The EMBO Journal vol.10 no.6 pp.1347 - 1354, 1991

FGFR-4, a novel acidic fibroblast growth factor receptor with a distinct expression pattern

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Communicated by K. Alitalo

We have previously identified two novel members of the fibroblast growth factor receptor (FGFR) gene family expressed in K562 erythroleukemia cells. Here we report cDNA cloning and analysis of one of these genes, named FGFR-4. The deduced amino acid sequence of FGFR-4 is 55% identical with both previously characterized FGFRs, *flg* and *bek*, and has the structural characteristics of a FGFR family member including three immunoglobulin-like domains in its extracellular part. Antibodies raised against the carboxy terminus of FGFR-4 detected 95 and 110 kd glycoproteins with a protein backbone of 88 kd in COS cells transfected with a FGFR-4 cDNA expression vector. The FGFR-4 protein expressed in COS cells could also be affinity-labelled with radioiodinated acidic FGF. Furthermore, ligand binding experiments demonstrated that FGFR-4 binds acidic FGF with high affinity but does not bind basic FGF. FGFR-4 is expressed as a 3.0 kb mRNA in the adrenal, lung, kidney, liver, pancreas, intestine, striated muscle and spleen tissues of human fetuses. The expression pattern of FGFR-4 is distinct from that of *flg* and *bek* and the yet additional member of the same gene family, FGFR-3, which we have also cloned from the K562 leukemia cells. Our results suggest that FGFR-4 along with other fibroblast growth factor receptors performs cell lineage and tissue-specific functions.

Key words: immunoglobulin superfamily/oncogene/tyrosine kinase

Introduction

The fibroblast growth factors (FGFs) are multifunctional regulatory peptides with a great impact on studies of tumorigenesis, cardiovascular diseases, repair of tissue injury, neurobiology, and embryonic development. At present, seven members of this growth factor family are known (Abraham *et al.*, 1986; Jaye *et al.*, 1986; Delli Bovi *et al.*, 1987; Smith *et al.*, 1988; Yoshida *et al.*, 1987; Zhan *et al.*, 1988; Finch *et al.*, 1989; Marics *et al.*, 1989). Acidic and basic FGFs (aFGF and bFGF) are the first and best characterized family members. The *in vitro* effects of these two polypeptides appear to be similar and very complex: depending on the cell type FGFs can induce mitogenesis, support cell survival and either induce or inhibit cellular differentiation (see Burgess and Maciag, 1989). *In vivo*, FGFs have been shown to participate in mesoderm induction in *Xenopus* embryos (Slack *et al.*, 1987; Kimelman *et al.*, 1988) and in angiogenesis (Thomas *et al.*, 1985; Thompson *et al.*, 1989; Folkman and Klagsbrun, 1987).

The fibroblast growth factor receptors (FGFRs) also seem to be encoded by a gene family. The receptor for bFGF was originally affinity purified and cloned from chicken (Lee *et al.*, 1989; Pasquale and Singer, 1989). This receptor was highly homologous to the previously cloned partial sequence of the human *flg* tyrosine kinase (Ruta *et al.*, 1988, 1989) and presumably is the chicken homologue of the human gene. *bek* is another gene cloned from mouse, human and chicken cDNA, whose protein product is related to the *flg* receptor (Kornbluth *et al.*, 1988; Hattori *et al.*, 1990; Dionne *et al.*, 1990; Houssaint *et al.*, 1990; Pasquale 1990; Miki *et al.*, 1991). Both of these receptors have been shown to bind aFGF and bFGF (Dionne *et al.*, 1990; Houssaint *et al.*, 1990; Johnson *et al.*, 1990). Yet an additional tyrosine kinase sequence belonging to FGFR family has been identified from chicken (Cek2; Pasquale, 1990). Structural features shared by members of the FGFR family include glycosylated immunoglobulin-like (Ig) loops in the extracellular domain and a conserved tyrosine kinase domain split by a short kinase insert.

The biosynthesis of different gene products by differential splicing further increases the complexity of FGFRs. Predicted *flg* protein products with two or three Ig loops as well as a form lacking the intracellular tyrosine kinase domain have been reported (Johnson *et al.*, 1990; Reid *et al.*, 1990; Hou *et al.*, 1991). Also the published *bek* cDNAs differ, having two or three Ig loops (Dionne *et al.*, 1990; Hattori *et al.*, 1990; Houssaint *et al.*, 1990; Miki *et al.*, 1991). A major question is whether the various FGFRs differ in some functional aspects, such as ligand binding properties, regulation of their expression or substrate specificities.

We have previously identified by the polymerase chain reaction (PCR) cloning method several novel tyrosine kinases expressed in the K562 erythroleukemia cells (Partanen *et al.*, 1990). Two of these, JTK2 and JTK4, were closely related to the previously characterized members of the FGFR family. Here we report the cDNA cloning, initial structural and functional characterization of the protein product of the JTK2 gene, which we have renamed FGFR-4. Analysis of mRNA expression in human fetal tissues demonstrates considerable differences in the regulation of the members of FGFR family.

Results

Cloning and sequence analysis of FGFR-4

The FGFR-4 cDNA fragment obtained by PCR amplification of polyadenylated RNA from the K562 leukemia cells (Partanen *et al.*, 1990) was used as a molecular probe to

screen an oligo(dT) primed HEL leukemia cell cDNA library (Poncz *et al.*, 1987). Several clones were identified and the longest FGFR-4 clone (HE6) of ~2.8 kb was sequenced. Due to cross-hybridization we also identified several cDNA clones of the FGFR-3 (JTK4) gene. One of these (HE8) was used as probe for mRNA expression studies. The 5′ portion of the HE6 clone was amplified by PCR and used to obtain more 5′ cDNA sequences from a random primed K562 cell cDNA library.

The deduced amino acid sequence of the FGFR-4 cDNA and its comparison with the human *flg*, *bek* and FGFR-3 amino acid sequences (Ruta *et al.*, 1988; Isacchi *et al.*, 1990; Wennström *et al.*, 1991; Dionne *et al.*, 1990; Houssaint *et al.*, 1990; Hattori *et al.*, 1990; Keegan *et al.*, 1991) are

