Cancer genomics and genetics of FGFR2 (Review)

MASARU KATOH

Genetics and Cell Biology Section, National Cancer Center Research Institute, Tokyo 104-0045, Japan

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Abstract. FGFR2 gene encodes FGFR2b in epithelial cells, and FGFR2c in mesenchymal cells. FGFR2b is a high affinity receptor for FGF1, FGF3, FGF7, FGF10 and FGF22, while FGFR2c for FGF1, FGF2, FGF4, FGF6, FGF9, FGF16 and FGF20. Here genomics and genetics of FGFR2, and therapeutics targeted to FGFR2 will be reviewed. Single nucleotide polymorphisms (SNPs) of FGFR2 are associated with increased risk of breast cancer. Gene amplification or missense mutation of FGFR2 occurs in gastric cancer, lung cancer, breast cancer, ovarian cancer, and endometrial cancer. Genetic alterations of FGFR2 induce aberrant FGFR2 signaling activation due to release of FGFR2 from autoinhibition, or creation of FGF signaling autocrine loop. Class switch of FGFR2b to FGFR2c is associated with more malignant phenotype. FGF and canonical WNT signals synergize during mammary carcinogenesis, but counteract during osteogenesis and adipogenesis. Among PD173074, SU5402, and AZD2171 functioning as FGFR inhibitors, AZD2171 is the most promising anti-cancer drug. Cancer genomics and genetics are utilized to predict cancer-driving pathway for therapeutic optimization. FGFR2ome is defined as a complete data set of SNP, copy number variation (CNV), missense mutation, gene amplification, and predominant isoform of FGFR2. FGFR2ome analyses in patients with several tumor types among various populations should be carried out to establish integrative database of FGFR2 for the rational clinical application of FGFR2-targeted cancer therapy.

1. Introduction

FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, FGF7 (KGF), FGF8, FGF9, FGF10, FGF16, FGF17, FGF18, FGF19, FGF20, FGF21, FGF22, and FGF23 are secreted proteins belonging to the fibroblast growth factor (FGF) family (1,2). Mouse and rat Fgf19 are designated Fgf15 due to the lineage specific protein diversification of FGF19 orthologs in rodents (3). FGF signals regulate a variety of cellular processes during embryogenesis, adult tissue homeostasis, and carcinogenesis (4-7).

FGFR1, FGFR2, FGFR3 and FGFR4 are FGF receptors (FGFRs) with extracellular immunoglobulin-like (Ig-like) domains and cytoplasmic tyrosine kinase domain. Secreted FGFs are associated with heparan sulfate proteoglycans for the formation of high affinity complex with FGFRs. Ligand-dependent dimerization of FGFRs induces autophosphorylation of tyrosine residues to release FGFRs from autoinhibition. FRS2 and FRS3 are PTB-domain docking proteins binding to the juxtamembrane domain of FGF receptors. FRS2 and FRS3 are tyrosine phosphorylated by FGFRs to recruit GRB2 and SHP2 for the activation of MAPK and PI3K signaling cascades (6). PLCγ is recruited to phosphotyrosine residues on the C-terminal tail of activated FGFRs to catalyze PIP2 to DAG and IP3. DAG activates protein kinase C (PKC) signaling cascade, while IP3 induces Ca2+ release from endoplasmic reticulum for the following activation of Calmodulin-Calcineurin-NFAT signaling cascade.

Structure and alternative splicing of FGFR2 gene, FGFR2 signaling during embryogenesis and adult tissue homeostasis, cross-talk of FGFR2 signaling with other signaling cascades, genomics and genetics of FGFR2 in human cancer, and FGFR2-targeted therapeutics will be described in this review.

