF was tested in porcine skin models of full- and partial-thickness burns, increased epidermal thickness and follicular proliferation were again observed (Danilenko *et al.*, 1995a). Although the rate of reepithelialization also was increased, the effect was not marked. While these experiments established that recombinant KGF had significant biological effects on skin, the clinical benefit of these effects on wound healing remains uncertain. However, a report that local injection of liposomes containing KGF cDNA caused significant improvements in epidermal regeneration suggests another possible therapeutic approach with KGF to enhance wound healing (Jeschke *et al.*, 2002).

KGF was shown to increase hair follicle survival and regeneration after irradiation (Booth and Potten, 2000). KGF pretreatment increased the expression of p21, a protein that interrupts cell cycle progression. Thus, KGF may induce a more rapid onset or prolonged duration of cell cycle arrest that could allow for more efficient DNA repair and/or a decline in apoptosis within the hair follicle.

B. Lung

Because KGF was originally purified from the conditioned medium of a human embryonic lung fibroblast cell line (Rubin *et al.*, 1989) and was subsequently shown to be a mitogen for type II alveolar cells *in vitro* (Panos *et al.*, 1993), it was reasoned that the lung epithelium might represent a therapeutic target for KGF action. Normal adult lung expresses both KGF and FGFR2b mRNAs, suggesting that endogenous KGF is involved in the normal growth of type II alveolar cells (Ulich *et al.*, 1994). Intratracheal administration of recombinant KGF to lungs of adult rats resulted

in a dose-dependent proliferation of type II alveolar cells. This appeared histologically as a micropapillary epithelial cell hyperplasia in the form of monolayers of cuboidal epithelial cells lining the alveolar septae. The hyperplastic cells contained immunoreactive surfactant protein B and lamellar inclusions characteristic of surfactant-producing type II alveolar cells (Ulich *et al*., 1994).

A series of studies using rodent models established that KGF has remarkable cytoprotective effects in response to acute lung injury. Intratracheal administration of KGF was shown to provide significant protection from a variety of toxic exposures, including hyperoxia (Barazzone *et al*., 1999; Guo *et al*., 1998; Panos *et al*., 1995), acid instillation (Yano *et al*., 1996), α-napthylthiourea (ANTU, model of increased permeability pulmonary edema) (Guery *et al*., 1997; Mason *et al*., 1996), radiation (Yi *et al*., 1996), and bleomycin (Sugahara *et al*., 1998; Yi *et al*., 1996, 1998). Similar results were observed when KGF was administered intravenously, rather than intratracheally, in studies of bleomycin- and hyperoxia-induced lung injury (Guo *et al*., 1998) as well as ventilator-induced lung injury (Welsh *et al*., 2000). In these models, KGF-treated animals exhibited a dramatic, dose-dependent decrease in mortality and morbidity, as measured by criteria such as body weight and pulmonary function tests. In addition, KGF reduced the incidence or severity of histological changes associated with injury, including fibrosis, and physiological indices of lung injury, such as formation of pulmonary edema. Somewhat unexpectedly, KGF also reduced endothelial cell injury associated with ANTU (Guery *et al*., 1997; Mason *et al*., 1996) and hyperoxia (Barazzone *et al*., 1999). This beneficial effect on the endothelium may have been due to indirect mechanisms, as maintenance of the epithelium markedly limited the inflammatory response after toxic exposure.

The results from preclinical models have prompted the suggestion that KGF should be tested in clinical trials involving lung injury (Ware and Matthay, 2002). The efficacy of intravenous KGF in some of the animal models was noteworthy, as this would provide a more convenient route of administration than intratracheal delivery. However, the beneficial effects observed in lung injury models typically required pretreatment with KGF. Because the development of acute lung injury in humans is often unpredictable, KGF treatment before the initial insult usually would not be feasible. However, before dismissing the use of KGF in these clinical settings, it is important to acknowledge that in most of the mouse injury models, because of the small size of the lungs, tissue damage occurred throughout the organs. In the corresponding human conditions, damage often begins in localized regions of the lung. Whether prompt KGF administration would protect the surrounding tissue and thereby limit the extent of tissue damage remains a theoretical possibility that warrants further consideration.

C. Bladder

Systemic administration of KGF to rats and rhesus monkeys caused rapid and striking proliferation of the urothelium (Yi *et al*., 1995). Subsequently, KGF was shown to ameliorate cyclophosphamide (CP)-induced ulcerative hemorrhagic cystitis in rats. In this model, a single intravenous injection of KGF administered 24 h before treatment with CP almost completely prevented CP-induced ulcerative hemorrhagic cystitis (Fig. 3) (Ulich *et al*., 1997). The protective effect probably was due to the rapid proliferation of urothelial cells during and after chemotoxic injury, or to cytoprotective mechanisms (Ulich *et al*., 1997). Hemorrhagic cystitis occurs sporadically in patients after radiation therapy, but is most commonly observed after treatment with CP and its more urotoxic derivative, ifosfamide. They are thought to induce urothelial injury by multiple mechanisms that result from the accumulation of high urinary concentrations of these drugs and their highly reactive metabolites (Wagner, 1994). The incidence of CP-induced hemorrhagic cystitis varies from 2 to 40% depending on dose and duration of therapy (Foad and Hess, 1976). This study provided a clear demonstration that KGF could be of benefit in ameliorating the toxic effects associated with cancer treatment regimens, and as such foreshadowed the development of this application as the primary clinical indication for KGF. Importantly, the opportunity to give KGF before the chemotherapy regimen maximized its protective effects.

D. Gastrointestinal Tract

KGF and FGFR2b mRNAs are expressed throughout the entire GI tract, indicating that the gut can both synthesize and respond to KGF (Housley *et al*., 1994). Administration of recombinant KGF to adult rats caused a marked increase in the proliferation of epithelial cells from the foregut to the colon, including hepatocytes. There also was a selective induction of mucin-producing goblet cells throughout the GI tract (Housley *et al*., 1994). The proliferative changes in the crypts of the small intestine that resulted from prolonged exposure to KGF appeared to be related to an increase in stem cell numbers and/or increases in the number of stem cells in the S phase of the cell cycle (Potten *et al*., 2001).

