Available online at www.sciencedirect.com

SCIENCE DIRECT

Experimental Cell Research 304 (2005) 417–431

Experimental Cell Research

www.elsevier.com/locate/yexcr

Cell responses to FGFR3 signalling: growth, differentiation and apoptosis

Corine G.M. L'Hôte*, Margaret A. Knowles

Cancer Research UK Clinical Centre, St James's University Hospital, Beckett Street, Leeds LS9 7TF, UK

Received 11 June 2004, revised version received 6 November 2004
Available online 16 December 2004

Abstract

FGFR3 is a receptor tyrosine kinase (RTK) of the FGF receptor family, known to have a negative regulatory effect on long bone growth. *Fgfr3* knockout mice display longer bones and, accordingly, most germline-activating mutations in man are associated with dwarfism. Somatically, some of the same activating mutations are associated with the human cancers multiple myeloma, cervical carcinoma and carcinoma of the bladder. How signalling through FGFR3 can lead to either chondrocyte apoptosis or cancer cell proliferation is not fully understood. Although FGFR3 can be expressed as two main splice isoforms (IIIb or IIIc), there is no apparent link with specific cell responses, which may rather be associated with the cell type or its differentiation status. Depending on cell type, differential activation of STAT proteins has been observed. STAT1 phosphorylation seems to be involved in inhibition of chondrocyte proliferation while activation of the ERK pathway inhibits chondrocyte differentiation and B-cell proliferation (as in multiple myeloma). The role of FGFR3 in epithelial cancers (bladder and cervix) is not known. Some of the cell specificity may arise via modulation of signalling by crosstalk with other signalling pathways. Recently, inhibition of the ERK pathway in achondroplastic mice has provided hope for an approach to the treatment of dwarfism. Further understanding of the ability of FGFR3 to trigger different responses depending on cell type and cellular context may lead to treatments for both skeletal dysplasias and cancer.

Keywords: FGFR3; FGF; Splice; Mutation; Achondroplasia; Multiple myeloma; Bladder cancer; Signalling; STAT

Introduction

Receptor tyrosine kinases (RTKs) comprise a large family of receptors, activated by ligand binding to their extracellular domain, that commonly induces dimerisation and activation of the intracellular kinase domain. Many common features are shared by RTKs: similar motifs in the extracellular domain, homology in the kinase domain and activation of the same docking proteins and signalling pathways. However, very different cell responses can be induced by RTK activation, e.g., cell migration, proliferation, altered survival or differentiation. The specificity of the effect depends on the cell type, its state of differentiation and also on its environment.

The fibroblast growth factor receptor (FGFR) family, represented by four major transmembrane receptors, plays an important role in normal angiogenesis and embryonic development. Germline activating mutations of *FGFR*s 1–3 are associated with a range of skeletal disorders [1,2] including the common forms of dwarfism. Changes in expression of FGFRs are implicated in tumour development in many organs and recent results indicate an important role for mutant FGFR3 in certain tumour types.

Apart from FGFR5 [3,4], a new member of the FGFR family, FGF receptors have an intracellular kinase domain split in two by an inserted sequence, similar to the PDGFR and VEGFR families. These three families of receptors also share an extracellular domain composed of immunoglobulin motifs, three in the case of FGFRs. Specific features of FGFRs are the presence of an acid box in the extracellular domain and, in FGFR1, FGFR2 and FGFR4, a HAV motif, characteristic of cell adhesion molecules. The FGFR3 transcript contains 19 exons and

* Corresponding author. Fax: +44 113 24 29 886.
E-mail address: Corine.LHote@cancer.org.uk (C.G.M. L'Hôte).

0014-4827/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.yexcr.2004.11.012

the protein product is encoded by exons 2 to 18 [5]. Several splice isoforms are known for each member of the FGFR family. In FGFRs 1–3, alternative splicing of the second half of the third Ig domain results in two isoforms, named IIIb and IIIc, which differ in their ligand-binding specificity (IIIa referring to the first half of the third Ig domain). FGFR3 IIIc is the isoform expressed in chondrocytes, the main cells affected in dwarfism, while IIIb is expressed by epithelial cells. However, many other splice forms have been also described, commonly missing some of the domains found in the full-length receptor. Their expression levels vary depending on the tissue and also during development.

At least 23 fibroblast growth factors (FGFs) are known so far. However, activation of FGFRs is not mediated by FGFs alone, but by a complex of FGFs with sulphated glycosaminoglycans (GAGs) such as heparin or heparan sulfate, sometimes referred to as low-affinity FGF receptors. Crystallisation of ternary complexes of extracellular ligand-binding domains of FGFR1 with FGF2 and heparin [6] or of FGFR2 with FGF1 and heparin [7] has confirmed the essential role of heparin, which interacts with both FGF and its receptor. Different heparan sulfate glycosaminoglycans (HSGAGs) can be required depending on the FGF–FGFR complex [8]. This constitutes another level of control of FGFR activation, which can therefore be influenced by the extracellular matrix and cell surface composition [9]. HSGAG can be carried by a peptidic chain to form heparan sulfate proteoglycans (HSPGs), including syndecans, which probably represent the major subfamily. Syndecans are able both to interact with extracellular matrix proteins and to signal. It has been shown that the cytosolic part of syndecan-4 is important in FGF2 signalling [10]. In addition, an effect of FGFR signalling on the extracellular matrix has been reported in stem cells, in which FGFR2 is involved in synthesis of the extracellular matrix proteins collagen IV and laminin-1 that are needed for epithelial cell differentiation [11].

FGFRs also associate with proteins involved in cell–cell interaction and through this mechanism may contribute to cancer metastasis [12]. FGFR1 and FGFR4 have been shown to interact with N-CAM and N-cadherin [13–15]. In breast cancer cell lines that express N-cadherins and FGFR1, FGF2 has been shown to induce matrix metalloproteinase-9 expression and cell invasion through matrigel-coated filters [15]. More indirectly, Notch, which is also involved in cell–cell interactions, has been shown to interact with FGF signalling pathways, its repression being associated with FGF-dependent transformation of NIH3T3 cells [16,17]. Taken together, it appears that cell–cell interaction can either attenuate FGFR signalling, thus repressing cell transformation [17], or maintain or increase it, thus contributing to transformation, e.g., by stimulating invasion [15]. It is possible that these contradictory effects depend on the signalling pathway(s) that FGFRs crosstalk with, underlining the importance of the cell type and cellular context when considering the cellular response to FGFR activation.

The number of possible ligands, splice isoforms and the modulation of signalling by extracellular matrix and/or other cell surface receptors, generate a high level of diversity and multiple possible ways to control FGFR activation that are likely to increase diversity in cellular effects.

FGFR3 and its ligands

FGFR3 IIIb [18–20] and IIIc isoforms are both activated by FGF1 (a known ligand for FGFRs 1–4) and by FGF9. However, this latter FGF is a much weaker ligand for IIIb than for IIIc and remains weaker than FGF1 [21,22]. In addition, FGFs 2, 4, 6, [23] and FGF8 [22] also bind to FGFR3 IIIc. More recently, FGF18 has been described as a potential additional ligand [24,25]. Indeed, FGF18−/− mice show a phenotype similar to *FGFR3*−/− mice, though with aspects that are more severe, which may reflect the added effects of reduced signalling by FGFRs 1 and 2 [24]. The number of known FGFs is still growing. FGF1 and FGF2 were the first to be isolated in 1973 and 1974, 10 years later, seven FGFs were known and in 2003 the family had gathered 23 members. It is possible therefore that a more specific ligand for FGFR3 IIIb remains to be discovered. With the large number of possible ligands binding with various affinities to the different FGFR3 splice isoforms, there is in theory a great number of combinations and possible effects. An interesting autoinhibitory mechanism regulating ligand binding that involves the first Ig domain and the following linker region has been reported for FGFR3 IIIc as well as for FGFR1 and FGFR2 [26]. Crystal structure analysis and real-time binding experiments, presented in this latter article, have shown that flexibility of the linker region allows interaction of the IgI domain with the second and third domain that are required for ligand binding.

Expression of FGFR3 in tissues and its biological roles

Although there is overlapping expression of the FGFRs in different tissues, there is also cell specificity and temporal variation of their expression during embryonic development [27,28]. FGFR3 is expressed in kidney, lung and brain [19], where in the latter it is present in the glial cells and astrocytes [29]. Expression has also been detected in cartilage (proliferating and hypertrophic chondrocytes), in the intestine [27,28], pancreas, and testis (see gene expression atlas: http://symatlas.gnf.org/SymAtlas/). Additionally, in mouse embryos, FGFR3 has been shown to be expressed in the cochlear duct and in the lens [30], where it is involved in differentiation [31]. Tissue-specific expression of the IIIb and IIIc isoforms has been described. Expression of FGFR3 IIIb appears to be associated with epithelial cells [20,32]. A switch from IIIb to IIIc can be induced by FGF1, possibly correlating with a loss of

epithelial phenotype [33]. In contrast, cytokines or growth factors such EGF, TGFβ and IL2, have been shown to up-regulate FGFR3 IIIb expression in the intestinal epithelial cell line Caco2 [34]. In mice, in vivo, FGFR3 is expressed in undifferentiated crypt epithelial cells, which could suggest a role of FGFR3 in intestinal cell proliferation [28].

Knowing where and when FGFR3 is expressed can give some clues to function, but more enlightening information has come from the generation of *Fgfr3* null mice (*Fgfr3*−/−) [35]. These mice show bone dysplasia with longer vertebral bodies and overgrowth of the long bones, associated with an increased number of proliferating chondrocytes during embryogenesis. This indicates that FGFR3 is a negative regulator of bone growth. It follows that the FGFR3 mutations associated with dwarfism must be activating mutations. Interestingly, such activating mutations, which clearly provide negative regulatory signals in the chondrocyte, are found not only in skeletal disorders but also in cancer.

FGFR3 mutations associated with skeletal disorders

Germline mutations in *FGFR3* are associated with skeletal disorders including several forms of dwarfism (Table 1; Fig. 1). The severity of the syndrome varies according to the mutation and probably its specific effect on the protein [36]. The most severe forms of chondrodysplasia are SADDAN (severe achondroplasia with developmental delay and acanthosis nigricans) and thanatophoric dysplasias (TDI and TDII), while hypochondroplasia is a milder form [37]. Mutations affecting codon 650 cause a range of conditions. K650M has been reported in TDI [38,39] and SADDAN [40,41] whereas K650E is reported only in TDII. Both of these mutations induce constitutive activation of the FGFR3 kinase but the effects on the patient differ and SADDAN syndrome individuals, though severely affected, are viable, while the TDII mutation is lethal at birth [40,42]. Other mutations of Lysine 650 to Asparagine or Glutamine that are associated with the milder condition, hypochondroplasia, show weaker autophosphorylation than K650M or K650E mutants [43]. FGFR3 mutations have also been located in the extracellular domain, transmembrane domain or at the stop codon (Table 1; Fig. 1). For example, the TDI phenotype is associated with mutations of the stop codon or mutations creating cysteines in the extracellular domain, either between IgII and IgIII (R248C, S249C) or between IgIII and the transmembrane domain (G370C, S371C, Y373C, G375C) [44]. These mutations, in the extracellular juxtamembrane region, introduce an unpaired cysteine, and have been shown to result in ligand-independent dimerisation of FGFR3 [36] as was suggested earlier [1] and demonstrated for FGFR2 [45]. In that case, loss of a cysteine, as well as other mutations, results in dimerisation and receptor activation. The situation may not be so simple for mutations of the stop codon [46] or the transmembrane domain. Indeed, it has been shown recently that FGFR3