A

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          sssssssssssssssssssssss                                    o
FGFR-4 MRLLLALLGVLLSVPGPPVLSLEASEEVELEPCLA-----PSLEQQEQELT-VA-LGQPV 53
flg    .WSWKC..FWAVL.TAT--.CTARPSPTLP.QAQ------.WGAPV.V.SFL.H-P.DLL 51
bek    .VSWGRFICLVVVTMAT--.CTARPSFSLV.DTTLEPEEP.TKY.IS.PEVY..AP.ESL 58
FGFR-3 .GAPACA.ALCVA.AIVAGA.S.SLGTEQRVVGR.AEVPG.EPG....---L.FGS.DA. 57
          x          o                          o o o x
FGFR-4 RLCCGRAERG----GHWYKEGSRLAPAGRVRGWRGRLEIASFLPEDAGRYLCLARGSMIV 109
flg    Q.R.RLRDDV--QSIN.LRD.VQ..ESN.T.ITGEEV.VQDSV.A.S.L.A.VTSSPSGS 109
bek    EVR.LLKDAA---VIS.T.D.VH.G.NN.TVLIGEY.Q.KGAT.R.S.L.A.T.SRTVDS 115
FGFR-3 E.S.PPPGG.PMGPTV.VKD.TG.V.SE..LVGPQ..QVLNASH..S.A.S.RQ.LTQR. 117
            g          aaaa
FGFR-4 LQ-NLTLITGDSLTSSNDDEDPK-SHRDPSNRHSYPQQ-----APYWTHPQRMEKKLHAV 162
flg    DTTYFSVNVS.A.P..E..D.DDD.SSEE-KETDNTKPNRMPV.....S.EK........ 168
bek    ETWYFMVNVT.AI-..G....DTDGAE.FVSEN.NNKR-----.....NTEK...R.... 169
FGFR-3 .C-HFSVRVT.AP-..G....GEDEAE.TGVDTG---------.....R.E..D...L.. 166
             o   x   g     o                                  o o o
FGFR-4 PAGNTVKFRCPAAGNPTPTIRWLKDGQAFHGENRIGGIRLRHQHWSLVMESVVPSDRGTY 222
flg    ..AK....K..SS.T.N..L....N.KE.KPDH....YKV.YAT..II.D......K.N. 228
bek    ..A.........G...M..M....N.KE.KQ.H....YKV.N.....I........K.N. 229
FGFR-3 ..A...R...........S.S...N.RE.R..H.....K....Q.............N. 226
          x                                  g       o   x
FGFR-4 TCLVENAVGSIRYNYLLDVLERSPHRPILQAGLPANTTAVVGSDVELLCKVYSDAQPHIQ 282
flg    ..I...EY...NHT.Q...V..............K.VAL..N..FM......P..... 288
bek    ..V...EY...NHT.H...V..............AST...G...FV............ 289
FGFR-3 ..V...KF....QT.T..................Q...L.....FH............ 286
          o     g                  g          g    o o o x
FGFR-4 WLKHIVINGSSFGAVGFPYVQVLKTADINSS--EVEVLYLRNVSAEDAGEYTCLAGNSIG 340
flg    .....EV...KI.PDNL....I....GV.TTDK.M...H.....F.............. 348
bek    .I..VEK...KY.PD.L..LK...A.GV.TTDK.I....I...TF.............. 349
FGFR-3 ....VEV...KV.PD.T...T.....GA.TTDK.L...S.H..TF.............. 346
                                   mmmmmmmmmmmmmmmmmmmmm
FGFR-4 LSYQSAWLTVLPEEDPTWTAAAPEARYTDIILYASGSLALAVLLLLAGLYRGQALHGRHP 400
flg    ..HH.......EALEER-P.VMTSPL.LE..I.CT.AFLISCMVGSVIV.KMKSGTKKSD 407
bek    I.FH........APGRE-KEITASPD.LE.AI.CI.VFLI.CMVVTVI.C.MKNTTKKPD 408
FGFR-3 F.HH....V...A.EELVE.DEAGSV.AG.LS.GV.FFLFILVVAAVT.CRLRSPPKKGL 406
                          r
FGFR-4 -RPPATVQKLS-RFPLARQFSLESGSSG--KSSSSLVR-GVRLSS-SGPALLAGLVSLDL 454
flg    FHSQMA.H..AKSI..R..VTVSAD..ASMN.GVL...-PS....-..TPM...VSEYE. 465
bek    FSSQPA.H..TK.I..R..VTVSAE..SSMN.NTP...ITT....TADTPM...VSEYE. 468
FGFR-3 --GSP..H.I.-....K..V....NA.--MS.NTP...I-A....-GEGPT..NVSELE. 459
             >        p p  p                        p
FGFR-4 PLDPLWEFPRDRLVLGKPLGEGCFGQVVRAEAFGMDPARPDQASTVAVKMLKDNASDKDL 514
flg    .E..R..L.....................L...I.L.KDK.NRVTK.......SD.TE... 525
bek    .E..K......K.T..............M...V.I.KDK.KE.V.........D.TE... 528
FGFR-3 .A..K..LS.A..T..............M...I.I.KD.AAKPV.........D.T.... 519
                                                              iiii
FGFR-4 ADLVSEMEVMKLIGRHKNIINLLGVCTQEGPLYVIVECAAKGNLREFLRARRPPGPDLSP 574
flg    S..I....M..M..K.........A...D........Y.S......Y.Q......LEYCY 585
bek    S.......M..M..K.........A...D........Y.S......Y........MEY.Y 588
FGFR-3 S.......M..M..K.........A...G.....L..Y.................L.Y.F 579
       iiiiiiiiii
FGFR-4 DGPRSSEGPLSFPVLVSCAYQVARGMQYLESRKCIHRDLAARNVLVTEDNVMKIADFGLA 634
flg    NPSHNP.EQ..SKD............E..A.K.......................... 645
bek    .IN.VP.EQMT.KD....T..L....E..A.Q..............N........... 648
FGFR-3 .TCKPP.EQ.T.KD............E..A.Q.......................... 639
            A
FGFR-4 RGVHHIDYYKKTSNGRLPVKWMAPEALFDRVYTHQSDVWSFGILLWEIFTLGGSPYPGIP 694
flg    .DI.........T.................I...........V..............V. 705
bek    .DINN.......T.............................V.M.............. 708
FGFR-3 .D..NL......T.............................V.................. 699
                                                  <
FGFR-4 VEELFSLLREGHRMDRPPHCPPELYGLMRECWHAAPSQRPTFKQLVEALDKVL-LAVSEE 753
flg    .....K..K......K.SN.TN...MM..D....V...........D..RIVA.TSNQ. 765
bek    .....K..K......K.AN.TN...MM..D....V...........D..RI.T.TTN.. 768
FGFR-3 .....K..K......K.AN.THD..MI.................D..R..TVTSTD. 759
       s      s
FGFR-4 YLDLRLTFGPYSPSGGDAS-STCSSS-DSVFSHDPLP--LGSSSFPF--GSGVQT      802
flg    ....SMPLDQ....FP.TRS.....GE......E...EEPCLPRH.AQLANRGLKRR  822
bek    ....SQPLEQ....YP.TR-.S...GD.....P..M.YEPCLPQY.HIN..-.K.    821
FGFR-3 ....SAP.EQ...G.Q.TP-.SS..GD....A..L..-----PAP.SSG..--R.    806
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B

