Review

Tyrosine kinase signalling in breast cancer Fibroblast growth factors and their receptors

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Received: 17 November 1999
Accepted: 21 February 2000
Published: 25 March 2000

Breast Cancer Res 2000, **2**:191–196

Abstract

The fibroblast growth factors [Fgfs (murine), FGFs (human)] constitute a large family of ligands that signal through a class of cell-surface tyrosine kinase receptors. Fgf signalling has been associated *in vitro* with cellular differentiation as well as mitogenic and motogenic responses. *In vivo*, Fgfs are critical for animal development, and some have potent angiogenic properties. Several Fgfs have been identified as oncogenes in murine mammary cancer, where their deregulation is associated with proviral insertions of the mouse mammary tumour virus (MMTV). Thus, in some mammary tumours of MMTV-infected mouse strains, integration of viral genomic DNA into the somatic DNA of mammary epithelial cells was found to have caused the inappropriate expression of members of this family of growth factors. Although examination of human breast cancers has shown an altered expression of FGFs or of their receptors in some tumours, their role in the causation of breast disease is unclear and remains controversial.

Keywords: breast cancer, fibroblast growth factor, mammary, receptor.

Introduction

There is a long history linking the inappropriate expression of Fgfs with breast cancer development. The evidence for their involvement in murine mammary cancer is strong, but in the human disease the evidence is weaker and relies heavily upon analogy with murine models to underpin the somewhat conflicting findings. Nevertheless, Fgfs show a multitude of properties *in vitro* that suggest that they have the potential to contribute to the induction, progression and metastasis of breast cancer. This short review provides an introduction to Fgfs, Fgf receptors, their role in murine mammary cancer and the evidence for their association with human breast cancer. The acronyms 'Fgf' and 'FGF' refer to the murine and human ligands, respectively.

Signalling through fibroblast growth factors and their receptors

In mammals, the Fgfs constitute a large family of about 20 structurally homologous ligands, which transduce signals through a class of cell-surface tyrosine kinase receptors (for review [1–4]). Most Fgfs are secreted polypeptides that typically have an amino-terminal signal sequence for export through the constitutive secretory pathway. Two notable exceptions are Fgf-1 and Fgf-2, however, which have a nuclear as well as a cytoplasmic localization and are secreted by novel but poorly understood mechanisms [5–10]. Fgfs also bind with a relatively high affinity to heparan sulphates, which in general are present as covalently linked side chains on cell-surface proteoglycans.

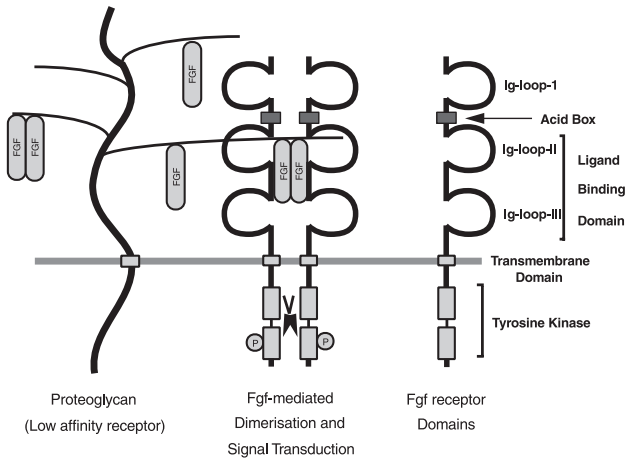
FGF/Fgf = fibroblast growth factor (human/murine); MMTV = mouse mammary tumour virus.

Thus, signal transduction requires the binding of Fgf to both heparan sulphate and Fgf receptor, to form a ternary signalling complex [11]. Because most cells have an abundance of proteoglycans on their surface, these cell-surface molecules also serve to limit the diffusion of secreted Fgfs to predominantly adjacent cells. Hence, these ligands function as important autocrine and paracrine signalling molecules.