2. Structure and alternative splicing of FGFR2 gene

Fgfr2 IIIb (Fgfr2b) and Fgfr2 IIc (Fgfr2c) are FGFR2 isoforms, which are almost identical except the latter half of the third Ig-like domain (8-10). Human FGFR2 gene at human chromosome 10q26 consists of 21 exons (Fig. 1A and B). Fgfr2b isoform consists of exons 1-6, 8, 9, 11-19, and 21, while Fgfr2c isoform consists of exons 1-6, 8, 10-19, and 21 (Fig. 1C). Exons 9 and 10, corresponding to the latter half of the third Ig-like domain, are used in FGFR2b and FGFR2c,
respectively (11). Splicing silencer and activator sequences within intron 8 is implicated in the regulation of exon 9 splicing (12). FGFR2 gene encodes FGFR2b and FGFR2c isoforms due to alternative splicing of mutually exclusive exons. FGFR2b isoform is predominantly expressed in epithelial cells, while FGFR2c isoform preferentially in mesenchymal cells. Ornitz et al. systemically investigated affinities of FGFS toward each FGFR isoform. FGFR2b is a high affinity receptor for FGF1, FGF3, FGF7, FGF10 and FGF22, while FGFR2c for FGF1, FGF2, FGF4, FGF6, FGF9, FGF16 and FGF20 (13,14).

3. FGFR2 signaling during embryogenesis and adult tissue homeostasis

FGF7 is secreted from various types of mesenchymal cells, such as fibroblasts, smooth muscle cells, endothelial cells, skin dermis, and γδ T cells (15,16). FGF7 transcription is up-regulated in mesenchymal cells by PDGF derived from platelets, and by IL-1, IL-1β and TNF-α derived from polymorphonuclear leukocytes or macrophages (17). Because FGFR2b is expressed on gastrointestinal epithelium, mammary gland epithelium, urothelium, and epidermal keratinocytes.
(18), FGF7-FGFR2b signaling is implicated in wound healing and mucosal repair of adult tissues.

FGf10 is secreted from mesenchymal cells during embryogenesis. Fgf10 activates Fgfr2b signaling in lung buds for epithelial migration toward Fgf10 producing mesenchymal cells through upregulation of Tgsp, Numb, Bmpr1a, Ctnnb1, Tacc2, Tm4sf3, Vnn1, and Myh7 (19,20). Fgf10 activates Fgfr2b signaling in developing glandular stomach accompanied by transcriptional upregulation of Gata4, Gata6, and Ihh (21), and in cecal bud to induce epithelial invasion into cecal mesenchymal tissue (22). Fgf10 also activates Fgfr2b signaling in surface ectoderm to initiate outgrowth of limb bud, and to generate apical ectodermal ridge for the limb formation (23). Fgf10 is secreted from mesoderm-derived cells to activate Fgfr2b signaling in adjacent endoderm- or ectoderm-derived cells during embryogenesis. Fgf10-Fgfr2b signaling is implicated in morphogenesis of embryonic tissues, such as lung, stomach, pancreas, eccum, limb, thymus, thyroid, pituitary gland, salivary gland, inner ear, teeth and skin.

Fgf22 is secreted from cerebellar granule cells to recruit axons projected from pontine and vestibular neurons expressing Fgfr2b (24). Fgf22-Fgfr2b signaling organizes synapse formation by inducing axon projection toward Fgf22 secreting cells.

FGFR2 gene is mutated in congenital skeletal abnormalities, such as Crouzon syndrome, Jackson-Weiss syndrome, Apert syndrome, Pfeiffer syndrome, and Beare-Stevenson syndrome (5,25-27). Missense mutations of FGFR2 gene are clustered to the hinge region of second and third Ig-like domains, the third Ig-like domain of FGFR2c type, and the tyrosine kinase domain (Fig. 1D). FGFR2 missense mutations within the hinge region and the third Ig-like domain alter ligand-receptor specificity of FGFR2, while those within the tyrosine kinase domain induce ligand independent FGFR2 activation.

Paznekas et al reported loss-of-function mutations of TWIST1/TWIST or gain-of-function mutations of FGFR2 or FGFR3 in Saethre-Chotzen syndrome, which is featured by craniosynostosis and limb anomalies (28). TWIST1 is a tissue specific transcription factor with basic helix-loop-helix (bHLH) domain binding to E-box within the promoter region of FGFR2 gene (29). TWIST1 homodimer induces FGFR2 upregulation, while heterodimer of TWIST1 and E2A-related proteins does not. TWIST1 regulates the expression of FGFR2 in a context-dependent manner based on the expression level of E2A-related proteins.