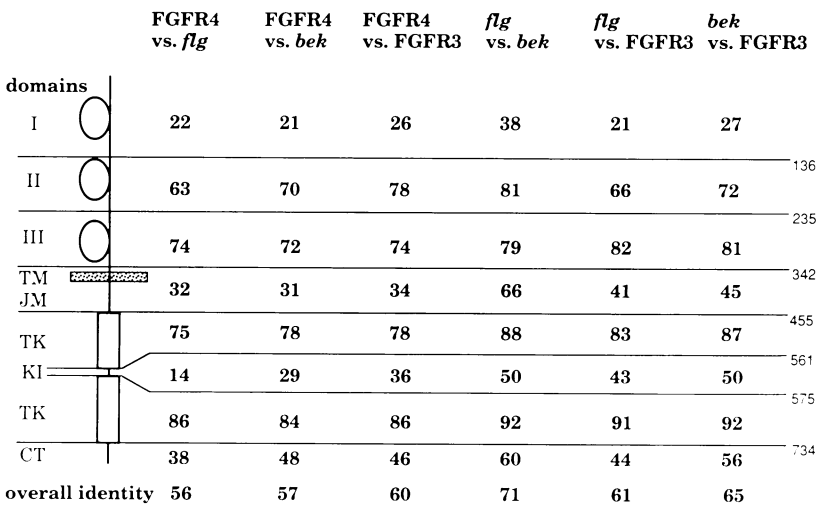

Fig. 1. A. Deduced amino acid sequence of FGFR-4 and its comparison with *flg*, *bek* and FGFR-3 sequences. Only residues of *flg*, *bek* and FGFR-3 which differ from those of FGFR-4 are shown. Dashes indicate absence of the corresponding amino acids. Marked in the FGFR-4 sequence are: the signal sequence (s), consensus residues of the immunoglobulin-like domains (o), including the cysteine residues predicted to participate in intrachain disulfide bonds (x), potential Asn (N)-linked glycosylation sites (g), the acidic region (a), putative transmembrane sequence (m), the tyrosine kinase region (> <), residues involved in ATP binding (p), kinase insert region (i), conserved tyrosyl residue corresponding to the c-*src* autophosphorylation site Y416 (A), possible Ser/Thr kinase phosphorylation site in the juxtamembrane region (r, Hou *et al.*, 1991) and conserved tyrosyl residues in the carboxyl terminal region (s). The FGFR-4 nucleotide sequence has been submitted to the EMBL database (accession no. X57205). **B.** A schematic structure of the known FGFRs, showing the degree of amino acid identify between different regions of the human FGFR-4, *flg*, *bek* and FGFR-3. The lines demarcating each of the regions are located at amino acid positions marked to the right end of each line. The boxes represent the tyrosine kinase domain (TK) split by the kinase insert region (KI) and the loops I–III represent the extracellular Ig-like domains. The transmembrane region (TM) has been stippled. JM, juxtamembrane region, CT, carboxy terminal tail.

shown in Figure 1A. The initiator methionine codon marked in the figure is surrounded by a satisfactory Kozak's consensus sequence (Kozak, 1984, data not shown) and followed by a putative hydrophobic leader peptide. The deduced FGFR-4 polypeptide is 802 amino acids long (for comparison, the length of the *flg* is 822, *bek* 821 and FGFR-3 806 amino acid residues), with a hydrophobic signal sequence of 24 (21, 21, 20) amino acids, an extracellular domain of 345 (355, 356, 353) amino acids, a hydrophobic sequence characteristic of a transmembrane domain of 21 (21, 21, 21) amino acids and an intracellular domain of 412 (425, 423, 410) amino acids. The tyrosine kinase domain of the intracellular portion is preceded by a juxtamembrane domain of 74 (78, 80, 73) amino acid residues and is split by a kinase insert region of 14 (14, 14 14) amino acids. The C-terminal tail is 59 (68, 64, 58) amino acid residues long. The extracellular portion contains the consensus sequences for three Ig domains (Williams and Barclay, 1988), which are typical for members of the FGFR family and also occur in receptors belonging to the PDGFR family (Ullrich and Schlessinger, 1990). Six potential N-linked glycosylation sites are located in the extracellular domain of FGFR-4, four of these sites align well with four of the nine, eight and seven sites used in *flg*, *bek* and FGFR-3, respectively. The stretch of eight and five consecutive acidic residues in the extracellular domains of *flg* and *bek*, respectively, correspond to four residues (DDED) in the FGFR-4.

Figure 1B shows a schematic structure and degree of homology of the FGFR-4, *flg*, *bek* and FGFR-3 protein products. The percentage of amino acid sequence identity between the corresponding domains of each receptor is marked beside the appropriate polypeptide regions. The homology between all four cloned FGFR genes is least in the extreme amino terminal domain (I; 21–38%), higher in the proximal Ig domains (II; 63–81% and III; 72–82%) and greatest in the tyrosine kinase domain (TK; 74–92%). The transmembrane (TM), juxtamembrane (JM) and kinase insert (KI) regions, as well as the carboxyl terminal tails (CT) have only little homology between FGFR-4 and the previously known receptors. Neither of the tyrosyl residues present in the kinase inserts of *flg* and *bek* have been conserved in FGFR-4; only one of them is present in FGFR-3. In general, the comparison of amino acid sequence similarities suggests that the *flg* and *bek*-type receptors are less closely related to FGFR-4 (56 and 57% identical, respectively) and to FGFR-3 (61 and 65% identical) than to each other (71% identical).

Expression of FGFRs in human fetal tissues

The FGFR-4 gene is expressed as an ~3.0 kb mRNA in various cell lines and tissues (Partanen *et al.*, 1990 and data not shown). The mRNA expression of the four cloned FGFR family members was analysed by Northern blotting and hybridization with specific cDNA probes in total RNA from 17–18 week human fetuses. To compare the expression patterns, the same blot was hybridized first with a FGFR-4 specific probe and then by a *flg*-specific probe. The probes were then stripped off and the filter was reprobed with fragments of FGFR-3 and *bek* cDNAs, with an intervening removal of signal. Interestingly, as can be seen from Figure 2, the FGFR-4, *flg* (4.4 and 4.8 kb), FGFR-3 (4.5 kb) and *bek* (4.4 kb) mRNAs appear to be independently regulated. The *flg* mRNA was highly expressed in the brain, skin, growth plates of developing bones and calvarial bones with lesser mRNA amounts in several other organs. Low amounts of FGFR-4 mRNA were present in the choroid plexus and meninges and intermediate levels in the kidney, intestine, pancreas, striated muscle, spleen and liver. Highest concentrations of the FGFR-4 mRNA were found in the adrenal and lung. In the adrenal, no FGFR-3 mRNA was seen and

the *flg* and *bek* mRNAs were expressed at barely detectable levels. On the other hand, no or very low amounts of FGFR-4 mRNA were present in the developing brain, calvarial bone, growth plates or skin, in contrast to the *flg*, FGFR-3 and *bek* mRNAs. The FGFR-3 mRNA was also abundant in the intestinum and lung, while high expression of *bek* mRNA was observed also in the choroid plexus.

Identification of the FGFR-4 polypeptides

For expression of the FGFR-4 protein, the coding region of the FGFR-4 cDNA was cloned under control of the SV40 promoter and transfected into COS-1 cells. Two days later the cells were labelled with [^{35}S]methionine and extracts immunoprecipitated with rabbit antisera against a β-galactosidase-FGFR-4 fusion protein expressed and purified from bacteria (see Materials and methods). Figure 3 shows an autoradiogram of the SDS−PAGE analysis of the resulting immunoprecipitates. COS cells transfected with the control vector (plasmid GSE) yielded a polypeptide of 120 kd with two anti-FGFR-4 antisera (lanes L3 and V3), while the same antisera precipitated additional polypeptides of 95 and 105−115 kd from cells transfected with the FGFR-4 expression vector (plasmid SV2SE, lanes V3 and L3). None of these polypeptides were precipitated by the corresponding immunogen-blocked or preimmune sera (lanes V0 and V3B). When the cells were labelled in the presence of tunicamycin to prevent protein glycosylation, the immunoprecipitation yielded only one specific polypeptide of 88 kd. The apparent molecular weight of this presumably unglycosylated FGFR-4 polypeptide fits well with the molecular weight calculated from its deduced amino acid sequence (87.6 kd). For comparison, when COS cells were transfected with a similar *flg* expression vector (SV7dFGFR-1), *flg*-specific antibodies (lane K91; Wennström *et al.*, 1991) immunoprecipitated polypeptides of 125 and 130−140 kd.

