Mechanism of Oncogenic Signal Activation by the
Novel Fusion Kinase FGFR3–BAIAP2L1

Yoshito Nakanishi, Nukinori Akiyama, Toshiyuki Tsukaguchi, Toshihiko Fujii, Yasuko Satoh, Nobuya Ishii, and Masahiro Aoki

Abstract

Recent cancer genome profiling studies have identified many novel genetic alterations, including rearrangements of genes encoding FGFR family members. However, most fusion genes are not functionally characterized, and their potentials in targeted therapy are unclear. We investigated a recently discovered gene fusion between FGFR3 and BAI1-associated protein 2-like 1 (BAIAP2L1). We identified 4 patients with bladder cancer and 2 patients with lung cancer harboring the FGFR3–BAIAP2L1 transfectant with Rat-2 fibroblast cells (Rat-2_F3-B). The FGFR3–BAIAP2L1 fusion had transforming activity in Rat2 cells, and Rat-2_F3-B cells were highly tumorigenic in mice. Rat-2_F3-B cells showed in vitro and in vivo sensitivity in the selective FGFR inhibitor CH5183284/Debio 1347, indicating that FGFR3 kinase activity is critical for tumorigenesis. Gene signature analysis revealed that FGFR3–BAIAP2L1 activates growth signals, such as the MAPK pathway, and inhibits tumor-suppressive signals, such as the p53, RB1, and CDKN2A pathways. We also established Rat-2_F3-B–BAR cells expressing an FGFR3–BAIAP2L1 variant lacking the Bin–Amphiphysin–Rvs (BAR) dimerization domain of BAIAP2L1, which exhibited decreased tumorigenic activity, FGFR3 phosphorylation, and F3-B–BAR dimerization, compared with Rat-2_F3-B cells. Collectively, these data suggest that constitutive dimerization through the BAR domain promotes constitutive FGFR3 kinase activation and is essential for its potent oncogenic activity. Mol Cancer Ther; 14(3); 704–12. ©2015 AACR.

Introduction

Chromosomal translocations/rearrangements are major drivers of tumorigenesis. Since the discovery of the BCR–ABL gene fusion in chronic myelogenous leukemia, several fusion kinases have been identified in hematologic and epithelial malignancies, including anaplastic lymphoma kinase (ALK) fusions, RET fusions, and ROS1 fusions (1–3). Generally, the partner proteins of fusion kinases possess dimerization domains, which promote kinase domain dimerization and constitutive activation. Several small-molecule inhibitors have been developed to treat patients with cancer with oncogenic fusion kinases. For instance, the ALK inhibitors crizotinib or alectinib have demonstrated excellent activity against cancers harboring EML4–ALK gene fusions (4, 5).

Normally, the FGFR receptor tyrosine kinase is transiently activated FGF ligand–mediated homo/heterodimerization. The FGFR signaling pathway is constitutively activated by genetic alterations, such as gene amplifications, point mutations, or chromosomal translocations/rearrangements, which promote cell growth, angiogenesis, cell migration, invasion, and metastasis (6). FGR1 amplification is a key genetic alteration in squamous cell lung carcinoma and hormone receptor–positive breast cancer (7, 8), whereas FGFR2 is amplified in gastric cancer (9). FGFR2 and FGFR3 point mutations are mainly observed in endometrial cancer and bladder cancer, respectively (10, 11). Since the first reports of FGFR1 and FGFR3 gene fusions in hematologic malignancies (12, 13), several novel chromosomal translocations/rearrangements of FGFRs have been discovered in patients with glioblastoma, bladder cancer, breast cancer, and cholangiocarcinoma by next-generation sequencing (NGS) technology (14–17).

A well-characterized gene fusion occurs between FGFR3 and the transforming acidic coiled-coil containing protein 3 (TACC3) gene. TACC3 contains a coiled-coil domain and exerts ligand-independent activation upon dimerization (17). The constitutively activated FGFR3–TACC3 protein can promote ERK and STAT3 signaling (16, 18). miR-99a targets the 3′-untranslated region (UTR) of FGFR3 to suppress FGFR3 expression in normal tissues. However, because the FGFR3 fusion loses its 3′-UTR, the fusion protein is highly expressed (19).