Table 1
Fgfr3 mutations associated with skeletal disorders

Mutated codon	Amino acid change	Phenotype	Location
248	Arg → Cys	Thanatophoric dysplasia I	Linker between IgII and IgIII
249	Ser → Cys	Thanatophoric dysplasia I	
250	Pro → Arg	Craniosynostosis	
328	Asn → Ile	Hypochondroplasia	IgIII
346	Gly → Glu	Achondroplasia	Linker between
370	Gly → Cys	Thanatophoric dysplasia I	IgIII and TM
371	Ser → Cys	Thanatophoric dysplasia I	
373	Tyr → Cys	Thanatophoric dysplasia I	
375	Gly → Cys	Achondroplasia	
380	Gly → Arg	Achondroplasia	Transmembrane
391	Ala → Glu	Crouzon syndrome	domain
538	Ile → Val	Hypochondroplasia	Tyrosine kinase
540	Asn → Lys	Hypochondroplasia	Domain I
	Asn → Ser		(TK-1)
	Asn → Thr		
650	Lys → Asn	Hypochondroplasia	Tyrosine kinase
	Lys → Gln	Hypochondroplasia	Domain II
	Lys → Glu	Thanatophoric dysplasia II	(TK-2)
	Lys → Met	SADDAN, TDI	
807	Stop → Arg	Thanatophoric	Stop codon
	Stop → Cys	dysplasia I	
	Stop → Gly		
	Stop → Leu		
	Stop → Trp		

Table modified from the human gene mutation database, see http://archive.uwcm.ac.uk/uwcm/mg/ns/1/127526.html for references.
Abbreviations: Ig, immunoglobulin-like domain; TM, transmembrane domain; SADDAN, severe achondroplasia with developmental delay and acanthosis nigricans.

with the G380R mutation in the transmembrane domain, which is associated with over 97% of achondroplasia cases, remains ligand-dependent for its dimerisation and activation. However, it differs from the wild-type (wt) protein in showing a delay in degradation following ligand binding [47]. It has been reported that ubiquitination of both G380R and K650E mutant receptors is not defective but that the degree of ubiquitination is related to the kinase activity of the receptor [48]. A recent study, confirming the prolonged half-life of the TDII mutant [49], has shown that in cells stably overexpressing mutant or wild-type FGFR3, the wild-type receptor is more abundant in lysosomes while FGFR3 G380R (ACH) or K650E (TDII) mutants are more abundant in the recycling endosomes. Such mis-sorting of mutant receptor could result in an amplification of signalling ability. It should be noted that the TDII form is not fully glycosylated [50], which could theoretically affect its localisation. Also, one mutation disrupting a glycosylation site (N328I) is associated with hypochondroplasia [51]. It affects the FGFR3 IIIc isoform but does not modify its three-dimensional structure. Mutations can also modify

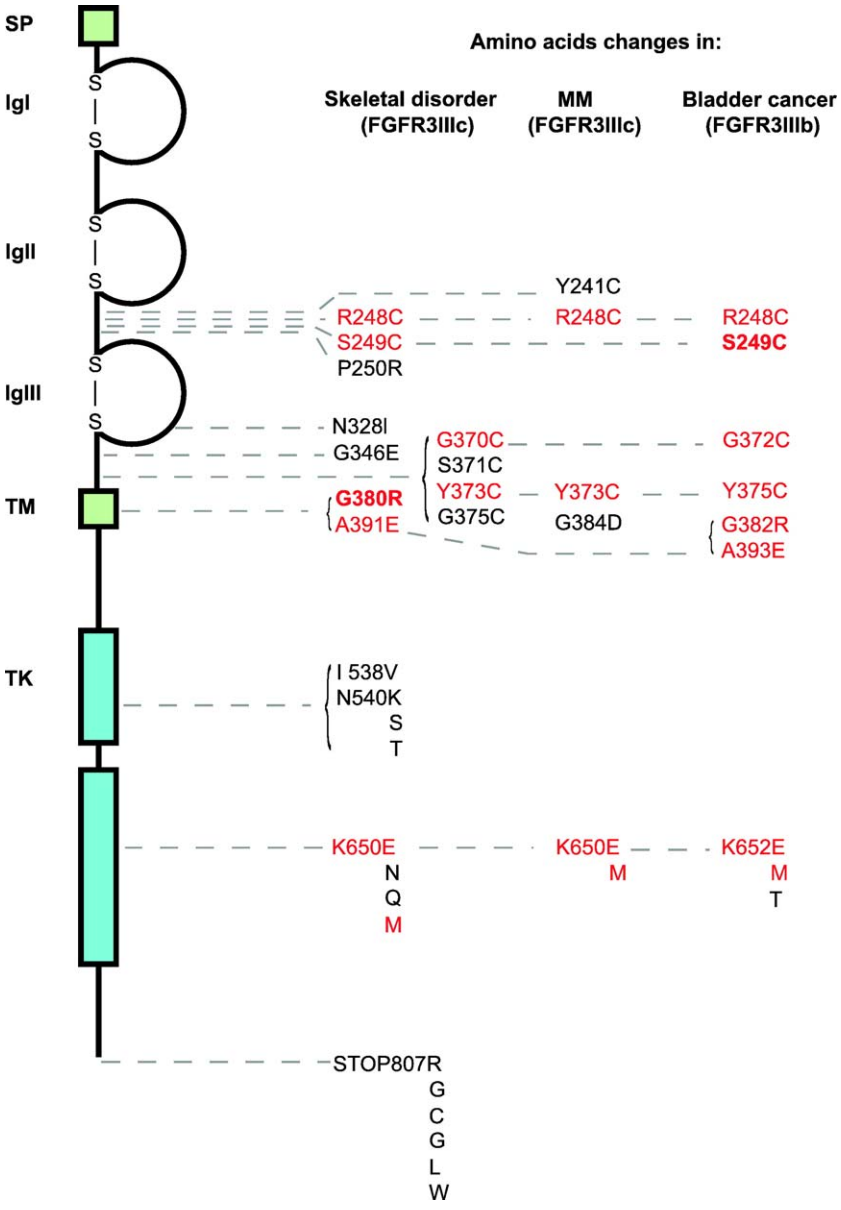

Fig. 1. Amino acid changes induced by mutations in *FGFR3*. Amino acid numbers are based on FGFR3 IIIc translation in skeletal disorders or MM, and on FGFR3 IIIb translation in bladder epithelial cells. Mutations shared between the three diseases are in red. In bold are the main *FGFR3* mutations reported in bladder tumours (S249C) and in achondroplasia (G380R), the most common form of chondrodysplasia. SP: signal peptide, Ig: immunoglobulin-like domain, TM: transmembrane domain, TK: split tyrosine kinase domain.

receptor specificity or affinity for ligand, as has been shown for the extracellular domain P250R mutation. This mutation is observed in both FGFR1 and FGFR2 at the analogous position, where it increases the binding to ligand, in particular FGF9 binding to FGFR1 and FGFR3 [52].

Effects of FGFR3 mutations on chondrocyte proliferation and differentiation

In the growth plate of developing long bones, cells progress through stages of proliferation and then hypertrophy, the terminal stage of differentiation that is normally associated with collagen type X synthesis, apoptosis and matrix calcification [53]. FGFR3 signalling plays a pivotal role in this process.

Much information has come from mouse models. *Fgfr3* knockout mice show increased length of the long bones and mice expressing activating mutations of FGFR3 show phenotypes reminiscent of human chondrodysplasia. Mice expressing the equivalent mutations to human G380R [54,55], G375C [56], K650E [57,58], K650M [59] and S371C [60] have been described. These show decreased chondrocyte proliferation and a reduced hypertrophic zone in the growth plates of postnatal mice. Disorganisation of the growth plate is severe in TDII mice [57,58], and can still be observed in ACH mice that present a more marked

phenotype than in humans [55]. Unexpectedly, in contrast to the inhibitory effect on the long bones, overgrowths of some cartilaginous tissues (rib, trachea and nasal septum) have been observed in mice with the corresponding human SADDAN (K644M) FGFR3 mutation [59]. This may indicate a difference in the effect of FGFR3 signalling in hyaline cartilage, which does not necessarily become ossified.

The effects of modulating FGFR3 signalling on chondrocyte proliferation appear to be different during the prenatal and the postnatal period. G380R mouse embryos show no difference in chondrocyte proliferation, while K644M and K644E embryos show increased proliferation during embryogenesis (peak proliferation at E18 and E15, respectively) [58,59]. Thus, it is proposed that FGFR3 can promote or inhibit proliferation depending on the embryonic developmental stage. However, decreased differentiation has been reported both prenatally and postnatally, indicating that this is the most likely primary cause of long bone retardation in all mouse models. Similarly, human foetuses with G380R, K650E, R248C, S249C or Y373C, show a reduced hypertrophic zone in vivo [61].

Findings from in vitro experiments on cultured chondrocytes indicate that cells from mutant mouse and human embryos can be stimulated to proliferate by FGFs. Unexpectedly, in culture, FGF2 and FGF9 induced mitogenic responses in human TDI foetal chondrocytes, similar to those of controls [62]. In contrast, rat chondrosarcoma cells (RCS) show reduced proliferation when exposed to FGF ligands for long periods, as might be expected of mature chondrocytes [63]. However, in these cells, expression of collagen X was not induced [64], possibly reflecting the absence of normal stromal or other environmental stimuli in vitro or that these tumour-derived cells are unable to recapitulate all features of the normal chondrocyte. Differentiation of RCS cells was accompanied by cytoskeletal alterations and rounding of the cells [63]. Shortly after FGF binding, tyrosine kinase activity was induced in focal adhesions and this co-localised with vinculin. Simultaneously, FGFR3 was removed from the focal adhesions that subsequently disassembled. These results suggest that modification of cell adhesion may be another effect of FGFR3 signalling. In accord with this, it has been reported that when the FGFR3 G380R mutant is expressed in the chondrocytic cell line CFK2, there is an alteration in the expression of integrins [65]. Could this effect on adhesion indirectly be responsible for the role of FGFR3 in induction of apoptosis in chondrocytes? This is not yet clear. However, the apoptotic effect of FGFR3 on chondrocytes is a chondrocyte-specific effect rather than an FGFR3-specific effect [66]. This latter study found that expression in transgenic mice of either the mutated FGFR3 G380R or of a chimeric receptor comprising the extracellular domain of FGFR3 G380R fused to the intracellular domain of FGFR1 led to the development of similar, dwarf mice. The similarity of signalling via both FGFR1 and FGFR3 intracellular domains in the bone suggests that chondrocytes are unique in their response and underlines the importance of cell type in response to a particular signal.

In vivo, FGFR3 plays a role not only in the growth plates during endochondral ossification but also in bone mineralisation by osteoblasts in the postnatal period. *Fgfr3*−/− mice have been reported to show osteopenia. These mice had decreased thickness of cortical bone and increased numbers of osteoblasts that failed to mineralise properly despite the presence of some markers of osteoblast differentiation in the cortical bone [67]. Stem cells, which will differentiate into osteoblasts, are brought by blood vessels invading the cartilage after apoptosis of hypertrophic chondrocytes. Interestingly, the growth plates of ACH transgenic mice and human TD foetuses were shown to have abnormal vascularisation and this seems to correlate with the severity of the disease [54,68]. Moreover, it has been reported recently [69] that *Fgfr3*−/− mice show a down-regulation of vascular endothelial growth factor (VEGF), an angiogenic factor produced by hypertrophic chondrocytes that is required for their apoptosis [70]. If activation of FGFR3 results in an angiogenic effect, this could also be an important factor in the development of cancer associated with *FGFR3* mutations.

FGFR3 mutations associated with cancer

Some of the missense mutations observed in achondroplasia are also associated with human multiple myeloma (MM) [71,72] and carcinomas of bladder and cervix [73]. Additionally, in colon carcinoma, two novel mutations have been identified: E322K and a nonsense mutation at nucleotide 849 [74]. No mutations in *FGFR3* have yet been found in prostate [75], skin, lung, stomach, brain or renal tumours [76,77], indicating that *FGFR3* mutations seem to be specific to a few cancers.