Reports that KGF was upregulated in IBD implied that endogenous KGF was at least partially responsible for the intestinal crypt hyperplasia seen in the regenerative response to this chronic condition (Bajaj-Elliott *et al*., 1997; Brauchle *et al*., 1996; Finch *et al*., 1996). Subsequent work demonstrated that exogenous KGF ameliorated mucosal injury in several models of colitis (Byrne *et al*., 2002; Egger *et al*., 1999; Zeeh *et al*., 1996). As previously noted, KGF null mice were more susceptible than their wild-type

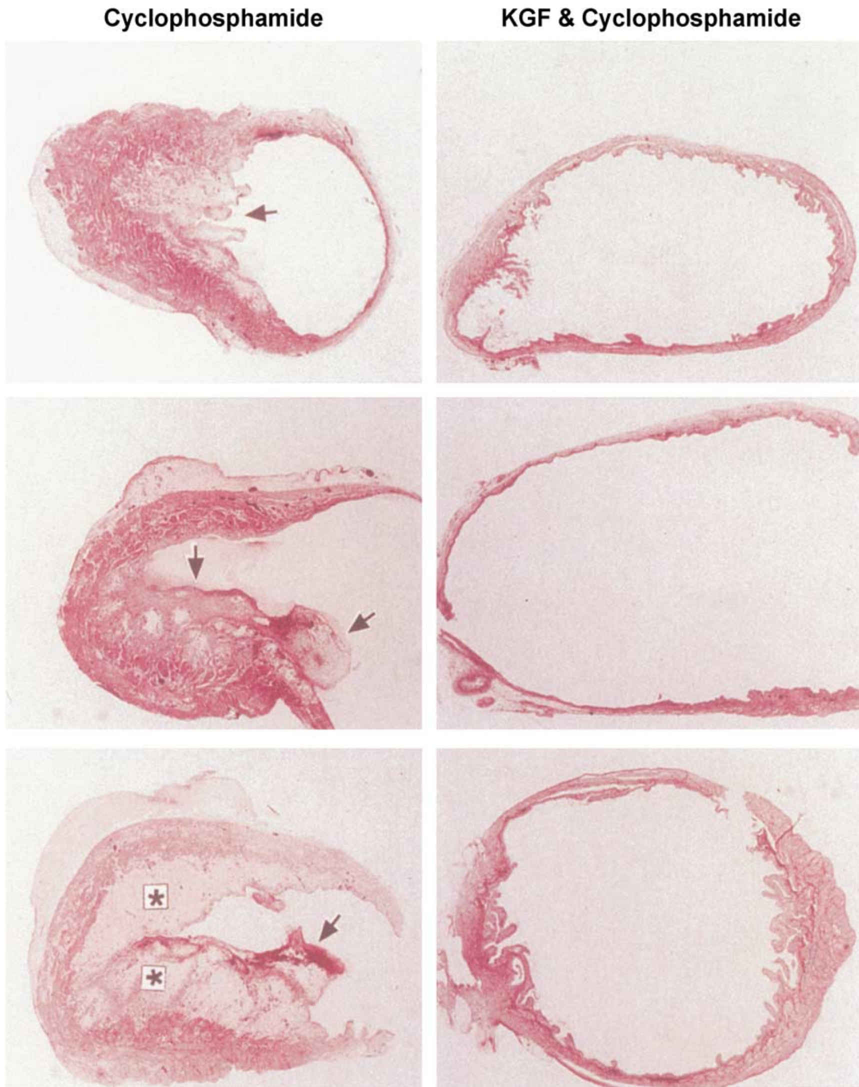


Fig. 3 Representative bladder H&E cross-sections from saline- or KGF-pretreated cyclophosphamide-challenged rats. Specimens from saline-pretreated rats (*left*) were characterized by severe hemorrhagic lesions (arrows) and extensive submucosal edema (*). The bladders of rats pretreated with KGF (5 mg/kg) 24 h before cyclophosphamide administration were largely free of ulcerations and edema (*right*). (From Ulich *et al*., 1997, Fig. 1, p. 473.)

