Review

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

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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 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 Caenorhabditis 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.

**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 insertion 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
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.

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.

References
Articles of particular interest have been highlighted as:
• of special interest
•• of outstanding interest

1. Basilico C, Moscatelli D: The FGF family of growth-factors and oncogenes. Adv Cancer Res 1992, 59:115–165.
2. Johnson D, Williams L: Structural and functional diversity in the FGF receptor multigene family. Adv Cancer Res 1993, 60:1–41.
3. McKeefan WL, Wang F, Kan M: The heparan-sulfate fibroblast growth-factor family: diversity of structure and function. Prog Nucleic Acid Res Mol Biol 1998, 59:135–176.
4. Klint P, Claesson-Welsh L: Signal transduction by fibroblast growth factor receptors. Frontiers Biosci 1999, 4:165–177.
5. Bugler B, Amalric F, Prats H: Alternative initiation of translation determines cytoplasmic or nuclear localization of basic fibroblast growth factor. Mol Cell Biol 1991, 11:573–577.
6. Renko M, Quarto N, Morimoto T, Rifkin D: Nuclear and cytoplasmic localization of different basic fibroblast growth factor species. J Cell Physiol 1990, 144:108–116.
7. LaVallee TM, Tarantini F, Gamble S, et al: Synaptotagmin-1 is required for fibroblast growth-factor-1 release. J Biol Chem 1998, 273:22217–22223.
12. Ornitz DM, Xu JS, Colvin JS, et al: Receptor specificity of the fibroblast growth-factor family. J Biol Chem 1996, 271:15292–15297.

13. Peters K, Werner S, Williams L: Two FGF receptor genes are differentially expressed in epithelial and mesenchymal tissues during limb formation and organogenesis in the mouse. Development 1992, 114:233–243.

14. Orr-Uttreyer A, Bedford M, Burakova T, et al: Developmental localization of the splicing alternatives of fibroblast growth-factor receptor-2 (FGFR2). Dev Biol 1993, 158:475–486.

15. Peters K, Ornitz D, Werner S, Williams L: Unique expression pattern of the fgf-receptor-3 gene during mouse organogenesis. Dev Biol 1993, 159:423–430.

16. Partanen J, Armstrong E, Makela TP, et al: A novel endothelial-cell surface-receptor tyrosine kinase with extracelular epidermal growth-factor homology domains. Mol Cell Biol 1992, 12:1698–1707.

17. Stark K, McMahon J, McMahon A: FGFR-4, a new member of the fibroblast growth factor receptor family, expressed in the definitive endoderm and skeletal muscle lineages of the mouse. Development 1991, 113:641–651.

18. Plontnikov A, Schlessinger J, Hubbard S, Mohammadi M: Structural basis of fgf receptor dimerization and activation. Cell 1999, 98:641–650. A structural analysis of Fgf binding to its receptor is presented, showing that two Fgf molecules bind two receptor elements in a configuration that may accommodate the known interaction with a heparan sulphate moiety. The paper also gives further insight into the parameters that control Fgf binding specificity.

19. Werner S, Smola H, Liao X, et al: The function of KGF in morphogenesis of epithelium and reepithelialization of wounds. Science 1994, 266:819–822.

20. Yamaguchi TP, Rossant J: Fibroblast growth-factors in mammalian development. Curr Opin Genet Dev 1995, 5:485–491.

21. Martin G: The roles of FGFS in the early development of vertebrate limbs. Genes Dev 1998, 12:1571–1586.

22. DeVore DL, Horvitz HR, Stern MJ: An FGF receptor signaling pathway is required for the normal-cell migrations of the sex myoblasts in C-elegans hermaphrodites. Cell 1995, 83:611–620.

23. Glazer L, Shilo B-Z: The Drosophila FGF-R homolog is expressed in the embryonic tracheal system and appears to be required for directed tracheal cell extension. Genes Dev 1991, 5:697–705.

24. DeMoorloze L, Dickson C: Skeletal disorders associated with fibroblast growth-factor receptor mutations. Curr Opin Genet Dev 1997, 7:378–385.

25. Nusse R, Varmus HE: Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. Cell 1982, 31:99–109.

26. Nusse R, Brown A, Papkoff J, et al: A new nomenclature for int-1 and related genes: the Wnt gene family. Cell 1991, 64:231–232.

27. Peters G, Brookes S, Smith R, Dickson C: Tumorigenesis by mouse mammary tumor virus: evidence for a common region for provirus integration in mammary tumors. Cell 1983, 33:369–377.

28. Peters G, Lee A, Dickson C: Concerted activation of two potential proto-oncogenes in carcinomas induced by mouse mammary tumour virus. Nature 1986, 320:628–631.

29. Stamp G, Fanti V, Poulsom R, et al: Nonuniform expression of a mouse mammary tumor virus-driven int-2/Fgf-3 transgene in pregnancy-responsive breast tumors. Cell Growth Differ 1992, 3:929–938.

30. Ornitz D, Cardill R, Kuo A, Leder P: Int-2, an autocrine and/or ultra-short-range effector in transgenic mammary tissue transplants. J Natl Cancer Inst 1992, 84:897–902.