MM, a B-cell neoplasm, provided the first indication that FGFR3 could act as an oncogene. Here, the chromosomal translocation t(4;14)(p16.3;q32), which results in ectopic expression of FGFR3 (from der 14) and immunoglobulin heavy chain-MMSET transcripts from the der 4 [71,72], has been described in 10–25% of patients. This translocation is found not only in MM but also in monoclonal gammopathy of undetermined significance (MGUS), which is believed to be a precursor for MM [78]. Thus, the translocation is considered to be an early genetic alteration in MM development. However, the role of FGFR3 expression in these conditions is not entirely clear. A study of MM and MGUS patient samples has shown that although all 67 MM cases with t(4;14) expressed FGFR3, none of 13 MGUS cases did, suggesting that although translocation may be the primary event, increased FGFR3 expression may not occur until later [79]. In fact, increased expression of FGFR3 is not universal in MM [80–82]. MMSET (from der4) is universally expressed in tumours with the translocation but FGFR3 is not expressed in approximately 30% of cases with

t(4;14) and in some cases the der14 is lost during tumour progression [81,82]. Clearly, this does not indicate that FGFR3 is irrelevant but it does raise the intriguing possibility that the role of FGFR3 may change during MM progression. Where FGFR3 is expressed in MM, FGFR3 IIIc appears to be the major isoform, but some tumours express FGFR3 IIIb [83] or both isoforms [79]. So far, neither isoform has been associated with specific characteristics of MM tumours or cell lines.

In some cases of MM, the translocated FGFR3 is mutated, mostly in late stage disease. However, it should be noted that *FGFR3* has also been found mutated in a MM tumour not showing the t(4;14) translocation [84]. In that case, it is possible that constitutive activation of FGFR3 would compensate for the absence of overexpression. A recent study of 150 newly diagnosed cases reported that FGFR3 overexpression was present in 16% but mutated in only two cases (1.3%) [83]. Taken together, this indicates that ~15% of patients have a t(4;14) and 6% of these have a potential activating mutation of the receptor. Interestingly, Sibley et al. [79] analysed samples taken 13 months apart from the same patient, and identified a K650E mutation only in the late sample, reinforcing the concept that *Fgfr3* mutation contributes late in the multistage disease process. *FGFR3* mutations found in MM tumours (Fig. 1) include R248C [84], K650E, K650M [71] and Y241C [83], which has not been seen in chondrodysplasia. In MM cell lines, mutations include K650E in OPM2, Y373C in KMS-11 [71,72,85] and G384D in KMS-18. The presence of a mutation appears to be an adverse prognostic factor [81]. It seems that overexpression of wild-type FGFR3 could be sufficient to lead to MM but its constitutive activation could be giving a stronger proliferative signal. This is in agreement with experimental transformation systems, in which wild-type FGFR3 is less potent than mutant receptor. For example, overexpression of wild-type FGFR3 in NIH3T3 cells does not induce focus formation in vitro or tumorigenicity in nude mice in contrast to the constitutive active kinase K650E mutant [85]. However, in vitro transduction of mouse bone marrow cells with a retrovirus encoding the wild-type receptor followed by transplantation into mice does lead to delayed lymphoma/leukaemias after approximately 1 year. Again, mutant receptor is more potent in this assay [86]. It is possible that in MM, overexpression of cyclin D1 [87] and/or mutations in other oncogenes could compensate for the weak proliferative or transforming signal from the wild-type receptor. Indeed, oncogenic mutations of Ras genes (*KRAS* and *NRAS*) have been observed in 40% of MM. These and *FGFR3* mutations appear to be mutually exclusive, suggesting that there may be an overlapping function [85].

The results described above have led to the investigation of FGFR3 as a valid target for therapy. It has been shown that inhibition of FGFRs using tyrosine kinase inhibitors (PD173074 and SU5402) induces cell death of the FGFR3 expressing cell lines KMS-11 and OPM-2, confirming the importance of FGFR3 in MM [88].

Among the different types of cancer screened, bladder cancer appears to have the highest frequency of *FGFR3* mutations. Screening for mutations has been carried out by several laboratories. The percentage detected varies from 31% to 60% of bladder tumour samples (Table 2). The most frequent mutation in bladder tumours and the only mutation found so far in cervical carcinoma, is S249C, a TDI mutation. The most frequent mutation in achondroplasia, G380R, has only been detected in a few bladder tumours [89]. The mechanisms by which mutations occur in the germline or somatically in the bladder, may be different, possibly reflecting a different balance between replicative errors and induced DNA damage. Bladder cancer is well known to be associated with exposure to environmental carcinogens, tobacco smoking being estimated to be responsible for almost half of bladder cancer cases [90]. However, the major influence on the frequency of certain mutations may be the lethal effect of the most highly activating *FGFR3* mutations in the germline, an effect that may well be the converse in cancer where high activity of FGFR3 is likely to give increased selective advantage. Interestingly, mutation localisation has been shown to have different effects on the level of FGFR3 constitutive phosphorylation and also on ligand dependence [36]. Such differences may confer an advantage in particular contexts and may explain the differences in mutation frequencies observed between diseases.

Most bladder cancers (90%) are transitional cell carcinomas (TCC). These tumours can be superficial or invasive with different degrees of invasion (tumour stage). They are also defined by their histologic grade or degree of anaplasia [91,92]. Analysis of the prognostic value of *FGFR3*

Table 2
FGFR3 mutations found associated with bladder carcinoma (based on a translation of IIIb isoform)

Number of tumour samples	76	63	286	81
Mutation %	35	41	60	31
Bibliographic reference	[73]	[139]	[103][a]	[93]
Mutations found in tumours	R248C S249C G372C	 S249C G372C Y375C	R248C S249C G372C Y375C A393E	R248C S249C G372C Y375C
	K652E	K652E	K652E K652M K652T	K652E K652M
Mutations in cell lines		S249C K652E		
Most common mutation in tumours (% of mutations)	S249C (56%)	S249C (81%)	S249C (69%)	S249C (44%)

[a] In an earlier publication, Van Rhijn et al. [89] have also reported another mutation, G382R.

mutations has shown an association with superficial bladder tumours of lower grade [93–95], rather than with high-grade, invasive tumours [93,96]. An apparent association with a lower recurrence rate has also been reported [96]. The *FGFR3* mutation-associated effects underlying these observations are not yet clear, but one possibility is that there is a major effect on urothelial differentiation, perhaps in combination with a survival signal. It is interesting to note that cyclin D1 up-regulation, reported in RCS cells treated with FGF, has also been found in low-stage, low-grade bladder tumours [97–100]. It is associated with a papillary pattern of differentiation [101] and possibly with better survival [97,102]. Could a link exist between FGFR3, cyclin D1 and cell differentiation in bladder? Since both *FGFR3* mutation and cyclin D1 expression are linked to superficial papillary tumours, it is possible that FGFR3 signalling maintains a differentiated phenotype that is incompatible with the development of more aggressive tumours. This idea was also proposed following the recent finding that *TP53* and *FGFR3* mutations are almost mutually exclusive in bladder tumours [95,103]. It seems possible that *FGFR3* mutations are an early event in the development of superficial papillary bladder cancer that in most cases may protect from progression to muscle invasion. Currently, the signalling pathway(s) downstream of FGFR3 in normal urothelial cells are not known, nor those stimulated in TCC cells with mutation. Similarly, nothing is yet known about the major effects of *FGFR3* mutation on urothelial cell phenotype. This information will be pivotal for a clear understanding of the consequences of mutation of the receptor during tumour development.

Association of *FGFR3* mutations with an early stage of tumour development or a less aggressive form of disease in bladder cancer contrasts with the situation in MM, where *FGFR3* mutation appears to be associated with tumour progression [85]. The mechanism of activation is also different in these two types of cancers where MM commonly shows overexpression of wild-type FGFR3 as the result of a chromosomal translocation whereas only point mutation has been found in bladder cancer. This is likely to reflect the propensity of haematopoietic cells to generate translocations, which are rarely found in solid tumours. The low frequency of mutations in MM has not allowed a clear assessment of mutation spectrum, but S249C, the most frequent mutation in the bladder, has not yet been reported in MM. However, S248C has been found, and this could have a similar effect. A major difference between these two types of cancer and chondrodysplasia, in which FGFR3 activation leads to apoptosis, is that in MM, it is the inhibition of FGFR3 kinase activity that results in growth arrest, cell differentiation and apoptosis [104], pointing again to cell-type-specific responses to FGFR3 signalling. It should also be noted that bladder epithelial cells express FGFR3 IIIb (our unpublished results) in contrast to chondrocytes, which express the IIIc isoform and thus these cell types could respond differently to FGFs. However, this splicing event is thought unlikely to affect cell-type-specific responses in the context of mutant receptors with constitutive FGF-independent activation. Nevertheless, splicing of the wild-type receptor can be associated with cell differentiation and also with tumour development.

Alternative FGFR3 splice isoforms and their relationship to differentiation and malignancy

In chondrocytes, differential *FGFR3* splicing may be a marker of differentiation state. An alternative splice form, lacking the acid box, is expressed in the undifferentiated chondrocyte cell line ATDC5, while full-length *FGFR3* is not. Moreover, the mouse pro-B cell line BaF3 that expresses this splice variant has a higher mitogenic response to FGF2, FGF4 and FGF8 than cells expressing full-length FGFR3 [105]. Further studies show that stimulation of full-length FGFR3-transfected ATDC5 cells with FGF1 induces cell rounding, while in cells expressing FGFR3 with the acid box deletion, a change in morphology, but no rounding up, is observed [106], indicating that the full-length and acid box-spliced FGFR3 perform different functions.

Differential splicing is also observed during malignant transformation (Fig. 2). In breast epithelial cells, FGFR3 has been shown to localise in the nucleus, likely due to the expression of a soluble isoform, missing the second half of the third immunoglobulin loop encoded by exon 8 or 9 and the transmembrane domain (exon 10) [107]. Later work comparing normal and malignant breast tissues has shown a preferential nuclear localisation of FGFR3 in tumour epithelium [108]. The same spliced isoform is expressed by the human squamous cell carcinoma cell line DJM-1 and a human osteoblast-like cell line (SaOS-2), and here it has been found to be secreted as a dimer in the medium [109,110]. No function has yet been defined for this soluble isoform. In colorectal cancer, transcripts splicing out exons 8 and 9 or including both of them have been described [111]. Additionally, the use of a cryptic site inside exon 7, resulting in aberrant splicing from within exon 7 up to exon 10, has been observed in primary tumours and cancer cell lines. This latter splicing event creates a frameshift that causes premature termination of the reading frame. It is proposed that the mechanism by which these alternative transcripts are generated may compete with the generation of wild-type transcripts, possibly reducing a negative effect of FGFR3 on proliferation [111]. A possible negative effect on FGFR3 IIIc wt, has been described at the protein level, in tumour cell lines expressing a splice isoform with a deletion in exon 7, also lacking the second half of the third Ig domain and the transmembrane domain. This isoform (FGFR3IIIS) appeared to have a positive effect on cell proliferation in a Ewing's sarcoma cell line [112]. Similarly, aberrant splicing of the third Ig domain within exon 7 has been described in a multiple myeloma patient without the t(4;14) translocation [84]. Is it possible that these deletions

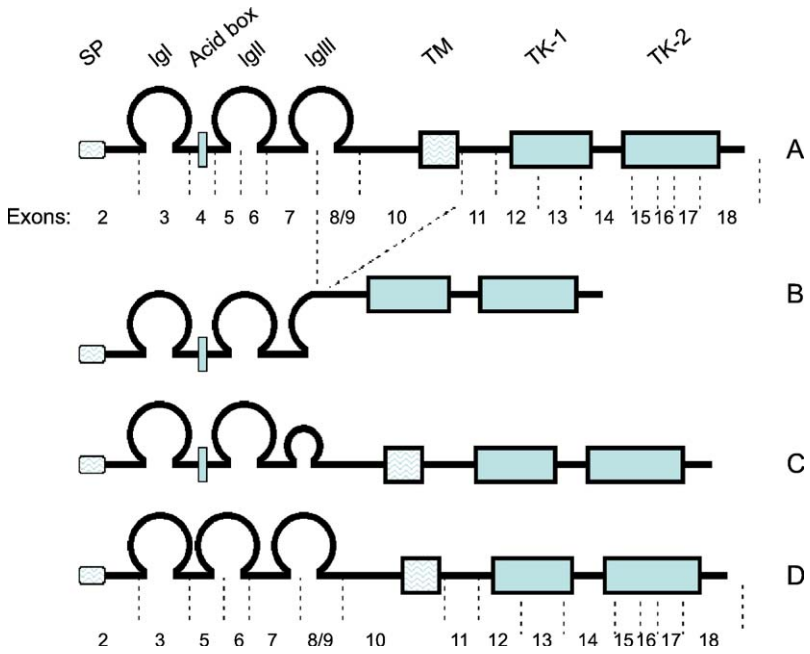

Fig. 2. Putative *FGFR3* isoforms described in cancer that result from an alternative splicing and remain in frame [5]. SP: signal peptide, Ig: immunoglobulin-like domain, TM: transmembrane domain, TK: tyrosine kinase domain. (A) Full-length wt FGFR3 *IIIb* (exon 8) or *IIIc* (exon9). (B) Isoform splicing out exons 8, 9 and 10, resulting in a soluble form of FGFR3 [107–110]. (C) IIIc isoforms with partial deletions at the end of exon 7 [84]. (D) Isoform lacking the acid box encoded by exon 4 [105].

have the same effect as a constitutively active mutation or could contribute to tumorigenesis by altering ligand-binding specificity? The current high level of interest in the role of FGFR3 in cancer will undoubtedly lead to answers to these questions in the near future.