Crosslinking of [^{125}I]aFGF to FGFRs expressed in COS cells

For covalent crosslinking, transfected COS cells were incubated with [^{125}I]aFGF in the presence or absence of a 50-fold excess of unlabelled aFGF or bFGF. After washing to remove unbound ligand the cells were treated with the covalent crosslinker disuccinimidyl suberate, extracted and analysed by SDS−PAGE and autoradiography. Untransfected COS cells or cells transfected with the pSV2EGFR/*neu* vector (Lehväslaiho *et al.*, 1989) showed a barely visible, fuzzy band in the 100 kd region (Figure 4A). This, however, was abolished by an excess of unlabelled aFGF, suggesting that it was derived from low amounts of endogenous FGFR on the COS cell surface. COS

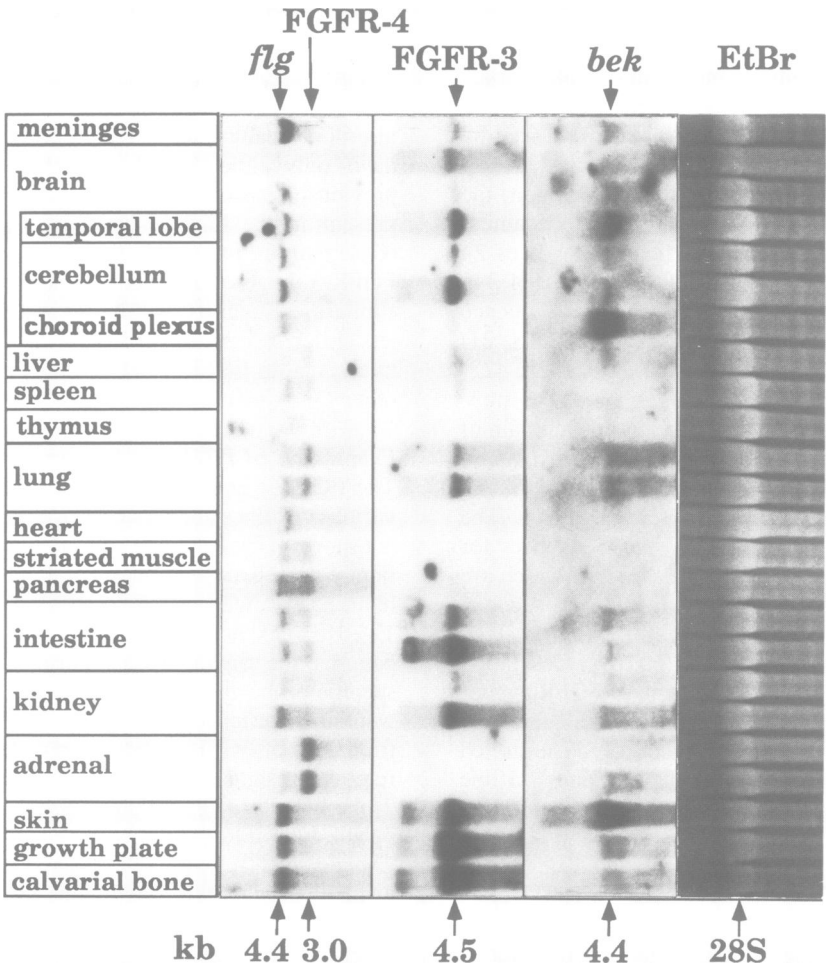

Fig. 2. Expression of the FGFR-4, FGFR-3, *flg* and *bek* genes in various tissues of 17−18 week old human fetuses. Total RNA was analysed as described in Materials and methods; for some tissues samples from two different fetuses are shown. The bottom panel shows the ethidium bromide (EtBr)-stained and UV-photographed RNA gel. The identity of the high mol. wt band seen in FGFR-3 hybridization is not known.

cells transfected with the FGFR-4 expression vector SV2SE showed a broad band of 90−95 kd and a less intense band of 140 kd. However, only the 140 kd protein could be immunoprecipitated with the antiserum against the FGFR-4 carboxy terminus (Figure 4B). The *flg* expression vector yielded a broad band of 105−110 kd and a weak band of 160 kd, which is consistent with the size difference between the *flg* and FGFR-4 proteins. Both of the *flg* and FGFR-4 signals were competed off by excess of unlabelled aFGF. In contrast, bFGF abolished only the *flg* signals completely, while somewhat diminished signals for FGFR-4 were retained. In the same experiment, both FGFs completely abolished the crosslinking of [^{125}I]aFGF to a major 160 kd and a minor 110 kd receptor on the surface of the Tera-2 teratocarcinoma cells, which were used as further controls.

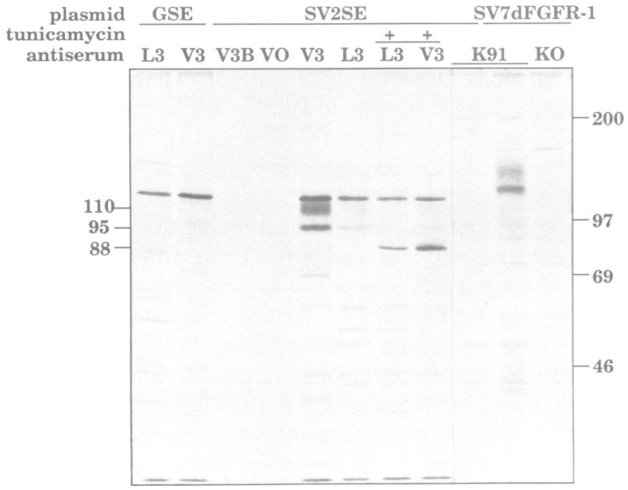

Fig. 3. Analysis of the FGFR-4 protein expressed in COS-1 cells. The SV40 early promoter-driven expression vector for FGFR-4 (SV2SE) or *flg* (SV7dFGFR-1) and a control plasmid (GSE) were transfected into COS-1 cells. Two days later the cells were metabolically labelled and immunoprecipitated with antisera against FGFR-4 (from two rabbits, marked L and V; 0, preimmune serum, 3 refers to third bleed; B, serum blocked with immunogen) or *flg* protein (lanes K91, immune serum; lane K0, preimmune serum). Where indicated the cells were labelled in the presence of tunicamycin to prevent N-linked glycosylation. The mol. wt markers are given on the right and apparent mol. wts of the FGFR-4 polypeptides on the left (in kd).

Analysis of aFGF and bFGF binding

The quantitation of specific binding of iodinated aFGF and bFGF to FGFR-4 and *flg*(FGFR-1)-transfected COS-cells is shown in Figure 5. The cells were incubated with various concentrations of iodinated FGFs and the cell bound radioactivity was measured. As can be seen from Figure 5A, aFGF binds to both receptors in a similar manner, whereas bFGF only binds to *flg*, but not to FGFR-4 (Figure 5B). Similar results were obtained from three independent experiments. Scatchard analyses of aFGF binding to FGFR-4 transfected cells (Figure 5C) allowed the estimation of K_d values, which ranged from 200 to 600 pM in the three different experiments. The corresponding values for aFGF binding to transfected COS cells expressing the *flg*-type receptor fell within the same range (data not shown).