The Fgf receptors are encoded by four genes (*Fgfr-1* to *Fgfr-4*), but because of alternative splicing of *Fgfr-1*, *Fgfr-2* and *Fgfr-3*, seven prototype receptors are generated [2,3]. Each prototype receptor has a different ligand-binding capacity and tissue distribution [12•,13–17]. The receptors are composed of an external part that consists of two or three immunoglobulin-like domains, and a transmembrane element that extends to a cytoplasmic tyrosine kinase (Fig. 1). The two membrane proximal immunoglobulin-like domains (loops 2 and 3) comprise the ligand-binding domain. Upon binding of the ligand, it appears that the Fgf receptor complexes dimerize, in conjunction with a heparan sulphate moiety, and the tyrosine kinase is activated through autophosphorylation [18••]. These events facilitate the binding of second messenger proteins, which in turn activate various intracellular signalling pathways (for review [4]). It should be noted, however, that additional alternative splicing, that does not alter the Fgf-binding domain, generates several other Fgf receptor forms that are assumed to serve some as yet undefined function. For example, it is common to find Fgf receptors with only the second and third immunoglobulin-like domains, which may or may not extend to the very acidic region (acid box) that lies between immunoglobulin loops 1 and 2 (see Fig. 1).

In culture, the cellular consequences of Fgf stimulation are quite varied. For example, many are broad-spectrum mitogens, and some induce cell motility, or alter the state of cellular differentiation (for review [1,3]). *In vivo*, some Fgfs have potent angiogenic properties, and others have been implicated in tissue remodeling, such as that required for wound repair [19]. The majority of Fgfs are expressed during embryonic development in precise, but often overlapping spatially and temporally restricted patterns [20,21]. Thus, it has become evident that the Fgfs have essential functions in many aspects of animal development, which range from myoblast migration in *Caenorhabolitis elegans* and tracheal formation in *Drosophila*, to inductive and patterning roles in formation of the mammalian limb [20–23]. Moreover, genetic linkage analysis has found that three Fgf receptor genes are the underlying cause of several human skeletal dysplasias and a number of autosomal-dominant craniosynostosis syndromes (for review [24]). Therefore, from their known properties and functions, it might be predicted that deregulation of this intercellular signalling system could contribute to other human pathologies, including the growth, survival and metastatic spread of tumours.

Figure 1



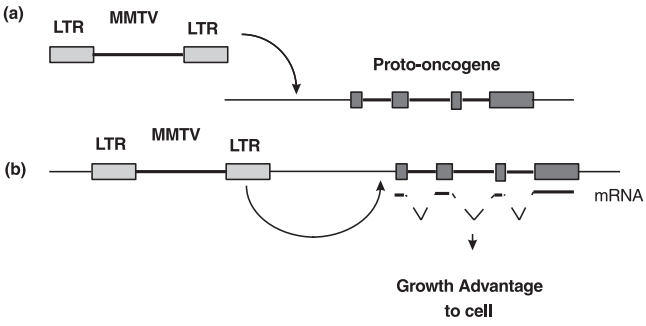
Fgf receptor structure and Fgf signalling. Structural domains of an Fgf receptor are shown on the right of the panel. Fgf signal transduction is initiated upon binding the Fgf ligand in conjunction with heparan sulphate to form a ternary complex. The result is autophosphorylation and activation of the tyrosine kinase, which facilitates second messenger signalling through phosphotyrosine-dependent and -independent interactions with the cytoplasmic portion of the receptor.

Identification of fibroblast growth factors as potent oncogenes for the mammary gland

The females of several inbred strains of mice show a very high incidence of mammary cancer. For most of these mouse strains, the major factor that predisposes the mice to mammary tumours is the presence of the MMTV. This retrovirus replicates primarily in the mammary epithelium, shedding its progeny into the milk of lactating mothers, so that the virus is acquired by their offspring as a congenital infection. Because retroviruses replicate through a DNA intermediate that integrates into the host cell genome, all retroviruses can be considered as insertional mutagens. The viral DNA appears to integrate in an essentially random manner, so that only on rare occasions does it cause a mutation that leads to a growth advantage for an infected cell, with the potential for it to ultimately progress to frank neoplasia (Fig. 2). Such events are likely to be extremely rare for any individually infected cell, but very large numbers of cells in the mouse mammary gland become infected, so most female mice will by chance eventually contain a cell that has acquired an oncogenic mutation.