FGFR3 signalling and transcriptional consequences

In the FGFR family, the archetype is FGFR1 and its signalling has been studied in great detail, particularly signalling via the Ras-MAPK pathway. The close homology of FGFRs 1 and 3 indicates possible similarities and this has provided clues to mechanisms of FGFR3 signalling. Receptor dimerisation leads to autophosphorylation of the receptors on tyrosine residues that are highly conserved between FGFRs. There are seven autophosphorylation sites in FGFR1, five of which are conserved in FGFR3 [113,114]. These are potential binding sites for Src homology 2 (SH2) or phosphotyrosine binding (PTB) domains of signalling proteins.

An essential protein in FGFR signalling is FRS2 [115], a lipid-anchored multidocking protein that is phosphorylated following binding to FGFR, and then transduces signals via GRB2/Sos to the PI 3-kinase (PI3K) and Ras/MAPK pathways. Phospholipase Cγ (PLCγ) can bind directly to FGFR1, on a phosphotyrosine (pY766) [116] that is conserved in FGFR3 (Y760) and once activated, hydrolyses PtdIns(4,5)P_2 to generate the second messengers Ins(1,4,5)P_3 and diacylglycerol. Some of what is known about FGFR1 and FRS2 signalling can be applied to FGFR3, as illustrated by a comparative study of signalling induced by myristylated and activated cytoplasmic regions of FGFR1, R3 and R4 carrying a TDII-like mutation [117]. This showed that they all caused NIH3T3 cell transformation, phosphorylation of Shp2, PLCγ, ERK1/2 and Stat3 and PI-3 kinase activation. However, specific responses to FGFR3 have also been described. For example, it has been reported that FGFR3 is a much poorer inducer of neurite outgrowth in PC12 cells than FGFR1 and in this context, primarily signals independently of the Ras pathway. Although PLCγ was stimulated to the same extent by FGFR1 and FGFR3, neurite differentiation was not induced in the absence of sustained Ras-MAPK signalling by FGFR3 [118]. The mitogenic responses of the cytoplasmic domains of FGFR1 and FGFR3 differ. When transfected into BaF3 cells, FGFR3 is weakly mitogenic compared to FGFR1, but a chimeric receptor with the extracellular domain of FGFR3 and intracellular domain of FGFR1 is as mitogenic as FGFR1, even though the extracellular domain of FGFR3 has a lower binding affinity for FGF1 [119]. This is in agreement with the finding that the activated cytosolic part of FGFR1 has a higher transforming activity than FGFR3 in NIH3T3 cells [117,120]. Differences in downstream signalling may explain the specific responses of the two receptors.

FGFR3 signalling has been extensively studied in chondrocytes. In chondrodysplasia, the level of up-regulation of certain signalling molecules correlates with the severity of the syndrome and therefore with the mutation in *FGFR3* that causes it [61]. Knock-in mice with TDII *FGFR3* mutation show up-regulation of STAT1, STAT5a and STAT5b as well as the cell cycle inhibitors p16, p18 and p19 [57]. Interestingly, STAT5a is not expressed by the

hypertrophic chondrocytes, [57] and it is STAT1, which is phosphorylated, that is required for inhibition of chondrocyte proliferation [121]. Indeed, it has been shown that FGF1 does not inhibit chondrocyte proliferation in *Stat1*−/− mice (Fig. 3). Moreover, TDII FGFR3 induces a constitutive STAT1 phosphorylation in transfected HEK293 cells [50]. In vitro, RCS cells have been used to examine the chondrocyte response to FGFs. Here, FGFR3 phosphorylation and inhibition of proliferation corresponding to a G1 arrest [63,122] are associated with up-regulation of STAT1, STAT3, p21, cyclin D1, cyclin D2 [63,121,122], Rb dephosphorylation and inactivation of cyclin E-cdk2 [122]. Down-regulation of a transcription factor, known to be an inhibitor of differentiation (Id1), is also described [63]. More recently, expression microarrays were exploited to examine the transcriptional effects of FGF1 in more detail [64]. This study confirmed that the up-regulation of p21 is associated with inhibition of cyclin E-cdk2, which corresponds with Rb and p130 dephosphorylation that are more likely to be involved in maintenance rather than initiation of growth arrest. Moreover, no increase in p21 expression was observed in *FGFR3* TDII knock-in mice and no rescue of the TD phenotype was reported when TDII FGFR3 was expressed in p21 null mice [57], confirming that if p21 is needed to maintain the growth arrest [64] it is probably not the major player. The microarray study of RCS cells also showed repression of E2F target genes and a down-regulation of the Id1-3 transcription factors. These events were followed by alterations in expression of genes associated with hypertrophic differentiation similar to those seen in mice expressing mutant FGFR3, confirming that the

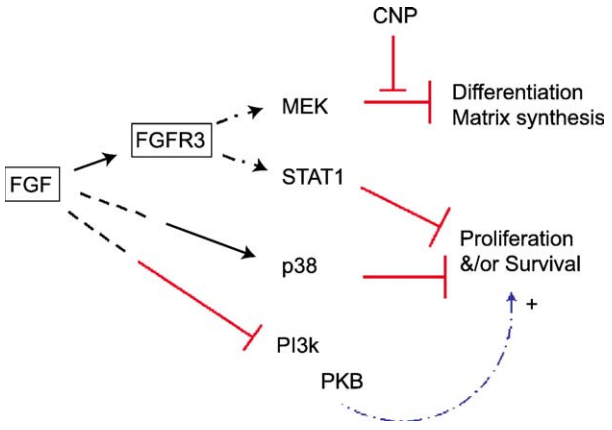

Fig. 3. Specific FGF and FGFR3 signalling in chondrocytes. The figure is derived mainly from Refs. [129,131,132]. TDII FGFR3 leads to up-regulation of STAT1 that is involved in apoptosis of chondrocytes, as no inhibition of proliferation is observed in *Stat1*−/− mice. PI-3 kinase (PI3K) and PKB (Akt) have been shown to induce a survival or proliferative signal, which is inhibited by, FGF. p38, but not PKB, has been shown to be involved in growth arrest. However, a direct link with FGFR3 activation has not been demonstrated. It has also been reported that ACH FGFR3 inhibits matrix synthesis in chondrocytes. This inhibitory effect can be rescued in cells treated by or overexpressing the C-type natriuretic peptide, probably via activation of its guanylyl cyclase receptor.

growth arrest is concomitant with chondrocyte differentiation [64], leading finally to apoptosis. Among the different events cited above, up-regulation of cyclin D1 is surprising as it is usually not expected to be associated with G1 arrest and indeed it has been reported previously that cyclin D1, which is activated by the transcription factors ATF-2 and CREB following TGFβ and PTHrP stimulation [123], is required for RCS proliferation [124]. However, it was also reported that *cyclin D1*−/− mice present a reduction in the size of the proliferative zone of the growth plate [124]. To add to this confusion, FGF-induced up-regulation of cyclin D1 has also been reported in MCF7 cells, where proliferation is inhibited by FGF2 [125] and during NGF-induced differentiation of the phaeochromocytoma cell line PC12 [126]. In several other cases, cyclin D1 expression has also been reported to be associated with growth arrest and differentiation [127,128]. Different states of cell differentiation could be important to define the role of cyclin D1 and that may apply particularly to proliferative or hypertrophic chondrocytes in vivo. Moreover, FGF induces a decrease in PKB (Akt) phosphorylation in chondrocytes [129] and specific inhibitors of PI3K or MAPK block the anti-apoptotic effect of IGF-1 [130], indicating that these pathways are important in cell proliferation and/or survival of chondrocytes (Fig. 3). It is likely, therefore, that the STAT pathway is the main pathway towards apoptosis in chondrocytes. However, the achondroplastic phenotype is not only the result of Stat1 activation but also of ERK activation [131]. Indeed, it has been reported that the achondroplasia phenotype can be rescued in mice overexpressing the C type natriuretic peptide (CNP), probably via activation of its guanylyl cyclase receptor, resulting in a decrease of ERK activation in $fgfr3^{Ach}$ mice. Interestingly, CNP does not increase proliferation but rescues the inhibition of extracellular matrix synthesis and therefore differentiation [131] (Fig. 3). An achondroplasia-like dwarfism can also result from constitutive activation of MEK1, which inhibits hypertrophy in chondrocytes [132]. Additionally, activation of p38, another MAP kinase that is required for hypertrophic chondrocyte differentiation in micromass culture [133], is also associated with FGF-induced RCS growth inhibition [129], though the main pathway leading to activation is unknown.

In cancer, if activation of FGFR3 by mutation or overexpression contributes to transformation, it is unlikely that FGFR3 will induce growth inhibition. Not many studies have yet examined FGFR3-specific signalling in cancer cell lines or primary cancer cells and none have examined the normal target cells (B-cells and urothelial cells) of the cancers in which FGFR3 is implicated. The Ras-MAPK and STAT pathways are major pathways mediating the oncogenic effects of FGFR3. Illustrating this is the fact that the TDII FGFR3 mutant (K650E) is transforming when overexpressed in NIH3T3 cells [85,117] and inhibition of Ras or Raf signalling by co-transfection with dominant-negative forms of Ras or Raf

reduces transformation efficiency in this assay [85]. Ligand-dependent MAPK activation, via FGFR3, has been observed in both chondrocytes [47] and MM cell lines carrying FGFR3 mutations [134]. Whether downstream differences in MAPK signalling can explain the different outcome of signalling in these different cell types is at present unknown. The importance of STAT activation in chondrocytes has led to its assessment in MM cell lines and transfected cell lines. Interestingly, differential activation of the STAT proteins has been observed, emphasising the importance of cell type. In MM cell lines expressing *FGFR3* mutants (G384D, K650E or Y373C), no activation of STAT1 or STAT3 is detected, while there is activation of MAP kinases. In contrast, 293T cells expressing the same *FGFR3* mutants show activation of STAT1 and STAT3 as well as phosphorylation of MAP kinases [134]. Perhaps this difference in STAT activation gives a clue to the different responses observed in chondrocytes and cancer cells.

Transformation of NIH3T3 cells is also associated with activation of Stat1 and Stat3 and seems to be mediated by FGFR3 tyrosine 724 [114,117]. Y724 is located in a consensus p85-binding motif, which mediates Shp2 and MAPK phosphorylation as well as PI 3-kinase activation [114]. Results from the same laboratory showed that Y770 has a negative effect on NIH3T3 transformation and PLCγ binding but does not seem to be involved in Shp2, STAT1 or STAT3 phosphorylation. This may function as a binding site for a negative regulator of FGFR3. It will be interesting to examine the phosphorylation status of this tyrosine residue in other FGFR3 transformation models. Finally, FGFR3 binds the adapter protein SH2-B via tyrosines 724 and 760, and phosphorylates it. This interaction is required for Stat5 activation and translocation to the nucleus [135]. It has also been shown that the FGFR3 juxtamembrane domain interacts with Pyk2, a focal adhesion kinase, whose activity is required for STAT5 phophorylation. However, FGFR3 (TDII) can override the requirement of Pyk2 for the activation of STAT5 [136]. STAT5, which is known to induce cyclin D1 expression, has been shown to be involved in cell proliferation and may represent a good candidate for downstream signalling in FGFR3-positive cancers [137].