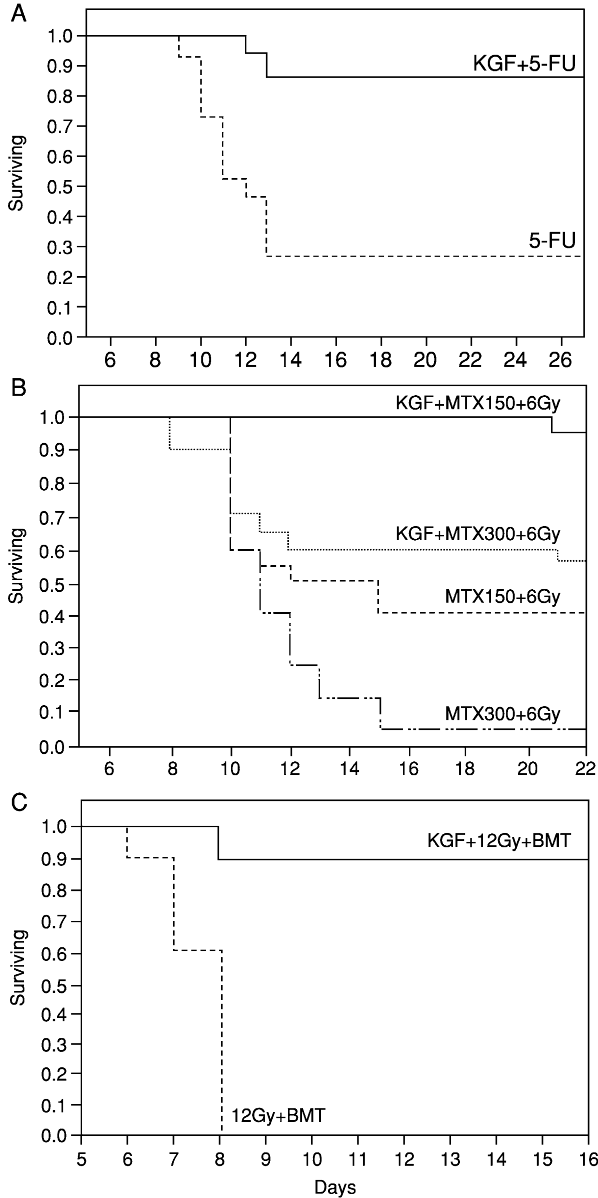


Fig. 4 KGF increased survival of mice treated with high doses of chemotherapy and/or irradiation. BDF_1 mice received (A) 5-fluorouracil (5-FU, 50 mg/kg per day, intraperitoneal) for 4 days (days 1–4; $n = 20$/group); (B) methotrexate (MTX, 150 or 300 mg/kg, intraperitoneal) 1 h before receiving 6 Gy of radiation (day 1; $n = 20$/group); or (C) 12 Gy of radiation from a cesium source (day 1) followed by a bone marrow transplant (BMT; $n = 20$/group), and

counterparts to dextran sulfate-dependent colonic injury, and healing was delayed in the absence of KGF (Chen *et al*., 2002). This implied that KGF has a specific role in mucosal protection and repair, not redundant with FGF10 or other FGFs. Additional reports provided evidence that KGF treatment enhanced the repair of colonic anastomoses in rats (Egger *et al*., 1998, 2001) and reduced the extent of intestinal atrophy in animals maintained on total parenteral nutrition (Yang *et al*., 2002).

Cytoablative doses of cancer chemotherapy and irradiation compromise the absorptive and barrier functions of the GI tract by killing rapidly dividing cells of the mucosa, thereby impairing normal cell renewal. Such treatments are often associated with mucositis, which is characterized by atrophy, ulceration, loss of barrier function, and infection. The beneficial effects of KGF in reducing chemotherapy and radiation-induced GI injury and mortality were first documented in a study that involved several mouse models (Farrell *et al*., 1998). Treatment with KGF (5 mg/kg per day for 3 days) before four daily doses of 5-fluorouracil (5-FU, 50 mg/kg per day) resulted in a striking improvement in survival (87 versus 27%, $p < 0.006$; Fig. 4A) and significant reduction in weight loss ($p < 0.0001$; Fig. 5A). However, KGF was not effective if given after 5-FU. The protective effect of KGF was not limited to a particular chemotherapeutic agent, as KGF pretreatment also reduced the weight loss associated with a single dose of carboplatin (Fig. 5B). In addition, KGF caused a marked improvement in survival of mice treated with a combination of total body irradiation (6 Gy) and either low (150 mg/kg) or high (300 mg/kg) doses of methotrexate (Fig. 4B). A decrease in weight loss of KGF-treated mice also was noted in this experiment (Fig. 5C). The most dramatic results were observed when mice were exposed to a lethal dose of total body irradiation (12 Gy) and supplemented with a bone marrow transplant. Whereas all the mice in the vehicle control group died within 8 days, 90% of the mice pretreated with KGF survived this toxic regimen (Fig. 4C).

Additional studies have focused on the ability of KGF to palliate the effects of aggressive cancer treatment regimens in the upper aerodigestive tract. Cells in the basal germinal layer of this tissue proliferate at a high rate. The reproductive capacity of the basal cells is impaired after radiation and chemotherapy, resulting in desquamation, ulceration, and subsequent infection as the epithelial barrier is lost. When mice were treated with either a single dose of 12 Gy or four daily doses of 4 Gy, there was a 30–40% decrease in average epithelial thickness of the tongue, esophagus, and

were pretreated with KGF (5 mg/kg per day) or vehicle for 3 days. Statistical differences assessed by Kaplan–Mayer survival analysis proved that in all of these experiments, pretreatment with KGF significantly reduced mortality. (From Farrell *et al*., 1998, Fig. 1, p. 934.)

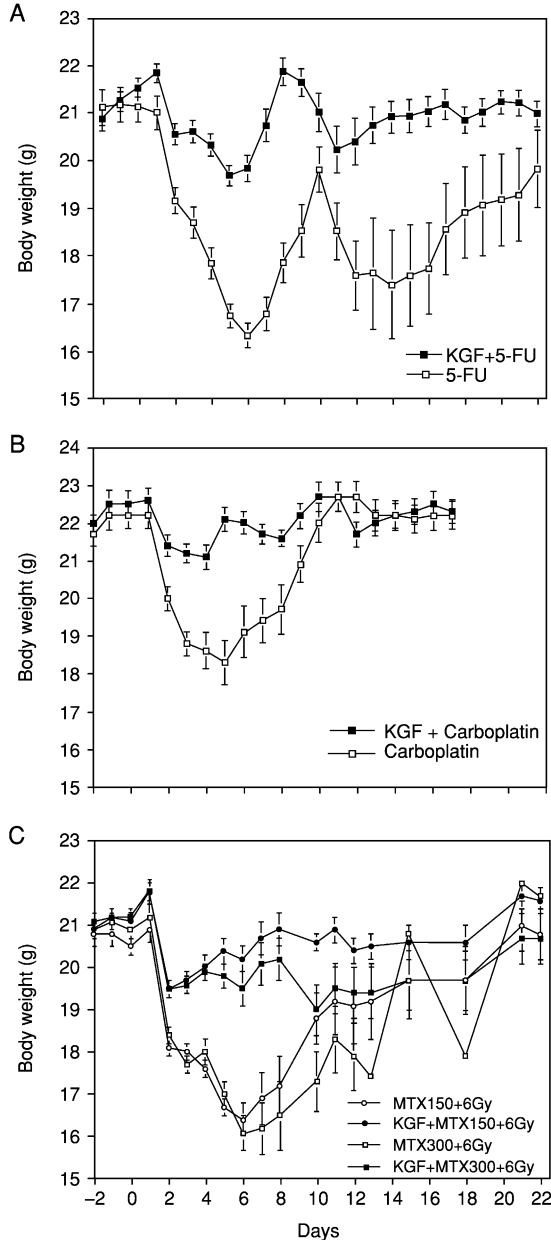


Fig. 5 KGF reduced weight loss in mice treated with chemotherapy and/or irradiation. BDF_1 mice received (A) 5-fluorouracil (5-FU, 50 mg/kg per day, intraperitoneal) for 4 days (days 1–4; $n = 15$/group); (B) carboplatin (125 mg/kg, intraperitoneal) (day 1; $n = 10$/group); or (C) methotrexate (MTX, 150 or 300 mg, intraperitoneal) 1 h before receiving 6 Gy of radiation

buccal mucosa 4 days after irradiation (Farrell *et al.*, 1999). This atrophy was reversed by KGF. Notably, in this model of tissue irradiation KGF was most effective when given after or during the toxic exposure, in contrast to the lung and the intestine where pretreatment was necessary for protection.