Discussion

The FGF growth factor−receptor system presents an interesting complexity revealed by molecular cloning of members of the FGF ligand and receptor families (Abraham *et al.*, 1986; Jaye *et al.*, 1986; Delli Bovi *et al.*, 1987; Smith *et al.*, 1988; Yoshida *et al.*, 1987; Zhan *et al.*, 1988; Finch *et al.*, 1989; Marics *et al.*, 1989; Lee *et al.*, 1989; Dionne *et al.*, 1990; Pasquale *et al.*, 1990; Miki *et al.*, 1991). At present, seven genes encoding FGF-like growth factors are known. However, only two different receptors have been reported from the human (*flg* and *bek*, Dionne *et al.*, 1990; Houssaint *et al.*, 1990) and three from the chicken FGFR family (cek1, cek2, cek3, Pasquale, 1990). We have identified from leukemia cell RNA two additional human genes belonging to the FGFR gene family (Partanen *et al.*, 1990). The protein product of one of these, the

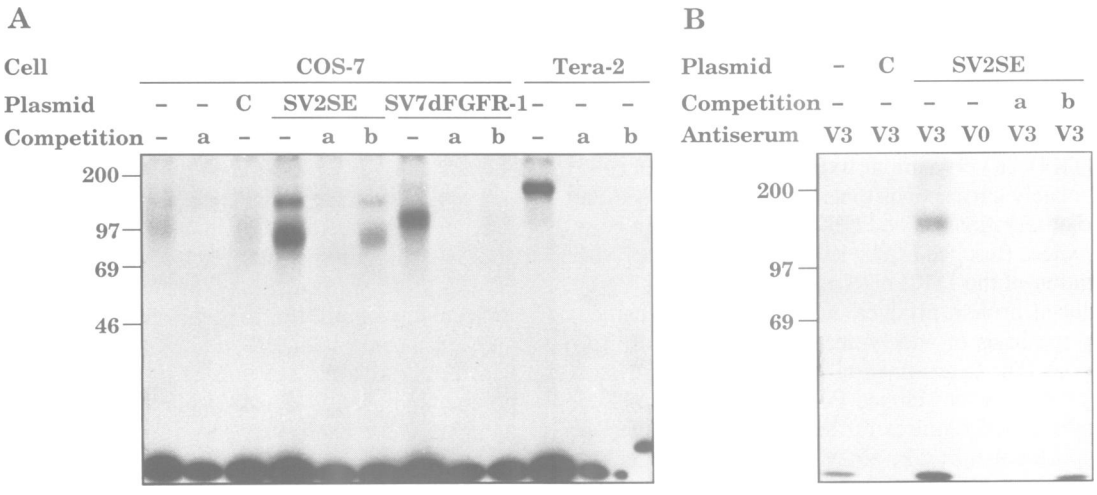

Fig. 4. Covalent crosslinking of acidic FGF to the FGFR-4 protein. COS-1 cells were transfected with expression vectors for FGFR-4 (SV2SE), *flg* (SV7dFGFR-1) or EGFR/*neu* chimaera (**C**; Lehväslaiho *et al.*, 1989). ^{125}I-labelled acidic FGF was crosslinked to the transfected COS cells and Tera-2 cells in the presence or absence of a 50-fold excess of acidic (a) or basic FGF (b). Aliquots of the cell lysates were analysed in SDS−PAGE (**A**); the remaining lysates were immunoprecipitated with antisera against FGFR-4 prior to analysis (**B**). The mol. wt markers are given on the left (in kd).

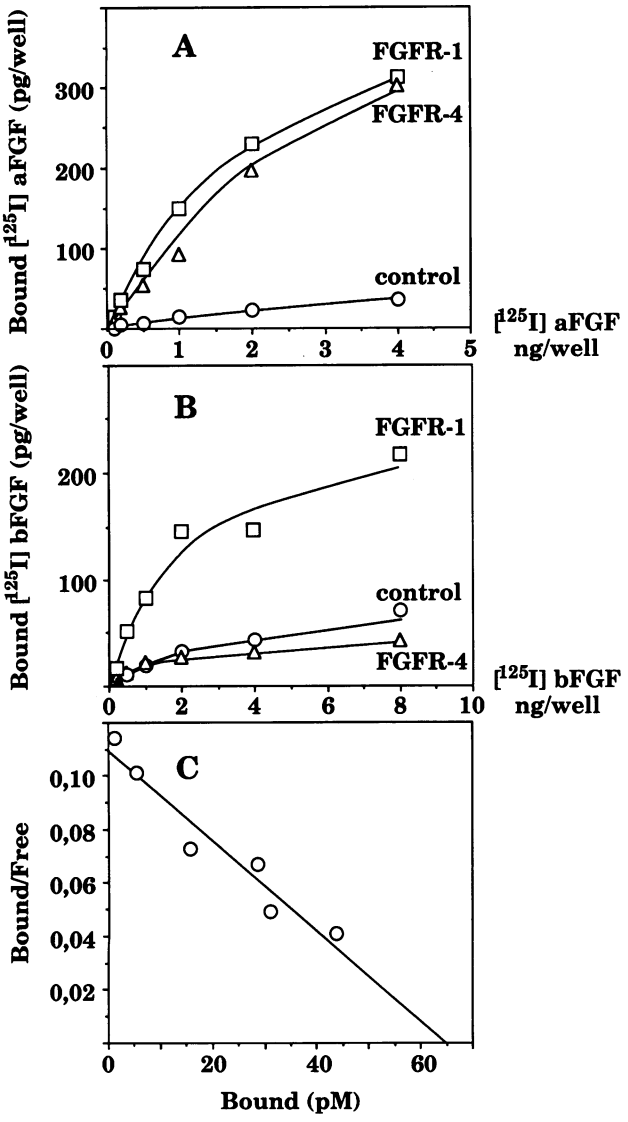

Fig. 5. Binding of aFGF and bFGF to FGFR-4 and *flg*-expressing COS cells. The FGFR-4 (SV2SE), *flg* (SVdFGFR-1) or control (pSV2EGFR7neu)-transfected COS cells were incubated with different concentrations of [^{125}I]aFGF (**A**) or [^{125}I]bFGF (**B**). Non-specific binding (~10% of the total) was estimated by parallel determinations with 100-fold excess of unlabelled ligand. Scatchard analysis of aFGF binding to FGFR-4 transfected cells is shown in (**C**).

FGFR-3 (JTK4), is very similar to that of the chicken cek2 gene and probably corresponds to its human homologue (our unpublished observations). The FGFR-3 cDNA has also been cloned elsewhere (Keegan *et al.*, 1991). Here we report the characterization of the JTK2 cDNA (Partanen *et al.*, 1990) and its deduced protein product, which we have renamed FGFR-4 on the basis of widely accepted nomenclature for FGF receptors. The determination of binding properties of different FGFs to various cloned FGF receptors is an issue of major interest and requires further studies. However, it has been reported that bFGF, aFGF and K-FGF all bind to the *flg* protein (Mansukhani *et al.*, 1990; Dionne *et al.*, 1990; Safran *et al.*, 1990) and bFGF and aFGF and KGF bind to *bek* (Dionne *et al.*, 1990; Houssaint *et al.*, 1990; Miki *et al.*, 1991). In addition to their FGF binding characteristics, the various receptors could differ in their substrate specificities or expression patterns.