Conclusions

FGFR3 germline missense mutations observed in skeletal disorders, result in constitutive activation of the receptor and/or inhibition of its degradation and lead to an inhibition of chondrocyte proliferation mediated via STAT1 [132],

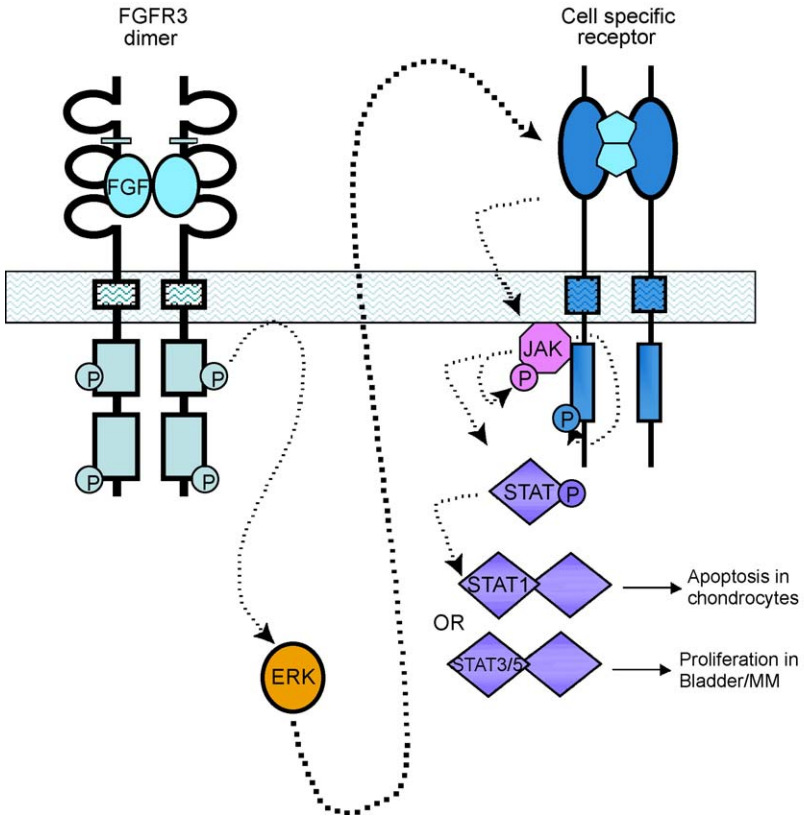

Fig. 4. Hypothesis for cell type specificity of FGFR3 signalling. The origin of STAT activation is not known. However, it has been reported that in endothelial cells, there is an indirect activation of STAT3 via the ERK pathway and stimulation of the platelet-activating factor receptor. This mechanism could apply to FGFR3-expressing cells. It would be then possible that in different cell types the second receptor activated indirectly via ERK is different and ultimately cell type specific. This hypothesis would explain a differential STAT activation and effect.

possibly a premature apoptosis [62] and to an inhibition of hypertrophic differentiation via MEK and ERK [131,132]. In cancer, the highest frequencies of sporadic *FGFR3* missense mutations are found with bladder cancer. Although no studies have yet been reported concerning FGFR3 signalling and cell responses in urothelial cells, expression of mutant FGFR3, as in MM, is expected to result in cell proliferation, in contrast to the situation in chondrocytes. It is notable that the chondrocyte response to FGFR3 activation has been shown to be cell-type specific rather than FGFR specific. However, expression of the IIIc isoform in chondrocytes rather than the IIIb isoform in epithelial cells cannot explain this difference, as MM cells express the IIIc isoform more often than IIIb. There are differences not only between chondrocytes and cancer cells but also between B cells and bladder cells. *FGFR3* mutations are implicated as an early event in the development of bladder tumours but are associated with advanced disease in MM and overexpression of wt FGFR3 appears to play a critical role in MM but as yet there is no evidence of it in bladder cancer. Currently, there is no experimental data to explain the differences seen between cell types in their response to FGF. Comparison of signalling pathways is not yet possible as information on FGFR3 signalling comes mainly from studies of activated FGFR3 in nonepithelial cells. A key finding is that activation of the ERK pathways is seen in all cell types studied. In contrast, differences have been reported for STAT activation in different cell types and this could be involved in cell-specific responses. In chondrocytes, STAT1 is activated but it is not known how far downstream of FGFR3 this occurs and it could be the result of an indirect response, perhaps via activation of a cell-type-specific receptor (Fig. 4). Supporting this idea is the fact that in endothelial cells, phosphorylation of STAT3, following FGF2 stimulation, has been shown to be indirect and linked to the release of platelet-activating factor and activation of its receptor [138]. It is tempting to link differences between growth arrest and cell proliferation to differences in activation of STAT1 and STAT3/5, particularly as hypertrophic chondrocytes show STAT1 activation, but no expression of STAT5a in contrast to proliferating chondrocytes [57]. However, NIH3T3 transformed by mutant FGFR3 also induce STAT1 phosphorylation [117]. The final cell response is likely to result from a balance between opposing signals. Indeed, tyrosines in the cytosolic part of FGFR3 have been shown to have different functions. For example, Y724 promotes transformation while Y770 inhibits it [114]. The puzzling and apparently contradictory effects of FGFR3 signalling in different cell types may not be resolved until specific cell systems are examined and compared in more detail.

The involvement of FGFR3 in two forms of human cancer presents a clear opportunity to develop novel diagnostic and therapeutic approaches. However, a clear understanding of the normal cellular function of FGFR3 in each cell type and of the exact contribution that aberrant signalling makes to tumorigenesis will be required for maximal exploitation of FGFR3 as a therapeutic target.

Acknowledgments

Work in this laboratory is supported by Cancer Research UK. We thank Wendy Kennedy and Darren Tomlinson for helpful comments on the manuscript.

References

[1] M.K. Webster, D.J. Donoghue, FGFR activation in skeletal disorders: too much of a good thing, Trends Genet. 13 (1997) 178–182.
[2] M.R. Passos-Bueno, W.R. Wilcox, E.W. Jabs, A.L. Sertie, L.G. Alonso, H. Kitoh, Clinical spectrum of fibroblast growth factor receptor mutations, Hum. Mutat. 14 (1999) 115–125.
[3] M. Sleeman, J. Fraser, M. McDonald, S. Yuan, D. White, P. Grandison, K. Kumble, J.D. Watson, J.G. Murison, Identification of a new fibroblast growth factor receptor, FGFR5, Gene 271 (2001) 171–182.
[4] I. Kim, S. Moon, K. Yu, U. Kim, G.Y. Koh, A novel fibroblast growth factor receptor-5 preferentially expressed in the pancreas(1), Biochim. Biophys. Acta 1518 (2001) 152–156.
[5] C. Wuchner, K. Hilbert, B. Zabel, A. Winterpacht, Human fibroblast growth factor receptor 3 gene (FGFR3): genomic sequence and primer set information for gene analysis, Hum. Genet. 100 (1997) 215–219.
[6] J. Schlessinger, A.N. Plotnikov, O.A. Ibrahimi, A.V. Eliseenkova, B.K. Yeh, A. Yayon, R.J. Linhardt, M. Mohammadi, Crystal structure of a ternary FGF–FGFR–heparin complex reveals a dual role for heparin in FGFR binding and dimerization, Mol. Cell 6 (2000) 743–750.
[7] L. Pellegrini, D.F. Burke, F. von Delft, B. Mulloy, T.L. Blundell, Crystal structure of fibroblast growth factor receptor ectodomain bound to ligand and heparin, Nature 407 (2000) 1029–1034.
[8] O. Ostrovsky, B. Berman, J. Gallagher, B. Mulloy, D.G. Fernig, M. Delehedde, D. Ron, Differential effects of heparin saccharides on the formation of specific fibroblast growth factor (FGF) and FGF receptor complexes, J. Biol. Chem. 277 (2002) 2444–2453.
[9] D. Berry, Z. Shriver, B. Natke, C.P. Kwan, G. Venkataraman, R. Sasisekharan, Heparan sulphate glycosaminoglycans derived from endothelial cells and smooth muscle cells differentially modulate fibroblast growth factor-2 biological activity through fibroblast growth factor receptor-1, Biochem. J. 373 (2003) 241–249.
[10] A. Horowitz, E. Tkachenko, M. Simons, Fibroblast growth factor-specific modulation of cellular response by syndecan-4, J. Cell Biol. 157 (2002) 715–725.
[11] X. Li, Y. Chen, S. Scheele, E. Arman, R. Haffner-Krausz, P. Ekblom, P. Lonai, Fibroblast growth factor signaling and basement membrane assembly are connected during epithelial morphogenesis of the embryoid body, J. Cell Biol. 153 (2001) 811–822.
[12] U. Cavallaro, G. Christofori, Cell adhesion and signalling by cadherins and Ig-CAMs in cancer, Nat. Rev., Cancer 4 (2004) 118–132.
[13] U. Cavallaro, J. Niedermeyer, M. Fuxa, G. Christofori, N-CAM modulates tumour-cell adhesion to matrix by inducing FGF-receptor signalling, Nat. Cell Biol. 3 (2001) 650–657.
[14] V.V. Kiselyov, G. Skladchikova, A.M. Hinsby, P.H. Jensen, N. Kulahin, V. Soroka, N. Pedersen, V. Tsetlin, F.M. Poulsen, V. Berezin, E. Bock, Structural basis for a direct interaction between