Further analysis was performed with an experimental model in which a 3 × 3 mm^2 area on the ventral surface of mouse tongue (strain C3H/Neu) was exposed to graded doses of X-rays, and the radiation dose resulting in ulcer formation in 50% of the mice (ED_{50}) was determined. The ED_{50} for single dose irradiation was 10.9 ± 0.7 Gy without KGF treatment. In contrast, when KGF was given subcutaneously at 5 mg/kg per day for 3–6 days the ED_{50} increased by a factor of 1.5 to 2.3. The maximum protective effect was observed when KGF was administered either from days −3 to +1 or from days 0 to +2, with an ED_{50} of 23.1 ± 5.7 or 24.9 ± 4.6 Gy, respectively ($p < 0.0001$ for both) (Dörr *et al.*, 2001). Similar beneficial effects were observed when KGF was administered to mice receiving five daily doses of 3 Gy (Dörr *et al.*, 2002a). Whereas mice became more radioresistant when treated with KGF before irradiation, maximal protection was obtained when KGF was given either during or both during and after irradiation, with the ED_{50} again more than doubling. Moreover, a single KGF injection administered either shortly before (day −1) or during (+4) the irradiation period (days 0–4) was similarly (5 mg/kg) or more (15 mg/kg) effective than multiple daily KGF doses. KGF treatment was not beneficial if initiated after ulceration already was present (typical latency period for ulcer formation in this model was ~10 days) (Dörr *et al.*, 2002b). Nonetheless, these data provided compelling evidence that KGF can reduce the damaging effects of X-rays in the oral cavity, and indicated that, in contrast to most other toxic exposures, prior treatment with KGF was not required to obtain mucoprotection.

E. Graft-versus-Host Disease and Thymus

Allogeneic bone marrow transplantation (BMT) is used in the treatment of a number of malignancies. Graft-versus-host disease (GVHD) represents a major transplant-related complication initiated when alloreactive T cells

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from a cesium source (day 1; $n = 20$/group), and were pretreated with KGF (5 mg/kg per day) or vehicle for 3 days. The changes in weight in (A) and (C) were analyzed by calculation of the percentage of change from baseline, using a linear mixed model for repeated measures. It includes the fixed effects of treatments, days of measurements, and the interaction treatment by day. Bars represent the standard error. In (B), the mean weights at the nadir were compared by Student *t* test. The data are expressed as means ± SE. In all of these experiments, pretreatment with KGF significantly reduced weight loss induced by cytoablative exposure. (From Farrell *et al.*, 1998, Fig. 2, p. 935.)

recognize histocompatibility antigens of the host tissue. Activation of inflammatory effector cells and secretion of cytopathic molecules result in tissue damage to the skin, GI tract, liver, lung, and immune system. Common prophylaxis includes the elimination of donor T cells from the host, or immunosuppression of the host that can result in the loss of graft-versus-leukemia activity and a high risk of relapse. Damage to the GI tract is thought to have a pivotal role in the pathophysiology of GVHD, augmenting the release of cytokines that promote inflammation and cytotoxic immune cell activities (Hill and Ferrara, 2000; Krenger *et al*., 1997). By reducing injury to the GI tract, particularly damage resulting from the chemoradiotherapy-conditioning regimens that precede BMT, it has been proposed that KGF might decrease the morbidity and mortality associated with GVHD (Hill and Ferrara, 2000; MacDonald and Hill, 2002; Wirth and Mertelsmann, 2002).

The ability of KGF to limit GVHD has been investigated in mouse allogeneic BMT models. Recipient mice were conditioned with total body irradiation before receiving bone marrow and spleen cells as the source of GVHD-inducing T cells. An increased survival of transplant recipients was observed when KGF was administered before total body irradiation, either alone or in conjunction with chemotherapy. KGF also ameliorated GVHD-related pathologic changes in liver, lung, and skin, but not in spleen, colon, or ileum (Panoskaltsis-Mortari *et al*., 1998). In a similar study, mice treated with KGF before total body irradiation exhibited increased leukemia-free survival, while GVHD in the GI tract was decreased (Krijanovski *et al*., 1999). Serum levels of lipopolysaccharide were decreased compared with controls, consistent with preservation of the GI epithelial barrier. There was a corresponding reduction in serum concentrations of TNF-α that presumably contributed to the diminished inflammatory activity. A subsequent investigation using T cell transfer into severe combined immunodeficient (SCID) mice revealed that KGF reduced GVHD even when mice were not subjected to a mucotoxic conditioning regimen (Panoskaltsis-Mortari *et al*., 2000b). KGF treatment was associated with an increase in the helper T cell type 2 (Th2) cytokine IL-13 and decreases in the inflammatory cytokines TNF-α and interferon-γ. This implied that, independent of any effect on limiting GVHD by reducing GI tract injury, KGF also has an immunomodulatory mechanism of action.