A number of studies have shown that the integrated virus alters the cell phenotype by causing inappropriate transcriptional activation of an adjacent host gene. This usually occurs through the action of its potent transcriptional enhancer elements or by bringing the transcription of the

Figure 2



Proto-oncogene activation by insertional mutagenesis of MMTV. **(a)** Insertion of viral genomic DNA into somatic cellular DNA in close proximity of a silent oncogene. **(b)** Inserted proviral DNA induces the transcription of the oncogene.

host gene under the control of the viral promoter. These types of mutation are dominant in the heterozygous condition. An advantage of the MMTV model of mammary cancer is that the provirus remains at the mutation site, and thereby acts as a tag to identify the linked somatic gene that is contributing to tumour induction. Hence, proviruses that locate to the same locus in several independent tumours mark the proximity of the candidate oncogene. Detailed analysis of proviral integration sites has led to the identification of several virally activated proto-oncogenes, that include three members of the Fgf family: *Int-2*/*Fgf-3*, *hst-1*/*Fgf-4* and *Fgf-8* (Fig. 2, Table 1).

Historically, the first proto-oncogene to be identified from analysis of MMTV-induced tumours was *Int-1*/*Wnt-1*, which was later found to be a homologue of the *Drosophila* segmentation polarity gene *wingless* [25,26]. This was closely followed by the discovery of *Fgf-3* at a second distinct locus [27]. Subsequently, many tumours were found to have MMTV insertions at both *Wnt-1* and *Fgf-3* [28]. Because insertions are thought to be largely a chance event, the discovery in individual tumours of insertions at both loci suggested that there must be a strong selection for both genes in tumour induction. The potent oncogenic effect of Wnt-1 and Fgf-3 was substantiated when transgenic mice, constitutively expressing either gene, were observed to develop multiple mammary tumours earlier than the original inbred strains harbouring MMTV [29–32]. Moreover, when a transgenic line expressing Wnt-1 was infected with MMTV, the mammary tumours that arose in these mice were found to have viral insertions at *Fgf-3* or the adjacent *Fgf-4* or *Fgf-8* locus, but not in the *Wnt-1* or *Wnt-3* loci [33–35]. This provided additional evidence for co-operation between these two oncogene families in mammary tumorigenesis.

Table 1

Oncogenes identified as common targets for activation by MMTV in mouse mammary tumours

Proto-oncogenes activated by MMTV	References
Fgf-3 (*Int-2*)	Peters *et al* 1983 [27]
Fgf-4 (*hst-1*)	Peters *et al* 1989 [60]
Fgf-8	MacArthur *et al* 1995 [35]
Wnt-1 (*Int-1*)	Nusse and Varmus 1982 [25]
Wnt-3	Roelink *et al* 1990 [61]
Wnt-10b	Lee *et al* 1995 [58]
Notch-4 (*Int-3*)	Gallahan *et al* 1987 [59]

Fibroblast growth factors and their receptors in human breast cancer

The identification of Fgfs as oncogenes in murine mammary cancer prompted the examination of human breast tumours for alterations in the structure and expression of these loci. Interestingly, *Fgf-3* and *Fgf-4*, which are only a few kilobases apart on mouse chromosome 7, show synteny with human chromosome 11 band q13. Examination of the *FGF-3*/*FGF-4* locus by Southern blotting analysis showed that approximately 15% of human breast tumour DNA had readily detectable levels of somatic amplification in this region. Analysis of RNA, however, revealed that neither *FGF-3* or *FGF-4* were transcribed in the vast majority of these tumours. Subsequently, the cyclin D_1 gene (*CCND1*) was found to be closely linked to *FGF-3*/*FGF-4*, invariably forming part of the same amplicon. From studies carried out by several groups, there is now a consensus that *CCND1* is the important active oncogene in this region, and is therefore implicated in many human breast tumours (for review [36]). Thus, for the great majority of human breast tumours showing *FGF-3*/*FGF-4* amplification, these genes are merely passengers on the same amplicon as *CCND1* and are not implicated in the disease process.