FGFR1 and NCAM and evidence for a regulatory role of ATP, Structure (Camb.) 11 (2003) 691–701.
[15] K. Suyama, I. Shapiro, M. Guttman, R.B. Hazan, A signaling pathway leading to metastasis is controlled by N-cadherin and the FGF receptor, Cancer Cell 2 (2002) 301–314.
[16] C.H. Faux, A.M. Turnley, R. Epa, R. Cappai, P.F. Bartlett, Interactions between fibroblast growth factors and Notch regulate neuronal differentiation, J. Neurosci. 21 (2001) 5587–5596.
[17] D. Small, D. Kovalenko, R. Soldi, A. Mandinova, V. Kolev, R. Trifonova, C. Bagala, D. Kacer, C. Battelli, L. Liaw, I. Prudovsky, T. Maciag, Notch activation suppresses fibroblast growth factor-dependent cellular transformation, J. Biol. Chem. 278 (2003) 16405–16413.
[18] A. Avivi, A. Yayon, D. Givol, A novel form of FGF receptor-3 using an alternative exon in the immunoglobulin domain III, FEBS Lett. 330 (1993) 249–252.
[19] A.T. Chellaiah, D.G. McEwen, S. Werner, J. Xu, D.M. Ornitz, Fibroblast growth factor receptor (FGFR) 3. Alternative splicing in immunoglobulin-like domain III creates a receptor highly specific for acidic FGF/FGF-1, J. Biol. Chem. 269 (1994) 11620–11627.
[20] B. Murgue, S. Tsunekawa, I. Rosenberg, M. deBeaumont, D.K. Podolsky, Identification of a novel variant form of fibroblast growth factor receptor 3 (FGFR3 IIIb) in human colonic epithelium, Cancer Res. 54 (1994) 5206–5211.
[21] D. Hecht, N. Zimmerman, M. Bedford, A. Avivi, A. Yayon, Identification of fibroblast growth factor 9 (FGF9) as a high affinity, heparin dependent ligand for FGF receptors 3 and 2 but not for FGF receptors 1 and 4, Growth Factors 12 (1995) 223–233.
[22] S. Santos-Ocampo, J.S. Colvin, A. Chellaiah, D.M. Ornitz, Expression and biological activity of mouse fibroblast growth factor-9, J. Biol. Chem. 271 (1996) 1726–1731.
[23] M. Kanai, M. Goke, S. Tsunekawa, D.K. Podolsky, Signal transduction pathway of human fibroblast growth factor receptor 3. Identification of a novel 66-kDa phosphoprotein, J. Biol. Chem. 272 (1997) 6621–6628.
[24] Z. Liu, J. Xu, J.S. Colvin, D.M. Ornitz, Coordination of chondrogenesis and osteogenesis by fibroblast growth factor 18, Genes Dev. 16 (2002) 859–869.
[25] N. Ohbayashi, M. Shibayama, Y. Kurotaki, M. Imanishi, T. Fujimori, N. Itoh, S. Takada, FGF18 is required for normal cell proliferation and differentiation during osteogenesis and chondrogenesis, Genes Dev. 16 (2002) 870–879.
[26] S.K. Olsen, O.A. Ibrahimi, A. Raucci, F.M. Zhang, A.V. Eliseenkova, A. Yayon, C. Basilico, R.J. Linhardt, J. Schlessinger, M. Mohammadi, Insights into the molecular basis for fibroblast growth factor receptor autoinhibition and ligand-binding promiscuity, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 935–940.
[27] G. Patstone, E.B. Pasquale, P.A. Maher, Different members of the fibroblast growth factor receptor family are specific to distinct cell types in the developing chicken embryo, Dev. Biol. 155 (1993) 107–123.
[28] A. Vidrich, J.M. Buzan, C. Ilo, L. Bradley, K. Skaar, S.M. Cohn, Fibroblast growth factor receptor-3 is expressed in undifferentiated intestinal epithelial cells during murine crypt morphogenesis, Dev. Dyn. 230 (2004) 114–123.
[29] N.P. Pringle, W.P. Yu, M. Howell, J.S. Colvin, D.M. Ornitz, W.D. Richardson, Fgfr3 expression by astrocytes and their precursors: evidence that astrocytes and oligodendrocytes originate in distinct neuroepithelial domains, Development 130 (2003) 93–102.
[30] K. Peters, D. Ornitz, S. Werner, L. Williams, Unique expression pattern of the FGF receptor 3 gene during mouse organogenesis, Dev. Biol. 155 (1993) 423–430.
[31] V. Govindarajan, P.A. Overbeek, Secreted FGFR3, but not FGFR1, inhibits lens fiber differentiation, Development 128 (2001) 1617–1627.
[32] C. Wuechner, A.C. Nordqvist, A. Winterpacht, B. Zabel, M. Schalling, Developmental expression of splicing variants of fibroblast growth factor receptor 3 (FGFR3) in mouse, Int. J. Dev. Biol. 40 (1996) 1185–1188.
[33] E. Scotet, E. Houssaint, Exon III splicing switch of fibroblast growth factor (FGF) receptor-2 and -3 can be induced by FGF-1 or FGF-2, Oncogene 17 (1998) 67–76.
[34] M. Kanai, I. Rosenberg, D.K. Podolsky, Cytokine regulation of fibroblast growth factor receptor 3 IIIb in intestinal epithelial cells, Am. J. Physiol. 272 (1997) G885–G893.
[35] C. Deng, A. Wynshaw-Boris, F. Zhou, A. Kuo, P. Leder, Fibroblast growth factor receptor 3 is a negative regulator of bone growth, Cell 84 (1996) 911–921.
[36] R. Adar, E. Monsonego-Ornan, P. David, A. Yayon, Differential activation of cysteine-substitution mutants of fibroblast growth factor receptor 3 is determined by cysteine localization, J. Bone Miner. Res. 17 (2002) 860–868.
[37] Z. Vajo, C.A. Francomano, D.J. Wilkin, The molecular and genetic basis of fibroblast growth factor receptor 3 disorders: the achondroplasia family of skeletal dysplasias, Muenke craniosynostosis, and Crouzon syndrome with acanthosis nigricans, Endocr. Rev. 21 (2000) 23–39.
[38] W.R. Wilcox, P.L. Tavormina, D. Krakow, H. Kitoh, R.S. Lachman, J.J. Wasmuth, L.M. Thompson, D.L. Rimoin, Molecular, radiologic, and histopathologic correlations in thanatophoric dysplasia, Am. J. Med. Genet. 78 (1998) 274–281.
[39] H. Kitoh, S.G. Brodie, K.G. Kupke, R.S. Lachman, W.R. Wilcox, Lys650Met substitution in the tyrosine kinase domain of the fibroblast growth factor receptor gene causes thanatophoric dysplasia Type I. Mutations in brief no. 199, Online, Hum. Mutat. 12 (1998) 362–363.
[40] P.L. Tavormina, G.A. Bellus, M.K. Webster, M.J. Bamshad, A.E. Fraley, I. McIntosh, J. Szabo, W. Jiang, E.W. Jabs, W.R. Wilcox, J.J. Wasmuth, D.J. Donoghue, L.M. Thompson, C.A. Francomano, A novel skeletal dysplasia with developmental delay and acanthosis nigricans is caused by a Lys650Met mutation in the fibroblast growth factor receptor 3 gene, Am. J. Hum. Genet. 64 (1999) 722–731.
[41] G.A. Bellus, M.J. Bamshad, K.A. Przylepa, J. Dorst, R.R. Lee, O. Hurko, E.W. Jabs, C.J. Curry, W.R. Wilcox, R.S. Lachman, D.L. Rimoin, C.A. Francomano, Severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN): phenotypic analysis of a new skeletal dysplasia caused by a Lys650Met mutation in fibroblast growth factor receptor 3, Am. J. Med. Genet. 85 (1999) 53–65.
[42] M.K. Webster, P.Y. D'Avis, S.C. Robertson, D.J. Donoghue, Profound ligand-independent kinase activation of fibroblast growth factor receptor 3 by the activation loop mutation responsible for a lethal skeletal dysplasia, thanatophoric dysplasia type II, Mol. Cell. Biol. 16 (1996) 4081–4087.
[43] G.A. Bellus, E.B. Spector, P.W. Speiser, C.A. Weaver, A.T. Garber, C.R. Bryke, J. Israel, S.S. Rosengren, M.K. Webster, D.J. Donoghue, C.A. Francomano, Distinct missense mutations of the FGFR3 lys650 codon modulate receptor kinase activation and the severity of the skeletal dysplasia phenotype, Am. J. Hum. Genet. 67 (2000) 1411–1421.
[44] F. Rousseau, V. el Ghouzzi, A.L. Delezoide, L. Legeai-Mallet, M. Le Merrer, A. Munnich, J. Bonaventure, Missense FGFR3 mutations create cysteine residues in thanatophoric dwarfism type I (TD1), Hum. Mol. Genet. 5 (1996) 509–512.
[45] S.C. Robertson, A.N. Meyer, K.C. Hart, B.D. Galvin, M.K. Webster, D.J. Donoghue, Activating mutations in the extracellular domain of the fibroblast growth factor receptor 2 function by disruption of the disulfide bond in the third immunoglobulin-like domain, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 4567–4572.
[46] F. Rousseau, P. Saugier, M. Le Merrer, A. Munnich, A.L. Delezoide, P. Maroteaux, J. Bonaventure, F. Narcy, M. Sanak, Stop codon FGFR3 mutations in thanatophoric dwarfism type 1, Nat. Genet. 10 (1995) 11–12.

[47] E. Monsonego-Ornan, R. Adar, T. Feferman, O. Segev, A. Yayon, The transmembrane mutation G380R in fibroblast growth factor receptor 3 uncouples ligand-mediated receptor activation from down-regulation, Mol. Cell. Biol. 20 (2000) 516–522.
[48] E. Monsonego-Ornan, R. Adar, E. Rom, A. Yayon, FGF receptors ubiquitylation: dependence on tyrosine kinase activity and role in downregulation, FEBS Lett. 528 (2002) 83–89.
[49] J.Y. Cho, C. Guo, M. Torello, G.P. Lunstrum, T. Iwata, C. Deng, W.A. Horton, Defective lysosomal targeting of activated fibroblast growth factor receptor 3 in achondroplasia, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 609–614.
[50] P.M. Lievens, E. Liboi, The thanatophoric dysplasia type II mutation hampers complete maturation of fibroblast growth factor receptor 3 (FGFR3), which activates signal transducer and activator of transcription 1 (STAT1) from the endoplasmic reticulum, J. Biol. Chem. 278 (2003) 17344–17349.
[51] A. Winterpacht, K. Hilbert, C. Stelzer, T. Schweikardt, H. Decker, H. Segerer, J. Spranger, B. Zabel, A novel mutation in FGFR-3 disrupts a putative N-glycosylation site and results in hypochondroplasia, Physiol. Genomics 2 (2000) 9–12.
[52] O.A. Ibrahimi, F. Zhang, A.V. Eliseenkova, R.J. Linhardt, M. Mohammadi, Proline to arginine mutations in FGF receptors 1 and 3 result in Pfeiffer and Muenke craniosynostosis syndromes through enhancement of FGF binding affinity, Hum. Mol. Genet. 13 (2004) 69–78.
[53] A. White, G. Wallis, Endochondral ossification: a delicate balance between growth and mineralisation, Curr. Biol. 11 (2001) R589–R591.
[54] O. Segev, I. Chumakov, Z. Nevo, D. Givol, L. Madar-Shapiro, Y. Sheinin, M. Weinreb, A. Yayon, Restrained chondrocyte proliferation and maturation with abnormal growth plate vascularization and ossification in human FGFR-3(G380R) transgenic mice, Hum. Mol. Genet. 9 (2000) 249–258.
[55] Y. Wang, M.K. Spatz, K. Kannan, H. Hayk, A. Avivi, M. Gorivodsky, M. Pines, A. Yayon, P. Lonai, D. Givol, A mouse model for achondroplasia produced by targeting fibroblast growth factor receptor 3, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 4455–4460.
[56] L. Chen, R. Adar, X. Yang, E.O. Monsonego, C. Li, P.V. Hauschka, A. Yayon, C.X. Deng, Gly369Cys mutation in mouse FGFR3 causes achondroplasia by affecting both chondrogenesis and osteogenesis, J. Clin. Invest. 104 (1999) 1517–1525.
[57] C. Li, L. Chen, T. Iwata, M. Kitagawa, X.Y. Fu, C.X. Deng, A Lys644Glu substitution in fibroblast growth factor receptor 3 (FGFR3) causes dwarfism in mice by activation of STATs and ink4 cell cycle inhibitors, Hum. Mol. Genet. 8 (1999) 35–44.
[58] T. Iwata, L. Chen, C. Li, D.A. Ovchinnikov, R.R. Behringer, C.A. Francomano, C.X. Deng, A neonatal lethal mutation in FGFR3 uncouples proliferation and differentiation of growth plate chondrocytes in embryos, Hum. Mol. Genet. 9 (2000) 1603–1613.
[59] T. Iwata, C.L. Li, C.X. Deng, C.A. Francomano, Highly activated Fgfr3 with the K644M mutation causes prolonged survival in severe dwarf mice, Hum. Mol. Genet. 10 (2001) 1255–1264.
[60] L. Chen, C. Li, W. Qiao, X. Xu, C. Deng, A Ser(365)—Cys mutation of fibroblast growth factor receptor 3 in mouse downregulates Ihh/PTHrP signals and causes severe achondroplasia, Hum. Mol. Genet. 10 (2001) 457–465.
[61] L. Legeai-Mallet, C. Benoist-Lasselin, A. Munnich, J. Bonaventure, Overexpression of FGFR3, Stat1, Stat5 and p21(Cip1) correlates with phenotypic severity and defective chondrocyte differentiation in FGFR3-related chondrodysplasias, Bone 34 (2004) 26–36.
[62] L. Legeai-Mallet, C. Benoist-Lasselin, A.L. Delezoide, A. Munnich, J. Bonaventure, Fibroblast growth factor receptor 3 mutations promote apoptosis but do not alter chondrocyte proliferation in thanatophoric dysplasia, J. Biol. Chem. 273 (1998) 13007–13014.
[63] O. Rozenblatt-Rosen, E. Mosonego-Ornan, E. Sadot, L. Madar-Shapiro, Y. Sheinin, D. Ginsberg, A. Yayon, Induction of chondrocyte growth arrest by FGF: transcriptional and cytoskeletal alterations, J. Cell Sci. 115 (2002) 553–562.
[64] L. Dailey, E. Laplantine, R. Priore, C. Basilico, A network of transcriptional and signaling events is activated by FGF to induce chondrocyte growth arrest and differentiation, J. Cell Biol. 161 (2003) 1053–1066.
[65] J.E. Henderson, M.C. Naski, M.M. Aarts, D. Wang, L. Cheng, D. Goltzman, D.M. Ornitz, Expression of FGFR3 with the G380R achondroplasia mutation inhibits proliferation and maturation of CFK2 chondrocytic cells, J. Bone Miner. Res. 15 (2000) 155–165.
[66] Q. Wang, R.P. Green, G. Zhao, D.M. Ornitz, Differential regulation of endochondral bone growth and joint development by FGFR1 and FGFR3 tyrosine kinase domains, Development 128 (2001) 3867–3876.
[67] G. Valverde-Franco, H. Liu, D. Davidson, S. Chai, H. Valderrama-Carvajal, D. Goltzman, D.M. Ornitz, J.E. Henderson, Defective bone mineralization and osteopenia in young adult FGFR3−/− mice, Hum. Mol. Genet. 13 (2004) 271–284.
[68] S. Cormier, A.L. Delezoide, C. Benoist-Lasselin, L. Legeai-Mallet, J. Bonaventure, C. Silve, Parathyroid hormone receptor type 1/Indian hedgehog expression is preserved in the growth plate of human fetuses affected with fibroblast growth factor receptor type 3 activating mutations, Am. J. Pathol. 161 (2002) 1325–1335.
[69] N. Amizuka, D. Davidson, H. Liu, G. Valverde-Franco, S. Chai, T. Maeda, H. Ozawa, V. Hammond, D.M. Ornitz, D. Goltzman, J.E. Henderson, Signalling by fibroblast growth factor receptor 3 and parathyroid hormone-related peptide coordinate cartilage and bone development, Bone 34 (2004) 13–25.
[70] H.P. Gerber, T.H. Vu, A.M. Ryan, J. Kowalski, Z. Werb, N. Ferrara, VEGF couples hypertrophic cartilage remodeling, ossification and angiogenesis during endochondral bone formation, Nat. Med. 5 (1999) 623–628.
[71] M. Chesi, E. Nardini, L.A. Brents, E. Schrock, T. Ried, W.M. Kuehl, P.L. Bergsagel, Frequent translocation t(4;14)(p16.3;q32.3) in multiple myeloma is associated with increased expression and activating mutations of fibroblast growth factor receptor 3, Nat. Genet. 16 (1997) 260–264.
[72] R. Richelda, D. Ronchetti, L. Baldini, L. Cro, L. Viggiano, R. Marzella, M. Rocchi, T. Otsuki, L. Lombardi, A.T. Maiolo, A. Neri, A novel chromosomal translocation t(4;14)(p16.3;q32) in multiple myeloma involves the fibroblast growth-factor receptor 3 gene, Blood 90 (1997) 4062–4070.
[73] D. Cappellen, C. De Oliveira, D. Ricol, S. de Medina, J. Bourdin, X. Sastre-Garau, D. Chopin, J.P. Thiery, F. Radvanyi, Frequent activating mutations of FGFR3 in human bladder and cervix carcinomas, Nat. Genet. 23 (1999) 18–20.
[74] J.H. Jang, K.H. Shin, J.G. Park, Mutations in fibroblast growth factor receptor 2 and fibroblast growth factor receptor 3 genes associated with human gastric and colorectal cancers, Cancer Res. 61 (2001) 3541–3543.
[75] B. Naimi, A. Latil, P. Berthon, O. Cussenot, No evidence for fibroblast growth factor receptor 3 (FGFR-3) R248C/S249C mutations in human prostate cancer, Int. J. Cancer 87 (2000) 455–456.
[76] M. Karoui, H. Hofmann-Radvanyi, U. Zimmermann, A. Couvelard, C. Degott, L. Faridoni-Laurens, J.C. Ahomadegbe, S. Gazzeri, E. Brambilla, T. Clerici, P. Charbonnier, C. Tresallet, E. Mitry, C. Penna, P. Rougier, C. Boileau, J.P. Thiery, B. Nordlinger, B. Franc, F. Radvanyi, No evidence of somatic FGFR3 mutation in various types of carcinoma, Oncogene 20 (2001) 5059–5061.
[77] K. Sibley, P. Stern, M.A. Knowles, Frequency of fibroblast growth factor receptor 3 mutations in sporadic tumours, Oncogene 20 (2001) 4416–4418.
[78] H. Avet-Loiseau, T. Facon, A. Daviet, C. Godon, M.J. Rapp, J.L. Harousseau, B. Grosbois, R. Bataille, 14q32 translocations and monosomy 13 observed in monoclonal gammopathy of undetermined significance delineate a multistep process for the oncogenesis