Allogeneic BMT also can result in lung injury not necessarily related to GVHD. It often manifests itself as a noninfectious, idiopathic pneumonia syndrome (IPS). KGF pretreatment hastened the repair of lung damage in a mouse allogeneic BMT model by stimulating type II alveolar cell proliferation, decreasing expression of the cytotoxic molecule granzyme B and the costimulatory ligand B7 that enhances formation of cytotoxic T lymphocytes, and increasing the production of Th2 cytokines (IL-4, IL-6, and

IL-13) (Panoskaltsis-Mortari *et al*., 2000a). A KGF-dependent increase in the expression of surfactant-A also contributes to the antiinflammatory effects of KGF in the lung after allogeneic BMT (Haddad *et al*., 2003; Yang *et al*., 2000).

Aside from the harm done to various organs as a consequence of GVHD, a major clinical concern after BMT is the development of satisfactory thymocyte function from the population of donor precursor cells. The success of the transplant is compromised in part by the deleterious effects of both the chemoradiotherapy-conditioning regimen and cytotoxic immunologic activity on the thymic epithelial cells (TECs). These cells have a critical role in promoting the differentiation of immature thymocytes by releasing IL-7. A surprising result in the SCID mouse experiment mentioned previously was the improvement in T cell alloengraftment that was associated with KGF treatment (Panoskaltsis-Mortari *et al*., 2000b). Later work established that KGF enhanced thymopoiesis by preserving the various TEC populations in the thymic cortex and medulla as well as TEC function (Min *et al*., 2002; Rossi *et al*., 2002). Moreover, the thymopoietic effects of KGF required TEC–thymocyte cross-talk mediated by IL-7 signaling (Min *et al*., 2002). This supported the view that improvement in thymic and peripheral T cell reconstitution after BMT was due to TEC cytoprotection by KGF (Min *et al*., 2002).

F. Physiological Mechanisms of Action

In Section III, we described many responses of epithelial cells to KGF. Presumably, the regenerative and protective effects of KGF in whole animals are attributable to a combination of these cellular responses: proliferation, migration, differentiation, antiapoptosis, and induction of enzymes that reduce oxidative stress. KGF stimulates the expression of a variety of additional factors that might also contribute to these processes, such as syndecan-1, a heparan sulfate proteoglycan (Maatta *et al*., 1999), caveolin-1, a gene involved in the regulation of various signal transduction pathways (Gassmann and Werner, 2000), the mitogen-regulated protein Mrp3 (or proliferin) (Fassett and Nilsen-Hamilton, 2001), as well as a number of enzymes involved in nucleotide biosynthesis (Gassmann *et al*., 1999) and a putative DNA helicase that conceivably could function in DNA repair (Frank and Werner, 1996).

The key effect of KGF in all the experimental injury models is preservation of the epithelial barrier. In the upper aerodigestive tract, this effect is manifested by a marked thickening of the squamous epithelium that results both from increased proliferation in the basal layer and reduced cell loss in the upper layers (Fig. 6). Desquamation is inhibited as a result of

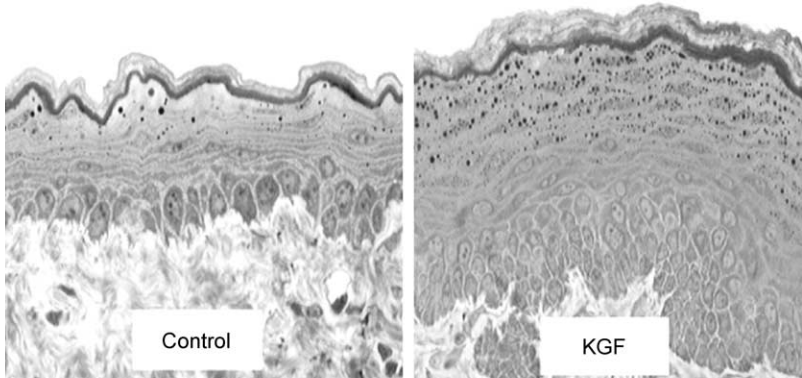

Fig. 6 Semithin sections of the ventral surface of the mouse tongue. Comparison of toluidine blue-stained sections from normal, control mice and mice that were treated with KGF (5 mg/kg, subcutaneous) for 3 days showed a KGF-dependent increase in overall epithelial thickness. Greater cellularity and an increase in the number and size of keratohyalin granules were evident. (From Farrell *et al.*, 2002, Fig. 1, p. 79.)

stimulation of cell–cell adhesion that is mediated by an increased number of desmosomes, which also are better developed (Farrell *et al.*, 1999). The mechanical barrier is further reinforced by a KGF-dependent increase in the number and size of keratohyalin granules (Farrell *et al.*, 1999) that are thought to strengthen the lipid permeability barrier (Squier and Kremer, 2001). In the intestines, KGF stimulates the formation of goblet cells (Housley *et al.*, 1994) that enhance the lumenal barrier by producing mucins and ITFs (Corfield *et al.*, 2000; Playford *et al.*, 1996). Presumably, KGF-dependent proliferation of intestinal crypt stem cells (Khan *et al.*, 1997; Potten *et al.*, 2001) augments repair after damage and thereby limits the period when the barrier might be breached. The proliferative response of type II alveolar cells to KGF has a similar effect in lung, but these cells also perform other valuable tasks related to barrier function. Surfactant from the type II cells reduces surface tension and thereby keeps the airways patent, facilitating the mechanics of respiration and decreasing the likelihood of infection (as noted previously, surfactant-A also has antiinflammatory activity). KGF sustains the permeability barrier of the respiratory epithelium by promoting alveolar fluid and ion transport via increased expression of Na^+,K^+-ATPase in type II cells (Borok *et al.*, 1998a; Guery *et al.*, 1997; Wang *et al.*, 1999b) and maintaining intercellular junctions (Barazzone *et al.*, 1999; Savla and Waters, 1998; Waters *et al.*, 1997; Welsh *et al.*, 2000). KGF downregulation of many interferon-induced genes in type II cells may reduce tissue damage resulting from interferon-dependent mechanisms (Dixon *et al.*, 2000; Prince *et al.*, 2001).