The structure of FGFR-4 further suggests that it serves as a cell surface receptor binding to members of the FGF-family. The predicted protein product of FGFR-4 is 56, 57% and 60% identical with *flg*, *bek* and FGFR-3 receptor polypeptides, respectively. In fact, the previously characterized family members are more closely related to each other than to FGFR-4. Yet FGFR-4 retains the structural features typical for FGFRs including three Ig domains containing several putative N-linked glycosylation sites. The homology between the extracellular domains of FGFR-4, *flg*, *bek* and FGFR-3 is greatest in the second and third Ig domains. This observation is of interest, as it has been reported that the *flg* protein lacking the first Ig domain binds basic and acidic FGF as avidly as the form containing all three Ig loops (Johnson *et al.*, 1990). Thus, the region implicated for FGF binding in *flg* is ~70% identical between FGFR-4 and *flg* as well as FGFR-4 and *bek*, whereas this region is ~80% identical between the *flg* and *bek* receptors.

The most divergent regions of the different receptors are the first Ig domains, the juxtamembrane domains, kinase inserts and carboxyl terminal tails. These differences might reflect divergent functions or loss of evolutionary constraints. Although dispensible for FGF binding, the first Ig loop might serve some specialized function in the different receptors. It is possible that these regions determine subtle differences in, e.g. ligand binding specificity. In PDGFR, phosphorylation of the KI region has been shown to regulate the binding of phosphatidylinositol 3-kinase by the receptor tyrosine kinase (Kazlauskas and Cooper, 1989). It is therefore of interest that the kinase insert of FGFR-4 does not contain the two tyrosine residues conserved between *flg* and *bek* (positions 583 and 585 in *flg* protein). Differences here could produce FGFRs with different substrate specificities. In general, domain swapping experiments between regions of greatest differences might reveal interesting insights into the specificity of the FGFRs.

When the FGFR-4 cDNA was expressed in COS cells, fuzzy bands suggesting several differentially glycosylated forms of the FGFR-4 protein were observed, which were somewhat smaller than the similarly expressed *flg* or *bek* polypeptides (Dionne *et al.*, 1990; Houssaint *et al.*, 1990; Wennström *et al.*, 1991). Inhibition of N-linked glycosylation with tunicamycin showed that ~7–22 kd of the apparent molecular weight of the FGFR-4 receptor polypeptides is due to carbohydrate chains; the remaining molecular mass fits well with the estimate deduced from the cDNA sequence.

The apparent molecular weights of the receptor polypeptides crosslinked to radioiodinated aFGF were 90–95 kd and 140 kd. The somewhat heterogeneous polypeptides of 90–95 kd may represent degradation products of the higher molecular weight form, as they were not recognized by our antiserum made against the FGFR-4 carboxyl terminus and because the relative amounts of the 140 kd and 90–95 kd polypeptides varied considerably from one experiment to another. These bands may result from the transient expression system used, because a similar broad band of 105–110 kd was seen in addition to a 160 kd polypeptide when the ligand was crosslinked to the *flg*-type receptor on COS cells, but not when Tera-2 cells expressing endogenous receptors were used (Wennström *et al.*, 1991; Dionne *et al.*, 1990). Addition of excess of unlabelled aFGF blocked the labelling of these components, but bFGF was not as effec-

tive as aFGF in blocking the binding to FGFR-4, although it completely blocked [^{125}I]aFGF binding to *flg* receptor and to the Tera-2 cell FGFRs. This suggested that bFGF is less effective than aFGF in binding to the FGFR-4.

Binding analyses showed that FGFR-4 and *flg* receptors bind aFGF with very similar affinities. The calculated K_ds are in the range of published values for *flg* and *bek* receptors (Houssaint *et al.*, 1990; Bottaro *et al.*, 1990; Miki *et al.*, 1991; Dionne *et al.*, 1990; Wennström *et al.*, 1991). The observed variation may result from different expression systems used or from differences in the receptor binding activity of iodinated FGF preparations. In contrast to the *flg* protein, FGFR-4 does not bind bFGF. This is a clear demonstration of functional differences between various FGFRs. Differential splicing seems also to generate receptors varying in the ligand specificity: the human and mouse *bek* receptors containing three and two Ig domains, respectively, appear to differ in their binding of bFGF (Dionne *et al.*, 1990; Miki *et al.*, 1991). We have not yet encountered differentially spliced forms of the FGFR-4 cDNA, but given the splicing variation of the *flg* receptor in three structural domains (Johnson *et al.*, 1990; Hou *et al.*, 1991) we are looking for FGFR-4 mRNA variants by RNase protection experiments.

Recently it has been reported that FGFs have a permissive role in primitive hematopoietic cell colony formation in culture (Gabbianelli *et al.*, 1990). In this regard it is interesting that the FGFR-4 and FGFR-3 cDNAs were cloned from erythroleukemia cells. Differences were observed in the fetal tissue-specific expression patterns of the four FGFR genes. Generally, FGFR-4 showed the greatest deviation from the expression patterns of the other three receptors. Previous analyses have indicated that in adult tissues the *flg* gene is expressed mainly in the central nervous system (Wanaka *et al.*, 1990) and that *bek* mRNA is found in adult mouse liver, lung, brain and kidney, but is absent from heart and spleen (Kornbluth *et al.*, 1988). FGFR-4 expression was particularly high in the fetal adrenals, where only minute amounts of *flg*, *bek* and FGFR-3 mRNAs were detected. Endocrine cells of the fetal adrenal are known to respond to FGF stimulation (Claude *et al.*, 1988; Stemple *et al.*, 1988). On the other hand, little or no FGFR-4 mRNA was found in the fetal brain, bone or skin, which expressed all other FGFR mRNAs. Only a weak FGFR-4 signal was observed in the cerebellum and choroid plexus. Although *in situ* hybridization experiments are needed to pinpoint the cells expressing the different FGFRs, the present experiments strongly suggest that some of the functions of the different FGFRs are tissue specific.

Materials and methods

Isolation and characterization of cDNA clones

An oligo-(dT) primed HEL cell cDNA library in bacteriophage λgt11 (kindly given by Dr Mortimer Poncz, Childrens Hospital of Philadelphia, PA) was screened with the JTK2 (FGFR-4) fragment PCR amplified from the polyadenylylated RNA of K562 leukemia cells (Partanen *et al.*, 1990). The longest FGFR-4 clone (HE6) was 2.8 kb in length, but it did not contain the most 5′ sequences of its protein coding region. Due to cross-hybridization this probe also detected cDNA clones of the JTK4 (FGFR-3) gene (including the clone HE8). The 5′ end of the HE6 clone (330 bp) was PCR amplified and this fragment was used for screening of a random-primed K562 cell cDNA library in λgt11 (Clontech). An overlapping FGFR-4 clone of 1.4 kb (5′2−1) was isolated, which extended further into the 5′ region. Positive plaques were identified and purified as described (Sambrook *et al.*, 1989). cDNA inserts of bacteriophage λ were isolated from a low melting point agarose gel, ligated into *Eco*RI-cleaved GEM3Zf(+) plasmid (Promega) and used to transform *Escherichia coli*. Both single and double stranded templates were used for sequencing by the dideoxynucleotide chain termination method (Sanger *et al.*, 1977). New sequencing primers were designed and synthesized according to the sequences obtained. All portions of the cDNAs were sequenced on both strands. Computer analysis of the sequences were done with the GCG package using Fasta and Wordsearch programs (Devereux *et al.*, 1983).

mRNA expression analyses

All specimens from fetal abortuses were obtained with permission of the joint ethical committee of the University Central Hospital and University of Turku (Turku, Finland). Total RNA was extracted as described (Sambrook *et al.*, 1989). 20 μg of total RNA samples were electrophoresed in agarose gels containing formaldehyde and blotted using standard conditions. The inserts of cDNA clones HE6 (FGFR-4), HE8 (FGFR-3), CD115 and CD116 (*flg* and *bek*, respectively, kindly provided by Drs C.Dionne and M.Jaye) were labelled by the random priming method and hybridized to the blot. Hydridization was carried out in 50% formamide, 5 × Denhardt's solution (100 × Denhardt's solution is 2% each of Ficoll, polyvinylpyrrolidone and bovine serum albumin), 5 × SSPE (3 M NaCl, 200 mM $NaH_2PO_4 \cdot H_2O$, 20 mM EDTA, pH 7.0), 0.1% SDS, and 0.1 mg/ml of sonicated salmon sperm DNA at 42°C for 18−24 h. The filter was washed at 65°C in 1 × SSC (150 mM NaCl, 15 mM sodium citrate, pH 7.0), 0.1% SDS and exposed to Kodak XAR-5 film.