of multiple myeloma. Intergroupe Francophone du Myelome, Cancer Res. 59 (1999) 4546–4550.
[79] K. Sibley, J.A. Fenton, A.M. Dring, A.J. Ashcroft, A.C. Rawstron, G.J. Morgan, A molecular study of the t(4;14) in multiple myeloma, Br. J. Haematol. 118 (2002) 514–520.
[80] F. Zhan, J. Hardin, B. Kordsmeier, K. Bumm, M. Zheng, E. Tian, R. Sanderson, Y. Yang, C. Wilson, M. Zangari, E. Anaissie, C. Morris, F. Muwalla, F. van Rhee, A. Fassas, J. Crowley, G. Tricot, B. Barlogie, J. Shaughnessy Jr., Global gene expression profiling of multiple myeloma, monoclonal gammopathy of undetermined significance, and normal bone marrow plasma cells, Blood 99 (2002) 1745–1757.
[81] J.J. Keats, T. Reiman, C.A. Maxwell, B.J. Taylor, L.M. Larratt, M.J. Mant, A.R. Belch, L.M. Pilarski, In multiple myeloma, t(4;14)(p16;q32) is an adverse prognostic factor irrespective of FGFR3 expression, Blood 101 (2003) 1520–1529.
[82] M. Santra, F. Zhan, E. Tian, B. Barlogie, J. Shaughnessy Jr., A subset of multiple myeloma harboring the t(4;14)(p16;q32) translocation lacks FGFR3 expression but maintains an IGH/MMSET fusion transcript, Blood 101 (2003) 2374–2376.
[83] O.N. Onwuazor, X.Y. Wen, D.Y. Wang, L. Zhuang, E. Masih-Khan, J. Claudio, B. Barlogie, J.D. Shaughnessy Jr., A.K. Stewart, Mutation, SNP, and isoform analysis of fibroblast growth factor receptor 3 (FGFR3) in 150 newly diagnosed multiple myeloma patients, Blood 102 (2003) 772–773.
[84] S. Soverini, C. Terragna, N. Testoni, D. Ruggeri, P. Tosi, E. Zamagni, C. Cellini, M. Cavo, M. Baccarani, S. Tura, G. Martinelli, Novel mutation and RNA splice variant of fibroblast growth factor receptor 3 in multiple myeloma patients at diagnosis, Haematologica 87 (2002) 1036–1040.
[85] M. Chesi, L.A. Brents, S.A. Ely, C. Bais, D.F. Robbiani, E.A. Mesri, W.M. Kuehl, P.L. Bergsagel, Activated fibroblast growth factor receptor 3 is an oncogene that contributes to tumor progression in multiple myeloma, Blood 97 (2001) 729–736.
[86] Z. Li, Y.X. Zhu, E.E. Plowright, P.L. Bergsagel, M. Chesi, B. Patterson, T.S. Hawley, R.G. Hawley, A.K. Stewart, The myeloma-associated oncogene fibroblast growth factor receptor 3 is transforming in hematopoietic cells, Blood 97 (2001) 2413–2419.
[87] P.L. Bergsagel, W.M. Kuehl, Critical roles for immunoglobulin translocations and cyclin D dysregulation in multiple myeloma, Immunol. Rev. 194 (2003) 96–104.
[88] E.K. Grand, A.J. Chase, C. Heath, A. Rahemtulla, N.C.P. Cross, Targeting FGFR3 in multiple myeloma: inhibition of t(4;14)-positive cells by SU5402 and PD173074, Leukemia 18 (2004) 962–966.
[89] B.W. Van Rhijn, A.A. Van Tilborg, I. Lurkin, J. Bonaventure, A. De Vries, J.P. Thiery, T.H. Van Der Kwast, E.C. Zwarthoff, F. Radvanyi, Novel fibroblast growth factor receptor 3 (FGFR3) mutations in bladder cancer previously identified in non-lethal skeletal disorders, Eur. J. Hum. Genet. 10 (2002) 819–824.
[90] S.E. Patton, M.C. Hall, H. Ozen, Bladder cancer, Curr. Opin. Oncol. 14 (2002) 265–272.
[91] UICC, TNM Classification of Malignant Tumors, Bladder, Union Internationale Contre le Cancer, Geneva, 1978.
[92] WHO, Histological typing of urinary bladder tumours, Int. Histol. Classif. Tumours, 1973.
[93] T. Kimura, H. Suzuki, T. Ohashi, K. Asano, H. Kiyota, Y. Eto, The incidence of thanatophoric dysplasia mutations in FGFR3 gene is higher in low-grade or superficial bladder carcinomas, Cancer 92 (2001) 2555–2561.
[94] C. Billerey, D. Chopin, M.H. Aubriot-Lorton, D. Ricol, S. Gil Diez de Medina, B. Van Rhijn, M.P. Bralet, M.A. Lefrere-Belda, J.B. Lahaye, C.C. Abbou, J. Bonaventure, E.S. Zafrani, T. van der Kwast, J.P. Thiery, F. Radvanyi, Frequent FGFR3 mutations in papillary non-invasive bladder (pTa) tumors, Am. J. Pathol. 158 (2001) 1955–1959.
[95] A.A. Bakkar, H. Wallerand, F. Radvanyi, J.B. Lahaye, S. Pissard, L. Lecerf, J.C. Kouyoumdjian, C.C. Abbou, J.C. Pairon, M.C. Jaurand, J.P. Thiery, D.K. Chopin, S. Gil Diez De Medina, FGFR3 and TP53 gene mutations define two distinct pathways in urothelial cell carcinoma of the bladder, Cancer Res. 63 (2003) 8108–8112.
[96] B.W. van Rhijn, I. Lurkin, F. Radvanyi, W.J. Kirkels, T.H. van der Kwast, E.C. Zwarthoff, The fibroblast growth factor receptor 3 (FGFR3) mutation is a strong indicator of superficial bladder cancer with low recurrence rate, Cancer Res. 61 (2001) 1265–1268.
[97] V.M. Tut, K.L. Braithwaite, B. Angus, D.E. Neal, J. Lunec, J.K. Mellon, Cyclin D1 expression in transitional cell carcinoma of the bladder: correlation with p53, waf1, pRb and Ki67, Br. J. Cancer 84 (2001) 270–275.
[98] Y. Takagi, M. Takashi, T. Koshikawa, T. Sakata, S. Ohshima, Immunohistochemical demonstration of cyclin D1 in bladder cancers as an inverse indicator of invasiveness but not an independent prognostic factor, Int. J. Urol. 7 (2000) 366–372.
[99] C.C. Lee, S. Yamamoto, K. Morimura, H. Wanibuchi, N. Nishisaka, S. Ikemoto, T. Nakatani, S. Wada, T. Kishimoto, S. Fukushima, Significance of cyclin D1 overexpression in transitional cell carcinomas of the urinary bladder and its correlation with histopathologic features, Cancer 79 (1997) 780–789.
[100] U. Wagner, K. Suess, T. Luginbuhl, U. Schmid, D. Ackermann, T. Zellweger, R. Maurer, G. Alund, H. Knonagel, M. Rist, P. Jordan, H. Moch, M.J. Mihatsch, T.C. Gasser, G. Sauter, Cyclin D1 overexpression lacks prognostic significance in superficial urinary bladder cancer, J. Pathol. 188 (1999) 44–50.
[101] Y. Suwa, Y. Takano, M. Iki, M. Takeda, T. Asakura, S. Noguchi, M. Masuda, Cyclin D1 protein overexpression is related to tumor differentiation, but not to tumor progression or proliferative activity, in transitional cell carcinoma of the bladder, J. Urol. 160 (1998) 897–900.
[102] A. Sgambato, M. Migaldi, B. Faraglia, G. De Aloysio, P. Ferrari, R. Ardito, C. De Gaetani, G. Capelli, A. Cittadini, G.P. Trentini, Cyclin D1 expression in papillary superficial bladder cancer: its association with other cell cycle-associated proteins, cell proliferation and clinical outcome, Int. J. Cancer 97 (2002) 671–678.
[103] B.W. van Rhijn, A.N. Vis, T.H. van der Kwast, W.J. Kirkels, F. Radvanyi, E.C. Ooms, D.K. Chopin, E.R. Boeve, A.C. Jobsis, E.C. Zwarthoff, Molecular grading of urothelial cell carcinoma with fibroblast growth factor receptor 3 and MIB-1 is superior to pathologic grade for the prediction of clinical outcome, J. Clin. Oncol. 21 (2003) 1912–1921.
[104] S. Trudel, S. Ely, Y. Farooqi, M. Affer, D.F. Robbiani, M. Chesi, P.L. Bergsagel, Inhibition of fibroblast growth factor receptor 3 induces differentiation and apoptosis in t(4;14) myeloma, Blood 103 (2004) 3521–3528.
[105] A. Shimizu, K. Tada, C. Shukunami, Y. Hiraki, T. Kurokawa, N. Magane, M. Kurokawa-Seo, A novel alternatively spliced fibroblast growth factor receptor 3 isoform lacking the acid box domain is expressed during chondrogenic differentiation of ATDC5 cells, J. Biol. Chem. 276 (2001) 11031–11040.
[106] A. Shimizu, Y. Takashima, M. Kurokawa-Seo, FGFR3 isoforms have distinct functions in the regulation of growth and cell morphology, Biochem. Biophys. Res. Commun. 290 (2002) 113–120.
[107] C.L. Johnston, H.C. Cox, J.J. Gomm, R.C. Coombes, Fibroblast growth factor receptors (FGFRs) localize in different cellular compartments. A splice variant of FGFR-3 localizes to the nucleus, J. Biol. Chem. 270 (1995) 30643–30650.
[108] C. Zammit, R. Barnard, J. Gomm, R. Coope, S. Shousha, C. Coombes, C. Johnston, Altered intracellular localization of fibroblast growth factor receptor 3 in human breast cancer, J. Pathol. 194 (2001) 27–34.
[109] M. Terada, A. Shimizu, N. Sato, S.I. Miyakaze, H. Katayama, M. Kurokawa-Seo, Fibroblast growth factor receptor 3 lacking the Ig IIIb and transmembrane domains secreted from human squamous cell carcinoma DJM-1 binds to FGFs, Mol. Cell. Biol. Res. Commun. 4 (2001) 365–373.