Preservation of the epithelial barrier has a multitude of beneficial consequences. The possibility of infection is reduced, and therefore inflammation and fibrosis become less likely. An intact oral epithelium enables the patient to continue eating, while a fully functional intestinal epithelium absorbs water and nutrients to maintain optimal nutritional status. When airways are free of edema they function well in gas exchange. Respiration is further enhanced by the production of surfactant that facilitates ventilation by allowing the airways to remain patent as the lungs expand and contract. These global effects of KGF treatment all derive from the maintenance of barrier and associated epithelial functions. They minimize the adverse sequelae of injury and ensure better health.

VII. CLINICAL TRIALS: AMELIORATION OF SEVERE ORAL MUCOSITIS

Preclinical data from rodent experiments demonstrated a potent cytoprotective effect of KGF after a variety of toxic exposures to the epithelia of lung, bladder, and GI tract. To test the clinical significance of these findings, the initial focus has been to determine whether KGF can reduce the duration of severe oral mucositis (OM) in patients receiving highly mucotoxic cancer therapies. Such patients commonly develop ulceration in their oral cavities and pain with swallowing that limits food intake and impairs speaking and sleeping. Loss of the epithelial barrier predisposes the patients to infection. These clinical complications often result in delays and/or reductions in chemotherapy or radiation that compromise the outcome of treatment regimens. Although existing palliative care is of some benefit, there are currently no effective remedies for OM and it remains an unmet clinical need (Duncan and Grant, 2003; Sonis, 1998; Squier and Kremer, 2001; Symonds, 1998).

The first KGF clinical trial was performed in normal human volunteers to establish that it was safe when taken in a dose range that had a clear biological effect on the oral epithelium (Serdar *et al*., 1997). Prior work with rhesus macaques had defined a range likely to include biologically active doses, given the similarity of anatomy between macaques and humans (Danilenko, 1999). Volunteers were divided into groups that received KGF or placebo (3:1) administered either as a single intravenous injection ($n = 4$), or a daily injection for three consecutive days ($n = 8$), with the size of the dose for each group varying from 0.2 to 20 μg/kg per day. Buccal biopsies were obtained from each of the trial participants for Ki67 immunostaining and counting of mitotic figures for evidence of epithelial proliferation. Three days of systemically administered KGF induced statistically

significant increases in proliferation with doses of 5–20 μg/kg per day. KGF administration was determined to be safe and well tolerated, with no significant adverse effects. On the basis of these results, trials were initiated in cancer patients. To assess the potential efficacy of KGF in ameliorating oral OM, it was used in conjunction with cancer treatment regimens associated with a high degree of oral mucosal injury.

A. Autologous Peripheral Blood Progenitor Cell Transplantation for Hematologic Malignancies

Myeloablative conditioning regimens associated with BMT or peripheral blood progenitor cell transplantation (PBPCT) typically have a substantial incidence of severe OM. A phase 1 trial was conducted in patients with Hodgkin's disease and non-Hodgkin's lymphoma who were receiving BEAM (BCNU, etoposide, Ara-C, and melphalan) chemotherapy before autologous PBPCT (Durrant *et al*., 1999). This was a randomized, placebo-controlled study in which patients received three daily doses of KGF intravenously, either before the start of chemotherapy or both before and after chemotherapy. Patients were divided into groups that received one of the following doses: 5, 20, 40, 60, or 80 μg/kg per day. Side effects of KGF consisted of mild–moderate erythema with or without edema, and an asymptomatic transient elevation of serum amylase and lipase. These results were consistent with those seen in normal volunteers, and generally more evident at higher doses of KGF.

The BEAM-conditioning regimen was characterized by a lower incidence of severe OM than most conditioning regimens for PBPCT. Using the World Health Organization (WHO) scale for OM (Table I), only 22% of the placebo patients experienced severe OM (grades 3 or 4) whereas 51% had ulcerative OM (grades 2–4). Although the numbers of patients in the KGF treatment groups were small and precluded definitive statistical analysis, it

Table I World Health Organization Grading Scale for Oral Mucositis[a]

Grade	Symptoms
0	None
1	Soreness/erythema
2	Erythema/ulcers/can eat solids
3	Ulcers/extensive erythema/requires liquid diet
4	Alimentation not possible

[a]Ulcerative oral mucositis consists of grades 2–4, while severe oral mucositis corresponds to grades 3 and 4. (Adapted from World Health Organization, 1979.)

was noteworthy that only 13% of patients receiving 60 μg/kg per day before BEAM had ulcerative OM. Moreover, the duration (mean $\pm$ SE) of ulcerative OM in this group was 0.8 ± 0.5 days, compared with 4.6 ± 1.8 days for the placebo group. Patients also reported a decrease in mouth and throat soreness as well as reduced use of analgesia. In view of these results, KGF was used at a dose of 60 μg/kg per day in subsequent clinical trials involving PBPCT.

A phase 2, double-blinded study was performed in patients treated for hematologic malignancies using a myeloablative regimen associated with more severe OM (Spielberger *et al.*, 2001). The conditioning regimen consisted of 12 Gy of total body irradiation, etoposide (60 mg/kg) and cyclophosphamide (75 or 100 mg/kg). One hundred and twenty-nine patients were randomly assigned to groups that received placebo or three daily 60 μg/kg intravenous doses of KGF before (pre) or both before and after (pre/post) conditioning. KGF treatment resulted in a highly significant decrease in the duration of severe OM, as the mean duration in the placebo group was 7.7 days but only 4.0 days in the pre/post KGF group ($p < 0.001$) and 5.0 days in the pre KGF group ($p < 0.04$). Patients reported a corresponding decrease in mouth/throat soreness and difficulty in swallowing, drinking, eating, talking, and sleeping. Moreover, those receiving KGF required less intravenous opioid analgesics and supplementation with total parenteral nutrition. KGF itself was well tolerated, with the same set of side effects previously described in the phase 1 trials.