4. Cross-talk with other signaling cascades

WNT family members are secreted glycoproteins playing key roles during embryogenesis and carcinogenesis (1,30-32). WNT signals are transduced to canonical and non-canonical signaling cascades in a context-dependent manner (33). Mammary carcinogenesis in MMTV-Wnt/1 transgenic mice is accelerated by MMTV integration around Fgf3-Fgf4 or Fgf8 loci, and that in MMTV-Fgf3 transgenic mice by MMTV integration around Wnt1-Wnt10b locus (34,35). WNT10B signaling promotes osteoblast differentiation to inhibit adipocyte differentiation, while FGF10-FGFR2 signaling inhibits osteoblast differentiation to promote adipocyte differentiation (36). Although FGF signals counteract canonical WNT signals during osteogenesis and adipogenesis, FGF and canonical WNT signals synergize during carcinogenesis (37,38).

Notch signals are implicated in the regulation of stem cell and progenitor cell populations through transcriptional activation of HES1, HES5, HEY1, HEY2 and HEYL genes (39-42). Fg signaling activation is followed by Notch and Wnt signaling activation during mouse somitogenesis, and then Notch signaling activation induces Hes1 expression to repress an Fgf inhibitor Dusp4 for the Fgf signaling re-activation (43,44). Fgfr2 and Notch ligand Jag2 are induced together by TP63 isoform ΔNp63 during thymicroc development (45). Fg10-Fgfr2b signaling in the developing gastric gland induces Hes1 upregulation, although the precise mechanism remains unclear (46). Cross-talk of FGFR2 and Notch signaling cascades plays a key role during embryogenesis.

The balance among FGF, WNT, Notch, and Hedgehog signaling networks is important for the maintenance of homeostasis among stem and progenitor cells. Disruption of the stem cell signaling network results in pathological conditions, such as congenital diseases and cancer (47).

5. Genomics and genetics of FGFR2 in cancer

Easton et al (48) and Hunter et al (49) reported the association between single nucleotide polymorphisms (SNPs) of FGFR2 gene and increased risk of breast cancer in a genome-wide association study, and Huijts et al confirmed the association by using candidate-gene approach (50). Five SNPs associated with breast cancer susceptibility are located within intron 2 of FGFR2 gene (Fig. 1B), however, the mechanism of mammary carcinogenesis in patients with those SNPs remains to be elucidated.

Missense mutation of FGFR2 gene occurs in breast cancer, gastric cancer, lung cancer, ovarian cancer, and endometrial uterine cancer (Fig. 1D). S267P mutation in gastric cancer, D283N and W920C mutations in lung cancer, S252W, K310R, A315T, S372C, and Y375C mutations in uterus cancer, and G272V mutation in ovarian cancer are clustered around the hinge region and the third Ig-like domain of FGFR2 (51-53). I457V, N495K, and K659E mutations in endometrial uterine cancer are clustered within the kinase domain of FGFR2 (53). Missense mutations of FGFR2 around the third Ig-like domain induce oncogenic FGFR2 activation due to the altered ligand-receptor specificity creating FGF autocrine loop, while those within the tyrosine kinase domain induce ligand independent FGFR2 activation due to the acquisition of ligand independency.

Gene amplification and overexpression of FGFR2 occurs in human breast cancer and gastric cancer (54,55). Exons 20 and 21 of FGFR2 gene are alternative last exons encoding the C-terminal region of FGFR2 isoforms. Wild-type FGFR2 transcripts with exon 21 are expressed in normal cells and most tumor cells, while aberrant FGFR2 transcripts with exon 20 are overexpressed in cases with FGFR2 gene amplification due to the exclusion of exon 21 from the FGFR2 amplicon (11). Y769, Y779, Y783, Y805, and Y812 of wild-type FGFR2 isoforms are lost in C-terminally deleted FGFR2 isoforms. Phosphorylated Y769 is the binding site for PLCγ and SHB adaptor molecule. Wild-type FGFR2b transiently phosphorylates FRS2 in a ligand-dependent manner, while C-terminally
deleted FGFR2b constitutively phosphorylates FRS2 in a ligand-independent manner (56). FGFR2 gene amplification leads to more malignant phenotype of tumors due to the acquisition of ligand independency as well as the alteration of quality and quantity of downstream signaling.