31. Muller W, Lee F, Dickson C, et al: The int-2 gene product acts as an epithelial growth factor in transgenic mice. EMBO J 1990, 9:907–913.

32. Tsukamoto AS, Grosschedl R, Guzman RC, Parslow T, Varmus HE: Expression of the int-1 gene in transgenic mice is associated with mammary gland hyperplasia and adeno-carcinomas in male and female mice. Cell 1989, 58:619–625.

33. Kwan H, Pecenka V, Tsukamoto A, et al: Transgenes expressing the wtnt-1 and int-2 protooncogenes cooperate during mammary carcinogenesis in doubly transgenic mice. Mol Cell Biol 1992, 12:147–154.

34. Shackleford GM, MacArthur CA, Kwan HC, Varmus HE: Mouse mammary-tumor virus-infection accelerates mammary carcinogenesis in wtnt-1 transgenic mice by insertional activation of int-2/fgf-3 and hst/fgf-4. Proc Natl Acad Sci USA 1993, 90:740–744.

35. MacArthur CA, Shankar DB, Shackleford GM: FGF-8, activated by proviral insertion, cooperates with the wtnt-1 transgene in murine mammary tumorigenesis. J Virol 1995, 69:2501–2507.

36. Lamme GA, Peters G: Chromosome 11q13 abnormalities in human cancer. Cancer Cells 1991, 3:413–420.

37. Penault-Llorca F, Bertucci F, Adelaide J, et al: Expression of FGF and FGF receptor genes in human breast-cancer. Int J Cancer 1995, 61:170–176.

38. Reif M, Leleuene S, Scott P, et al: Expression of the angiogenic factors vascular endothelial cell growth factor, acidic and basic fibroblast growth factor, tumor growth factor β-1, platelet-derived endothelial cell growth factor, placenta growth factor, and pleiotrophin in human primary breast cancer and its relation to angiogenesis. Cancer Res 1997, 57:963–969.

39. Smith K, Fox SB, Whitehouse R, et al: Upregulation of basic fibroblast last growth factor in breast carcinoma and its relationship to vascular density, oestrogen receptor, epidermal growth factor receptor and survival. Ann Oncol 1999, 10:707–713. This is a recent study of FGF-2 expression in human breast tumours, showing its expression was restricted to the stroma, and also that there was no observable relationship to microvascular count or EGF receptor status.

40. Colomer R, Aparicio J, Monero S, et al: Low levels of basic fibroblast growth factor (bFGF) are associated with a poor prognosis in human breast carcinoma. Br J Cancer 1997, 76:1215–1220.

41. Anandappa SY, Winstanley JHR, Leinster S, et al: Comparative expression of fibroblast growth-factor messenger-RNAs in benign and malignant breast disease. Br J Cancer 1994, 69:772–776.

42. Luqmani Y, Graham M, Coombs R: Expression of basic fibroblast growth factor, FGR1 and FGR2 in normal and malignant human breast, and comparison with other normal tissues. Br J Cancer 1992, 66:271–290.
mutations of FGFR3 in human bladder and cervix carcinoma. Br J Cancer 1997, 76:1621–1630.

50. Marsh SK, Bansal GS, Zammit C, et al: Increased expression of fibroblast growth factor 8 in human breast cancer. Oncogene 1999, 18:1053–1060.

51. Tanaka A, Furuya A, Yamasaki M, et al: High-frequency of fibroblast-growth-factor (FGF)-8 expression in clinical prostate cancers and breast tissues, immunohistochemically demonstrated by a newly established neutralizing monoclonal-antibody against FGF-8. Cancer Res 1998, 58:2053–2056.

52. Theillet C, Adelaide J, Louason G, et al: FGFR1 and PLAT genes and DNA amplification at 8p12 in breast and ovarian cancers. Genes Chromosomes Cancer 1993, 7:219–226.

53. Linder C, Bystom P, Engel G, et al: Correlation between basic fibroblast growth factor immunostaining of stromal cells and stromelysin-3 mRNA expression in human breast carcinoma. Br J Cancer 1998, 77:841–945.

54. Smith J, Yelland A, Baillie R, Coombes RC: Constitutive activation of fibroblast growth-factor receptors by point mutations in the extracellular, transmembrane, and kinase domains. J Biol Chem 1996, 271:25049–25057.

55. Webster MK, Donoghue DJ: Constitutive activation of fibroblast growth-factor receptor-3 by the transmembrane domain point mutation found in achondroplasia. EMBO J 1996, 15:320–327.

56. Jackson D, Bresnick J, Rosewell I, et al: Constitutive activation of fibroblast growth-factor receptor signaling has a role in lobuloalveolar development of the mammary-gland. J Cell Sci 1997, 110:1261–1268.

57. Lee FS, Lane TF, Kuo A, Shackleford GM, Leder P: Insertional mutagenesis identifies a member of the wnt gene family as a candidate oncogene in the mammary epithelium of int-2/FGF-3 transgenic mice. Proc Natl Acad Sci USA 1995, 92:2269–2272.

58. Gallahan D, Kozak C, Callahan R: A new common integration region (int-3) for mouse mammary tumor virus on mouse chromosome 17. J Virol 1987, 61:218–220.