A phase 3, double-blinded study involving the same conditioning regimen has been completed (Spielberger *et al.*, 2003). In this trial, 212 patients were randomly distributed (1:1) into a placebo group or a group receiving KGF intravenously at 60 μg/kg for 3 days both before and after the conditioning regimen. As in the phase 2 trial, there was a marked decrease in the mean duration of severe OM associated with KGF use: 10.4 days in the placebo group versus 3.7 days in the KGF group ($p < 0.001$). Moreover, the incidence of severe OM was significantly reduced (98% in the placebo group versus 63% in the KGF group, $p < 0.001$), as was the incidence of the most severe form, grade 4 (62% in the placebo group versus 20% in the KGF group, $p < 0.001$). The decline in morbidity was reflected in a reduced use of intravenous opioid analgesics (median milligrams of morphine equivalent in the placebo group was 512 mg versus 212 mg in the KGF group) and total parenteral nutrition (40% in the placebo group versus 11% in the KGF group). Assessment by the patients of their condition again correlated well with the clinical data. These results provided strong evidence that KGF was safe and effective in reducing the incidence and duration of severe OM in patients receiving high-dose chemoradiotherapy before autologous PBPCT for hematologic malignancies.

B. Solid Tumors

Various treatments for solid tumors, particularly those involving a combination of chemotherapy and radiation, also are characterized by a high incidence of severe OM. A phase 1 trial was performed with patients receiving 5-FU and leukovorin for advanced colorectal cancer (Meropol *et al.*, 2003). They were divided into groups treated with various doses of KGF similar to the routine employed in the phase 1 PBPCT trial involving patients with hematologic malignancies (Durrant *et al.*, 1999). As before, KGF was well tolerated with the same set of observed side effects. Combining the results from patients who had received KGF intravenously at >10 μg/kg for 3 days before chemotherapy, the data suggested KGF was effective in reducing the incidence of ulcerative OM (67% in the placebo group versus 43% in the KGF group, $p < 0.06$). To better evaluate the therapeutic potential of KGF in this setting, additional patients were randomly assigned to a placebo group or to a group receiving KGF intravenously at 40 μg/kg on days 1–3, followed by 5-FU (425 mg/m^2 per day) and leukovorin (20 mg/m^2 per day) on days 4–8 of a 28-day cycle (Clarke *et al.*, 2001). The entire treatment routine was repeated in a second cycle. The mean duration of ulcerative OM was markedly reduced by KGF (10.2 days in the placebo group versus 3.4 days in the KGF group, $p < 0.001$), as was its incidence (78% in the placebo group versus 32% in the KGF group, $p < 0.001$). Again, the side effects were similar to those previously mentioned. Considering the possibility that colorectal tumor cells might express FGFR2b and consequently could be affected by exogenous KGF, it is worth noting that there was no significant difference in the median survival of patients in the two treatment groups. This study was not continued because the incidence of severe, as opposed to ulcerative, OM in patients receiving this treatment regimen was relatively low. Furthermore, the use of 5-FU for colorectal carcinoma has been superseded by a new generation of chemotherapy agents that cause less OM. Nevertheless, these results demonstrated the potential utility of KGF in ameliorating OM in a solid tumor setting.

Another phase 1 trial of KGF involved a more mucotoxic regimen for patients with head/neck carcinoma (Brizel *et al.*, 2001). This entailed a combination of hyperfractionated radiation (1.25 Gy twice a day for a total of 72.5 Gy over <7 weeks) and concurrent chemotherapy (5-FU, 1000 mg/m^2 per day for 4 days, and cisplatin, 100 mg/m^2 during weeks 1 and 5), with a break in radiation during the fourth week. Different amounts of KGF were tested with a schedule consisting of three daily intravenous doses before the start of cancer therapy, and then weekly single doses for a total of 10 weeks. This comprised the largest cumulative dosing of KGF in patients, and the primary objective was to assess its safety. The side effects

in this study were similar to those observed in other contexts: erythema and flushing, and transient asymptomatic elevation of serum amylase and lipase. Moreover, there were no significant differences between patients receiving placebo or KGF with regard to various parameters of clinical response (1-year actuarial local-regional control, failure-free survival and survival). Thus, the prolonged administration of KGF in this cancer treatment regimen was safe for the patients.

On the basis of these findings, a phase 2 trial was initiated with patients being treated for head/neck carcinoma using a cancer treatment regimen similar to that employed in the phase 1 trial (Brizel *et al*., 2002). The only major difference was that in the phase 2 study patients received either a combination of hyperfractionated radiation and chemotherapy as previously described, or standard fractionation of radiation (2 Gy/day to 70 Gy) plus chemotherapy. Typically the former is associated with more severe acute OM, but fewer long-term sequelae in the oral cavity. KGF was given intravenously at a dose of 60 μg/kg per day for 3 days before the start of cancer therapy and then a weekly single 60-μg/kg dose for a total of 10 weeks. As in the phase 1 trial, preliminary analysis indicated that there were no serious side effects of KGF in this trial. There was no clear-cut evidence that KGF was effective in reducing OM in this trial. However, interim analysis suggested that the maximally tolerated KGF dose had not been reached (Brizel *et al*., 2002). Therefore, the use of higher doses and perhaps an alternative schedule of administration would be a consideration in future trials.

C. KGF and Cancer

The use of KGF in patients with malignancies of epithelial origin raises a theoretical concern about the possible impact of KGF on tumorigenesis. There is no definitive response to this question, as the implications of various articles are contradictory. Whereas FGFR2b expression is common in epithelial tumor cell lines, its coexpression with KGF is rare (Rubin *et al*., 1995). There are accounts of KGF and FGFR2b coexpression in tumor specimens, but the functional significance typically is undefined (Bansal *et al*., 1997; Imagawa *et al*., 2000; Ishiwata *et al*., 1998; Jacquemier *et al*., 1998; Otte *et al*., 2000; Watanabe *et al*., 2000; Zhang *et al*., 1998). Endometrial cancer cells reportedly expressed KGF and its receptor, and KGF stimulated cell proliferation, although the effect was small (Taniguchi *et al*., 2003). When autocrine expression systems were artificially engineered, one resulted in neoplasia after a long latency and high expression (Kitsberg and Leder, 1996) whereas another was associated with hyperplasia but not malignancy (Foster *et al*., 2002).