Preparation of antisera

cDNA fragments encoding 64 (V construct) or 120 (L construct) carboxy terminal amino acids were inserted into pEX3 and pEX2 bacterial expression vectors (Stanley and Luzio, 1985; a kind gift from Dr K.K.Stanley, EMBL, Heidelberg, FRG), respectively. The resulting β-galactosidase fusion proteins were produced in bacteria and partially purified by preparative SDS−PAGE. Polypeptide bands were cut out from the gel, minced, mixed with Freund's adjuvant and used for immunization of rabbits. Antisera were used after the third booster immunization. The *flg*-specific antipeptide antiserum has been characterized by Wennström *et al.* (1991).

Expression in COS-1 cells and immunoprecipitation

The full-length FGFR-4 coding sequence was constructed from two overlapping cDNA clones (HE6 and 5′2−1) using an internal *Afl*II site and inserted into the SVpoly mammalian expression vector (Stacey and Schnieke, 1990) as a *Sac*I−*Eco*RI fragment. Similar constructs were used for expression of *flg* protein (Wennström *et al.*, 1991) and EGFR/*neu* chimaera (Lehväslaiho *et al.*, 1989). The constructs were introduced into COS−1 cells by DEAE−dextran transfection method (McClutchan and Pagano, 1962). Two days after transfection the cells were labelled for 4 h with [^{35}S]methionine in the presence or absence of 10 μg/ml tunicamycin. The cells were washed with PBS and scraped into immunoprecipitation buffer (10 mM Tris pH 7.5, 50 mM NaCl, 0.5% sodium deoxycholate, 0.5% Nonidet P40, 0.1% SDS, 0.1 TIU/ml Aprotinin). The lysates were sonicated, centrifuged for 15 min at 10 000 *g* and incubated overnight on ice with the antisera. Protein A−sepharose (Pharmacia) was added and the incubation was continued for 30 min with rotation. The precipitates were washed four times with the immunoprecipitation buffer, once with PBS and once with water before analysis in SDS−PAGE.

FGF−receptor crosslinking

Confluent monolayers of transfected COS−1 cells in 90 mm culture dishes were washed twice with binding buffer (DMEM containing 0.1% gelatin and 50 mM HEPES, pH 7.5). About 25 ng of [^{125}I]aFGF in the binding buffer was added on ice and incubation continued for 90 min. The cells were washed once with binding buffer, twice with PBS and incubated at 4°C in PBS containing 0.3 mM DSS for 20 min. The cells were then washed once with 10 mM HEPES pH 7.5, 200 mM glycine, 2 mM EDTA and once with PBS, lysed in lysis buffer (20 mM HEPES, pH 7.5, 150 mM NaCl, 1% Triton X−100, 10% glycerol, 1.5 mM $MgCl_2$, 1.0 mM EDTA, 1 μg/ml aprotinin) and centrifuged for 10 min at 10 000 *g*. Aliquots of the supernatant were boiled in an equal volume of the electrophoresis sample buffer and analysed in SDS−PAGE. The rest of the lysates were immunoprecipitated with anti-FGFR-4 antiserum as described above.

Receptor binding assays

aFGF (a kind gift of Drs C.Dionne and M.Jaye) and bFGF (kindly provided by L.Bergonzoni, Farmitalia Carlo-Erba, Milan) were iodinated by the chloramine T method (Hunter and Greenwood, 1962) and the labelled FGFs

were purified by a heparin–Sepharose column. Specific activities obtained were 6.5 $\times 10^4$ for aFGF and 4.9 $\times$ 10^4 for bFGF. Transfected COS cells on gelatinized 24 well plates (4 $\times$ 10^5 cells/well) were placed on ice and washed twice with cold binding buffer + heparin (DMEM, 1 mg/ml BSA, 5U/ml heparin, 50 mM HEPES, pH 7.4) and incubated 2 h at +4°C with increasing amounts of iodinated FGFs in binding buffer without heparin. When aFGF was used the cells were washed three times with binding buffer, lysed in 0.3 M NaOH, and the solubilized radioactivity was determined with a gamma-counter. In the case of the bFGF the cells were washed three times with PBS and twice with 2 M NaCl, 20 mM HEPES pH 7.5. The receptor bound ligand was released by two washes with 2 M NaCl, 20 mM sodium acetate pH 4.0 (Moscatelli, 1987) and measured by gamma-counting. The same determinations were done in the presence of 100 fold excess of unbound ligand to estimate the nonspecific binding.

Acknowledgements

We thank Minna Ahlstedt, Elina Roimaa, Kirsi Pylkkänen, Hilkka Toivonen, Anne Polvi and Tapio Tainola for expert technical assistance. We kindly thank Drs Craig Dionne and Michael Jaye (Rhone-Poulenc Rorer Central Research, King of Prussia, PA, USA) for their generous gift of *flg* and *bek* plasmids and [^{125}I]aFGF, Dr Ralf Petterson for aFGF, Dr Laura Bergonzoni (Farmitalia Carlo Erba, Milan) for bFGF, Dr Gerard Grosveld (Erasmus University, Rotterdam) and Mortimer Poncz (Childrens Hospital, Philadelphia, PA) for cDNA libraries, Dr K.K.Stanley for pEX bacterial expression system and Dr Chris F.Graham (Oxford) for the Tera–2 cells. This study was supported by the Finnish Cancer Organizations, The Finnish Academy, The Sigrid Juselius and Finnish Cultural Foundations, Orion Corporation Research Fund, the Research and Science Foundation of Farmos.

References

Abraham,J.A., Whang,J.L., Tumulo,A., Mergia,A., Frieman, J., Gospodarowicz,D. and Fiddes,J.C. (1986) *EMBO J.*, **5**, 2523–2528.

Bottaro,D.P., Rubin,J.S., Ron,D., Finch,P.W., Florio,C. and Aaronson,S.A. (1990) *J. Biol. Chem.*, **265**, 12767–12770.

Burgess,W.H. and Maciag,T. (1989) *Annu. Rev. Biochem.*, **58**, 575–606.

Claude,P., Parada,I.M., Gordon,K.A., D'Amore,P.A. and Wagner,J.A. (1988) *Neuron* **1**, 783–790.

Delli Bovi,P., Curatola,A.M., Kern,F.G., Greco,A., Ittmann,M. and Basilico,C. (1987) *Cell* **50**, 729–739.

Devereux,J., Haeherli,P. and Smithies,O. (1983) *Nucleic Acids Res.*, **12**, 387–395.

Dionne,C.A., Crumley,G., Bellot,F., Kaplow,J.M., Searfoss,G., Ruta,M., Burgess,W.H., Jaye,M. and Schlessinger,J. (1990) *EMBO J.*, **9**, 2685–2692.