The introduction of reverse transcription polymerase chain reaction procedures has led to the detection of a number of FGFs and their receptors in normal and malignant breast tissue [37]. These studies are not sufficient to unambiguously implicate FGFs or their receptors as major players in the development of breast cancers, but they are suggestive of some involvement. FGF-2, which has angiogenic properties, has been the most extensively investigated member of the FGF family. The majority of studies have been on small to modest numbers of tumours, and the results are often conflicting. For example, some reports indicate that an increased amount of FGF-2 can be found in tumours compared with in normal breast tissue [38,39•], whereas others find no difference [40] or

lower levels [41–43]. Some of these studies show an association between higher FGF-2 levels and a better prognosis, however [39•,40]. Interestingly, the study by Smith *et al* [39•] examined the relationship between FGF-2 levels and microvessel count, but found no evidence for an angiogenic effect of FGF-2. Immunohistochemistry shows that most FGF-2 in breast tumours is found in association with the stromal component, and little or none has been reported in the cancer cells [39•,44]. Similar studies that investigated the presence of FGF-1 have found it in normal and malignant breast tissue, and again it appears to be reduced in the cancer cells [37,45–47]. In contrast to the conspicuous absence in human breast cancer of the two mouse mammary oncogenes FGF-3 and FGF-4, the situation appears to be different for FGF-8. Both reverse transcription polymerase chain reaction and *in situ* hybridization analyses indicate that elevated levels of FGF-8 are associated with a small subset of malignant breast tumours [48,49].

There are also a few reports that, in some breast cancers, FGF receptor genes are amplified, with *FGFR1* (approximately 20%) and *FGFR4* (approximately 30%) both providing a significant number of cases [37,50,51]. In addition, elevated expression of FGF receptors was detected using ligand-binding studies with iodinated FGF-2 and immunolocalization with an antibody to FGFR1 [47,52]. At present, although there are some intriguing correlations between the expression of FGFs or their receptors in breast cancer, the evidence that they play a major role is by no means compelling. A recent study [53••], however, found that a significant proportion of bladder and cervical carcinomas harbour point mutations in *FGFR3* that are similar to those that underlie thanatophoric dysplasia, a rare but severe skeletal abnormality of newborn children. Analysis of the mutant receptors has shown that they have acquired ligand independent activity [54–56]. Activating mutations of *FGFR1*, *FGFR2* and *FGFR3* have also been found in some craniosynostosis syndromes (for review [24]). Hence, it will be important to determine whether similar somatic mutations occur in breast cancers, thereby contributing to deregulation of proliferation, differentiation or cell motility.

Conclusion

Studies to date clearly show that inappropriate Fgf signalling in the mouse mammary gland leads to hyperplastic growth and eventually to frank neoplasia. Although there is evidence that FGFs and their receptors can be aberrantly expressed in human breast cancers, the findings between groups are inconsistent and there is no overwhelming evidence pointing to a major role for these molecules in either growth stimulation, or as potentiators of angiogenesis. There are several reasons why the present data are conflicting. The sample sizes analyzed are generally small, whereas the variation within each group is quite large, thereby reducing confidence in the conclusions. In some cases the controls for the breast cancer group were benign tumour samples, whereas for others tissue from reduction mammoplasty was used, making direct comparisons between studies difficult.

Although there are at least 20 members of the FGF family, the majority of studies have concentrated on FGF-1 and FGF-2. Other members of the family are under investigation for their potential involvement in breast cancer, however. Indeed, the results for FGF-8 suggest that it may be an important cytokine in mammary cancer. There is also good evidence that *FGFR1* and *FGFR4* are amplified in a number of breast tumours. It is not clear, however, whether amplification of these receptors contributes to tumour development, because there is little information on the expression and activity of these receptors. As most gene amplifications extend over 1–2 Mb of DNA, they often encompass several genes. Thus, the identity of a potential oncogene cannot be established without a rigorous analysis of the amplicon.

The role of FGF signalling in breast cancer remains contentious. However, given the widespread occurrence of this signalling pathway, with its diverse biological effects, it would be surprising if it was not involved in at least a subset of breast tumours. Two observations support the likelihood of this: first, its well-established involvement in murine mammary cancer; and second, the recent finding that point mutations in *FGFR3* have been detected in bladder and cervical carcinomas. In breast tissue, FGFR2 appears to be important for normal mammary gland development [57], but as yet there is no documented evidence that activating point mutations of this or any other FGF receptor occurs in human breast tumours.

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