Independent of autocrine stimulation of tumor cell growth, cancer cells are likely to be exposed to KGF released in a paracrine fashion from nearby stromal cells. However, exposure of FGFR2b-expressing tumor cells to either autocrine- or paracrine-derived KGF may not result in cancer progression. On the contrary, according to a rat Dunning tumor model of prostate cancer, FGFR2b expression was associated with a well-differentiated phenotype, whereas its loss correlated with a shift to more virulent behavior (Yan *et al.*, 1993). Moreover, KGF inhibited the growth of rat prostatic cancer cells in which FGFR2b expression had been restored by transfection (Matsubara *et al.*, 1998). However, investigations of human prostate cancer cells have yielded conflicting impressions: some have documented KGF and FGFR2b expression in human prostate cancer epithelial cells, including androgen-insensitive specimens (Leung *et al.*, 1997; McGarvey and Stearns, 1995), whereas others have reported that loss of FGFR2b expression correlated with androgen independence (Carstens *et al.*, 1997). Decreased expression of FGFR2b also was observed in a subset of human transitional cell carcinomas that exhibited an invasive phenotype (Diez de Medina *et al.*, 1997) and FGFR2b allegedly had tumor suppressive properties in human bladder cancer (Ricol *et al.*, 1999). A similar pattern was seen in salivary gland adenocarcinomas, as FGFR2b expression was reportedly absent and restoration induced differentiation and apoptosis rather than growth (Zhang *et al.*, 2001). K-SAM isoforms are FGFR2b derivatives with altered carboxy-terminal, cytoplasmic tails that arise from mRNA splice variants (Ishii *et al.*, 1995; Itoh *et al.*, 1994; Ueda *et al.*, 1999). K-SAM expression occurs in poorly differentiated gastric carcinomas and may contribute to their etiology (Hattori *et al.*, 1996; Ishii *et al.*, 1995; Itoh *et al.*, 1994), although the functional significance of K-SAM–KGF interactions in the tumor setting has not been elucidated.

The use of KGF for the treatment of OM in patients with tumors of epithelial origin raises two important questions. First, does KGF promote cancer cell growth? Second, would KGF have a cytoprotective effect on the cancer cells themselves, thus rendering them more resistant to cytoablative treatments intended to kill them? Typically when tumor cells have been treated with KGF *in vitro* or *in vivo*, there has been little or no effect on proliferation (Hille *et al.*, 2003; Ning *et al.*, 1998). Furthermore, preclinical studies demonstrated that histological changes associated with KGF administration to normal animals were rapidly reversed after withdrawal of KGF. By analogy, KGF administered on a transient basis in clinical situations would be unlikely to have permanent effects on tumor cell proliferation and/or progression. Similarly, KGF did not reduce the sensitivity of tumor cells to radiation or chemotherapy either *in vitro* or *in vivo* (Gibson *et al.*, 2002; Hille *et al.*, 2003; Ning *et al.*, 1998). Nonetheless, the potential

activity of KGF on epithelial tumors remains an important issue that should be closely monitored in clinical trials.

VIII. CONCLUDING REMARKS

KGF was isolated as an epithelial-specific mitogen and subsequently identified as a member of the FGF family. It is produced by cells of mesenchymal origin and acts in a paracrine manner on adjacent epithelial cells. Unlike most FGF family members, KGF utilizes only a small subset of the many FGFRs and splice variants, namely FGFR2b isoforms, which have been detected predominantly in epithelial cells. Despite widespread KGF expression during embryogenesis and in the adult, KGF null mice were viable, with only minor developmental abnormalities. However, the up-regulation of KGF after epithelial injury suggested that it had a major role in tissue repair, a view that has been reinforced by evidence that intestinal damage is worse and healing impaired in KGF null mice. Preclinical data from several animal models demonstrated that exogenous KGF could enhance the regenerative capacity of epithelial tissues and protect them from a variety of toxic exposures. The timing of KGF administration is critical for its beneficial effects, as pretreatment initiates several cellular responses that collectively strengthen the epithelial barrier and facilitate regeneration. These include stimulation of mitogenic, migratory, and DNA repair activity, and inhibition of apoptosis. KGF also induces the expression of genes involved in detoxification of reactive oxygen species and maintenance of tissue barrier functions. The availability of advanced transgenic and knockout technologies should provide the experimental tools with which to evaluate the contributions of many of these processes in mediating KGF effects in specific physiological and pathological settings.

The encouraging results from preclinical models led to testing of KGF in humans, to establish its efficacy in reducing tissue injury when exposure to harmful agents could be anticipated. Initial efforts have focused on the ability of KGF to decrease the duration and incidence of severe OM in patients receiving highly mucotoxic cancer therapies. A series of clinical trials have been performed in patients being treated with myeloablative chemoradiotherapy before autologous PBPCT for hematologic malignancies. These studies have culminated in a successful phase 3 trial in which KGF markedly reduced both the incidence and duration of the most severe form of OM, and decreased the use of painkillers and total parenteral nutrition while improving patient quality of life (Spielberger *et al.*, 2003). Similar investigations are underway to demonstrate and optimize the beneficial effects of KGF in patients being treated for solid tumors. In this

context, special attention should be given to the possibility that KGF might have activity on the tumor cells themselves. Thus far preclinical and clinical results suggest deleterious activity is unlikely, but patients should be carefully monitored for any adverse effects. By limiting damage to normal tissue, KGF has the potential not only to diminish the suffering of cancer patients, but it also might enable dosage escalation of therapeutic agents that could improve clinical responses.

On the basis of its success in ameliorating chemoradiotherapy-induced severe OM in humans and tissue damage in a variety of animal models, other potential clinical applications of KGF warrant consideration. Future investigation might address the ability of KGF to reduce mucositis in the lower GI tract that results from cancer treatment regimens. Control of GVHD in allogeneic stem cell transplantation is another potential indication for KGF, as is the prevention or containment of lung injury after a host of toxic exposures. Basic research has yielded important insights about the function of KGF. Now applied research is providing opportunities to enhance the normal capacity of the body to protect and heal itself through the use of this knowledge.

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