Finch,P.W., Rubin,J.S., Miki,T., Ron,D. and Aaronson,S.A. (1989) *Science* **245**, 752–755.

Folkman,J. and Klagsbrun,M. (1987) *Science*, **235**, 442–447.

Gabbianelli,M., Sargiacoma,M., Pelosi,E., Testa,U., Isacchi,G. and Peschle,C. (1990) *Science* **249**, 1516–1564.

Hattori,Y., Odagiri,H., Nakatani,H., Miyagawa,K., Naito,K., Sakamoto,H., Katoh,O., Yoshida,T., Sugimura,T. and Terada,M. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 5983–5987.

Hou,J., Kan,M., McKeehan,K., McBride,G., Adams,P. and McKeehan,W.L. (1991) *Science*, **251**, 665–668.

Houssaint,E., Blanquet,P.R., Champion-Arnaud,P., Gesnel,M.C., Torriglia,A., Courtois,Y. and Breathnach,R. (1990) *Proc. Natl Acad. Sci. USA*, **87**, 8180–8184.

Hunter,W.M. and Greenwood,F.C. (1962) *Nature*, **194**, 495–496.

Isacchi,A., Bergonzoni,L. and Sarmientos,P. (1990) *Nucleic Acids Res.*, **18**, 2616.

Jaye,M., Howk,R., Burgess,W., Ricca,G.A., Chiu,I.M., Ravera,M.W., O'Brien,S.J., Modi,W.S., Maciag,T. and Drohan,W.N. (1986) *Science* **233**, 541–545.

Johnson,D.E., Lee,P.L., Lu,J. and Williams,L.T. (1990) *Mol. Cell. Biol.*, **10**, 4728–4736.

Kazlauskas,A. and Cooper,J.A. (1989) *Cell*, **58**, 1121–1132.

Keegan,K., Johnson,D.E., Williams,L.T. and Hayman,M.J. (1991) *Proc. Natl Acad. Sci. USA*, **88**, 1095–1099.

Kimelman,D., Abraham,J.A., Haaparanta,T., Palisi,T.M. and Kirschner,M.W. (1988) *Science*, **242**, 1053–1058.

Kornbluth,S., Paulson,K.E. and Hanafusa,H. (1988) *Mol. Cell. Biol.*, **8**, 5541–5544.

Kozak,M. (1984) *Nucleic Acids Res.*, **12**, 857–872.

Lee,P.L., Johnson,D.E., Cousens,L.S., Fried,V.A. and Williams,L.T. (1989) *Science*, **245**, 57–60.

Lehväslaiho,H., Lehtola,L., Sistonen,L. and Alitalo,K. (1989) *EMBO J.*, **8**, 159–166.

Mansukhani,A., Moscatelli,D., Talarico,D., Levytska,V. and Basilico,C. (1990) *Proc. Natl Acad. Sci. USA*, **87**, 4378–4382.

Marics,I., Adelaide,J., Raybaud,F., Mattei,M.-G., Coulier,F., Planche,J., de Lapeyriere,O. and Birnbaum,D. (1989) *Oncogene*, **4**, 335–340.

McClutchan,J.H. and Pagano,J.S. (1968) *J. Natl Cancer Inst.*, **41**, 351–357.

Miki,T., Fleming,T.P., Bottaro,D.P., Rubin,J.S., Ron,D. and Aaronson,S.A. (1991) *Science*, **251**, 72–75.

Moscatelli,D. (1987) *J. Cell Physiol.*, **131**, 123–130.

Partanen,J., Mäkelä,T.M., Alitalo,R., Lehväslaiho,H. and Alitalo,K. (1990) *Proc. Natl Acad. Sci. USA*, **87**, 8913–8917.

Pasquale,E.B. (1990) *Proc. Natl Acad. Sci. USA*, **87**, 5812–5816.

Pasquale,E.B. and Singer,S.J. (1989) *Proc. Natl Acad. Sci. USA*, **86**, 5449–5453.

Poncz,M., Surrey,S., LaRocco,P., Weiss,M.J., Rappaport,E.F., Conway,T.M. and Schwartz,E. (1987) *Blood*, **69**, 219–223.

Reid,H.H., Wilks,A.F. and Bernard,O. (1990) *Proc. Natl Acad. Sci. USA*, **87**, 1596–1600.

Ruta,M., Howk,R., Ricca,G., Drohan,W., Zabelshansky,M., Laureys,G., Barton,D.E., Francke,U., Schlessinger,J. and Givol,D. (1988) *Oncogene*, **3**, 9–15.

Ruta,M., Burgess,W., Givol,D., Epstein,J., Neiger,N., Kaplow,J., Crumley,G., Dionne,C., Jaye,M. and Schlessinger,J. (1989) *Proc. Natl Acad. Sci. USA*, **86**, 8722–8726.

Safran,A., Avivi,A., Orr-Urtereger,A., Neufeld,G., Lonai,P., Givol,D. and Yarden,Y. (1990) *Oncogene*, **5**, 635–643

Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sanger,F., Nicklen,S. and Coulson,A.R. (1977) *Proc. Natl Acad. Sci. USA*, **74**, 5463–5467.

Slack,J.M.W., Darlington,B.G., Heath,J.K. and Godsave,S.F. (1987) *Nature*, **326**, 197–200.

Smith,R., Peters,G. and Dickson,C. (1988) *EMBO J.*, **7**, 1013–1022.

Stacey,A. and Schnieke,A. (1990) *Nucleic Acids Res.*, **18**, 2829.

Stanley,K.K. and Luzio,J.P. (1984) *EMBO J.*, **3**, 1429–1434.

Stemple,D.J., Mahanthappa,N.K. and Anderson,D.J. (1988) *Neuron*, **1**, 517–525.

Thomas,K.A., Rios-Candelore,M., Giminez-Gallego,G., DiSalvo,J., Bennett,C., Rodkey,J. and Fitzpatrick,S. (1985) *Proc. Natl Acad. Sci. USA*, **82**, 6409–6413.

Thompson,J.A., Haudenschild,C.C., Anderson,K.D., DiPietro,J.M., Anderson,W.F. and Maciag,T. (1989) *Proc. Natl Acad. Sci. USA*, **86**, 7928–7932.

Ullrich,A. and Schlessinger,J. (1990) *Cell*, **61**, 203–212.

Wanaka,A., Johnson,E.M.,Jr. and Milbrandt,J. (1990) *Neuron*, **5**, 267–281.

Wennström,S., Sandström,C. and Claesson-Welsh,L. (1991) *Growth Factors*, in press.

Williams,A.F. and Barclay,A.N. (1988) *Annu. Rev. Immunol.*, **6**, 381–405.

Yoshida,T., Miyagawa,K., Odagiri,H., Sakamoto,H., Little,P.F.R., Terada,M. and Sugimura,T. (1987) *Proc. Natl Acad. Sci. USA*, **84**, 7305–7309.

Zhan,X., Bates,B., Hu,X. and Goldfarb,M. (1988) *Mol. Cell. Biol.*, **8**, 3487–3495.

Received on January 7, 1991; revised on March 5, 1991

Note added in proof

The size of the affinity-labelled aFGF receptor in clones of NIH 3T3 cells overexpressing the FGFR-4 cDNA from an LTR-expression vector is 140 000. Thus, the 95 000 band in Figure 4, lane SV2SE– may be generated only in the short-term COS cell expression system.