Class switch of FGFR2b to FGFR2c occurs during progression of prostate cancer and bladder cancer (7,57). Prostate cancer and bladder cancer with decreased FGFR2b show poorer prognosis due to increased potential for invasion and metastasis. Re-expression of FGFR2b in prostate and bladder cancer cell lines results in decreased proliferation in vitro, and decreased tumorigenicity in vivo (58,59). Although the precise mechanism remains unclear, FGFR2 class switch during multi-stage carcinogenesis results in more malignant phenotype due to the altered ligand-receptor specificity creating the FGF autocrine loop.

6. Therapeutics targeted to FGFR

Protein kinases share the catalytic domain with conserved amino-acid sequence, and similar three-dimensional structure (60), and ATP mimetic compounds fitting into the ATP-binding pockets of target protein kinases have been searched for with the high-throughput technologies in the post-genome era (61,62). Protein kinases are now the second most popular class of drug target after G protein-coupled receptors (63).

PD173074 with the pyrido[2,3-d]pyrimidine core inhibits FGFR1 tyrosine kinase activity with IC50 value of 0.02 μM (64). SU5402 with the indolin-2-one core inhibits VEGFR2, PDGFRβ and FGFR1 tyrosine kinase activity with IC50 values of 0.02 μM, 0.51 μM and 0.03 μM, respectively (65). AZD2171 inhibits VEGFR2, PDGFRβ and FGFR1 tyrosine kinase activity with IC50 values of <0.001 μM, 0.005 μM, and 0.026 μM, respectively (66). PD173074 and SU5402 are targeted toward relatively narrow range of tyrosine kinases, while AZD2171 toward broad range of receptor tyrosine kinases. Small-molecule compounds specifically inhibiting the target kinase were previously searched for based on the hypothesis that narrow target window might reduce unexpected side effects; however, tumor cells resistant to the kinase inhibitor usually appear after the targeted molecular therapy due to the robustness of tumors. Therefore, small-molecule compounds with multiple targets have been recently developed to overcome the recurrence of drug resistant tumors (68,69).

In addition to small-molecule compound and monoclonal antibody, emerging technologies, such as RNA aptamer, peptide mimetic and synthetic miRNA, will be utilized for cancer therapeutics (67).

7. Perspectives

Cancer patients respond to treatment individually due to the divergences of tumor subtype and individual genotype. Cancer genomics and genetics are utilized to predict cancer-driving pathway for therapeutic optimization (68). Personalized medicine prescribing pathway-targeted therapeutics to cancer patients based on tumor subtyping and individual genotyping is believed to improve prognosis as well as quality of life (69,70).

Epigenomic data, genome sequence data, SNP data, copy number variation (CNV) data, and transcriptome microarray data are generated in the post-genome era by using high-throughput technologies, such as next generation sequencing, whole genome tiling array, and transcriptome microarray (68). These omics data are combined with clinical records to establish integrative databases in Europe, USA, and other regions to promote translational research in various fields (71,72).

‘FGFR2ome’ is here defined as a complete data set of SNP and CNV of FGFR2 in individual genome, missense mutation and gene amplification of FGFR2 in a primary tumor, and expression of FGFR2 isoforms with or without C-terminal deletion in a primary tumor. It is necessary to investigate FGFR2ome of patients with a variety of tumors, such as breast cancer, gastric cancer, ovarian cancer, endometrial cancer, and prostate cancer. Due to genetic diversity among populations, it is also necessary to investigate FGFR2ome in several populations in the world. Therefore, FGFR2ome analyses in patients with several tumor types in various populations should be carried out to establish integrative database of FGFR2 for the rational clinical application of FGFR2-targeted cancer therapy.

References

12. Carstens RP, Wagner EJ and Garcia-Blanco MA: An intronic splicing silencer causes skipping of the IIIb exon of FGFR2b constitutively phosphorylates FRS2 in a primary tumor. It is necessary to investigate FGFR2ome of patients with a variety of tumors, such as breast cancer, gastric cancer, ovarian cancer, endometrial cancer, and prostate cancer. Due to genetic diversity among populations, it is also necessary to investigate FGFR2ome in several populations in the world. Therefore, FGFR2ome analyses in patients with several tumor types in various populations should be carried out to establish integrative database of FGFR2 for the rational clinical application of FGFR2-targeted cancer therapy.