[110] J.H. Jang, Identification and characterization of soluble isoform of fibroblast growth factor receptor 3 in human SaOS-2 osteosarcoma cells, Biochem. Biophys. Res. Commun. 292 (2002) 378–382.
[111] J.H. Jang, K.H. Shin, Y.J. Park, R.J. Lee, W.L. McKeehan, J.G. Park, Novel transcripts of fibroblast growth factor receptor 3 reveal aberrant splicing and activation of cryptic splice sequences in colorectal cancer, Cancer Res. 60 (2000) 4049–4052.
[112] L.M. Sturla, A.E. Merrick, S.A. Burchill, FGFR3IIIS: a novel soluble FGFR3 spliced variant that modulates growth is frequently expressed in tumour cells, Br. J. Cancer 89 (2003) 1276–1284.
[113] M. Mohammadi, I. Dikic, A. Sorokin, W.H. Burgess, M. Jaye, J. Schlessinger, Identification of six novel autophosphorylation sites on fibroblast growth factor receptor 1 and elucidation of their importance in receptor activation and signal transduction, Mol. Cell. Biol. 16 (1996) 977–989.
[114] K.C. Hart, S.C. Robertson, D.J. Donoghue, Identification of tyrosine residues in constitutively activated fibroblast growth factor receptor 3 involved in mitogenesis, Stat activation, and phosphatidylinositol 3-kinase activation, Mol. Biol. Cell 12 (2001) 931–942.
[115] H. Kouhara, Y.R. Hadari, T. Spivak-Kroizman, J. Schilling, D. Bar-Sagi, I. Lax, J. Schlessinger, A lipid-anchored Grb2-binding protein that links FGF-receptor activation to the Ras/MAPK signaling pathway, Cell 89 (1997) 693–702.
[116] M. Mohammadi, A.M. Honegger, D. Rotin, R. Fischer, F. Bellot, W. Li, C.A. Dionne, M. Jaye, M. Rubinstein, J. Schlessinger, A tyrosine-phosphorylated carboxy-terminal peptide of the fibroblast growth factor receptor (Flg) is a binding site for the SH2 domain of phospholipase C-gamma 1, Mol. Cell. Biol. 11 (1991) 5068–5078.
[117] K.C. Hart, S.C. Robertson, M.Y. Kanemitsu, A.N. Meyer, J.A. Tynan, D.J. Donoghue, Transformation and Stat activation by derivatives of FGFR1, FGFR3, and FGFR4, Oncogene 19 (2000) 3309–3320.
[118] D.Y. Choi, J.J. Toledo-Aral, H.Y. Lin, I. Ischenko, L. Medina, P. Safo, G. Mandel, S.R. Levinson, S. Halegoua, M.J. Hayman, Fibroblast growth factor receptor 3 induces gene expression primarily through Ras-independent signal transduction pathways, J. Biol. Chem. 276 (2001) 5116–5122.
[119] M.C. Naski, Q. Wang, J. Xu, D.M. Ornitz, Graded activation of fibroblast growth factor receptor 3 by mutations causing achondroplasia and thanatophoric dysplasia, Nat. Genet. 13 (1996) 233–237.
[120] M.K. Webster, D.J. Donoghue, Enhanced signaling and morphological transformation by a membrane-localized derivative of the fibroblast growth factor receptor 3 kinase domain, Mol. Cell. Biol. 17 (1997) 5739–5747.
[121] M. Sahni, D.C. Ambrosetti, A. Mansukhani, R. Gertner, D. Levy, C. Basilico, FGF signaling inhibits chondrocyte proliferation and regulates bone development through the STAT-1 pathway, Genes Dev. 13 (1999) 1361–1366.
[122] T. Aikawa, G.V. Segre, K. Lee, Fibroblast growth factor inhibits chondrocytic growth through induction of p21 and subsequent inactivation of cyclin E-Cdk2, J. Biol. Chem. 276 (2001) 29347–29352.
[123] F. Beier, R.J. Lee, A.C. Taylor, R.G. Pestell, P. LuValle, Identification of the cyclin D1 gene as a target of activating transcription factor 2 in chondrocytes, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 1433–1438.
[124] F. Beier, Z. Ali, D. Mok, A.C. Taylor, T. Leask, C. Albanese, R.G. Pestell, P. LuValle, TGFbeta and PTHrP control chondrocyte proliferation by activating cyclin D1 expression, Mol. Biol. Cell 12 (2001) 3852–3863.
[125] H. Wang, M. Rubin, E. Fenig, A. DeBlasio, J. Mendelsohn, J. Yahalom, R. Wieder, Basic fibroblast growth factor causes growth arrest in MCF-7 human breast cancer cells while inducing both mitogenic and inhibitory G1 events, Cancer Res. 57 (1997) 1750–1757.
[126] G.Z. Yan, E.B. Ziff, NGF regulates the PC12 cell cycle machinery through specific inhibition of the Cdk kinases and induction of cyclin D1, J. Neurosci. 15 (1995) 6200–6212.
[127] E.K. Han, M. Begemann, A. Sgambato, J.W. Soh, Y. Doki, W.Q. Xing, W. Liu, I.B. Weinstein, Increased expression of cyclin D1 in a murine mammary epithelial cell line induces p27kip1, inhibits growth, and enhances apoptosis, Cell Growth Differ. 7 (1996) 699–710.
[128] J.S. de Jong, P.J. van Diest, R.J. Michalides, J.P. Baak, Concerted overexpression of the genes encoding p21 and cyclin D1 is associated with growth inhibition and differentiation in various carcinomas, Mol. Pathol. 52 (1999) 78–83.
[129] A. Raucci, E. Laplantine, A. Mansukhani, C. Basilico, Activation of the ERK1/2 and p38 mitogen-activated protein kinase pathways mediates fibroblast growth factor-induced growth arrest of chondrocytes, J. Biol. Chem. 279 (2004) 1747–1756.
[130] M. Koike, Y. Yamanaka, M. Inoue, H. Tanaka, R. Nishimura, Y. Seino, Insulin-like growth factor-1 rescues the mutated FGF receptor 3 (G380R) expressing ATDC5 cells from apoptosis through phosphatidylinositol 3-kinase and MAPK, J. Bone Miner. Res. 18 (2003) 2043–2051.
[131] A. Yasoda, Y. Komatsu, H. Chusho, T. Miyazawa, A. Ozasa, M. Miura, T. Kurihara, T. Rogi, S. Tanaka, M. Suda, N. Tamura, Y. Ogawa, K. Nakao, Overexpression of CNP in chondrocytes rescues achondroplasia through a MAPK-dependent pathway, Nat. Med. 10 (2004) 80–86.
[132] S. Murakami, G. Balmes, S. McKinney, Z. Zhang, D. Givol, B. de Crombrugghe, Constitutive activation of MEK1 in chondrocytes causes Stat1-independent achondroplasia-like dwarfism and rescues the Fgfr3-deficient mouse phenotype, Genes Dev. 18 (2004) 290–305.
[133] L.A. Stanton, S. Sabari, A.V. Sampaio, T.M. Underhill, F. Beier, p38 MAP kinase signalling is required for hypertrophic chondrocyte differentiation, Biochem. J. 378 (2004) 53–62.
[134] D. Ronchetti, A. Greco, S. Compasso, G. Colombo, P. Dell'Era, T. Otsuki, L. Lombardi, A. Neri, Deregulated FGFR3 mutants in multiple myeloma cell lines with t(4;14): comparative analysis of Y373C, K650E and the novel G384D mutations, Oncogene 20 (2001) 3553–3562.
[135] M. Kong, C.S. Wang, D.J. Donoghue, Interaction of fibroblast growth factor receptor 3 and the adapter protein SH2-B. A role in STAT5 activation, J. Biol. Chem. 277 (2002) 15962–15970.
[136] A.N. Meyer, R.F. Gastwirt, D.D. Schlaepfer, D.J. Donoghue, The cytoplasmic tyrosine kinase Pyk2 as a novel effector of fibroblast growth factor receptor 3 activation, J. Biol. Chem. 279 (2004) 28450–28457.
[137] I. Matsumura, T. Kitamura, H. Wakao, H. Tanaka, K. Hashimoto, C. Albanese, J. Downward, R.G. Pestell, Y. Kanakura, Transcriptional regulation of the cyclin D1 promoter by STAT5: its involvement in cytokine-dependent growth of hematopoietic cells, EMBO J. 18 (1999) 1367–1377.
[138] D.D. Deo, T.W. Axelrad, E.G. Robert, V. Marcheselli, N.G. Bazan, J.D. Hunt, Phosphorylation of STAT-3 in response to basic fibroblast growth factor occurs through a mechanism involving platelet-activating factor, JAK-2, and Src in human umbilical vein endothelial cells. Evidence for a dual kinase mechanism, J. Biol. Chem. 277 (2002) 21237–21245.
[139] K. Sibley, D. Cuthbert-Heavens, M.A. Knowles, Loss of heterozygosity at 4p16.3 and mutation of FGFR3 in transitional cell carcinoma, Oncogene 20 (2001) 686–691.