The FGFR3–BAI1-associated protein 2-like 1 (BAIAP2L1) fusion gene has not been identified in clinical tumor samples. Although FGFR3–BAIAP2L1 dimerization and increased ERK and STAT1 phosphorylation in FGFR3–BAIAP2L1-positive cells have been reported (18), the mechanisms of constitutive activation and associated signaling pathways are unclear. Therefore, we assessed the prevalence of the FGFR3–BAIAP2L1 fusion gene in clinical samples, studied its tumorigenic activity in vitro and in vivo, and investigated its signaling pathway. BAIAP2L1 has a Bin–Amphiphysin–Rvs (BAR) domain, which is the most conserved feature in...
amphiphysins. The BAR domain forms a crescent-shaped dimer that preferentially binds highly curved, negatively charged membranes (20). In the FGFR3–BAIAP2L1 fusion, the BAIAP2L1 fragment, including the BAR domain, is fused with the C-terminal domain of FGFR3, and therefore FGFR3–BAIAP2L1 retains the entire kinase domain of FGFR3. To clarify the role of BAR domain in the FGFR3–BAIAP2L1 fusion kinase, we compared the tumorigenic and dimerization activities of FGFR3–BAIAP2L1 and FGFR3–BAIAP2L1 lacking the BAR domain.

Materials and Methods

Reagents and cell lines

A 1-(1H-benzimidazol-5-yl)-5-aminopyrazole derivative, CH5183284/Debio 1347, was synthesized at Chugai Pharmaceutical Co., Ltd., as described previously (21). SW780, RT4, 3T3, 293, HT1376, and HCT116 were purchased from the American Type Culture Collection. All cell lines were obtained from Health Protection Agency Culture Collections. All cell lines were maintained in pathogen-free conditions. Stably transduced Rat-2 cells were prepared as described previously (21).

PCR, Sanger sequencing of FGFR3–BAIAP2L1 gene fusions, and break-apart FISH assays

Sequencing of clinical samples was conducted under Institutional Review Board–approved protocols conducted at Chugai Pharmaceutical Co., Ltd. cDNAs from clinical samples derived from patients with bladder cancer (n = 46), lung cancer (n = 83), head and neck cancer (n = 17), and gastroesophageal cancer (n = 18) were obtained from OriGene Technologies, Inc. or TriStar Technology Group, LLC. The information of primers of PCR is available in Supplementary Materials and Methods. For FGFR3 break-apart FISH experiments, 4-μm-thick formalin-fixed, paraﬁn-embedded sections were deparafﬁnized and treated with Pretreatment Reagent (Abbott) for 10 minutes at 95°C. Pepsin solution (ZytoVision) was added and samples were incubated at 37°C for 10 minutes. FGFR3 gene rearrangements were detected with the FGFR3 Split Dual Color FISH probe (GSP Lab., Inc.). Slides and probes were denatured simultaneously at 75°C for 10 minutes, followed by hybridization at 37°C overnight with Thermoflourite (Abbott Molecular, Inc.). Slides were washed with 0.3% NP-40/2x saline-sodium citrate at 72°C for 2 minutes and stained with DAPI (Life Technologies).

 Colony formation assays, spheroid formation assays, and xenograft transplantation studies

Rat-2 F3-B cells treated for 24 hours with either 0.1% DMSO or 1 μM CH5183284/Debio 1347 concentrations of 0.003 to 20,000 nmol/L and incubated at 37°C for 4 days. Subsequently, cell viabilities were measured using the CellTiter-Glo Luminescent Cell Viability Assay Kit. For in vivo efficacy studies, cells were implanted into mice as described above. After tumors reached approximately 200 to 300 mm³, animals were randomized into groups (4–5/group) and received oral CH5183284/Debio 1347 administration once per day.

Western blot analysis

Cells were treated with CH5183284/Debio 1347 or a solvent control (0.1% dimethyl sulfoxide; DMSO) for 2 hours and lysed in Cell Lysis Buffer (Cell Signaling Technology) containing protease and phosphatase inhibitors. For animal studies, xenograft tumors were homogenized using a BioMasher (K.K. Ashisuto) before lysis. Cell lysates were denatured with Sample Buffer Solution with Reducing Reagent for SDS-PAGE (Life Technologies) and resolved on precast 10% or 5% to 20% SDS-PAGE gels (Wako Pure Chemical Industries, Ltd.). After electrophoresis, Western blot analysis was performed as described (23). Antibody references are available in Supplementary Materials and Methods.

RNA-Seq and expression analysis

Cellular RNA was extracted using the RNeasy Mini Kit (Qiagen, Inc.). Quality assessment, poly-A selection, and sequencing with a HiSeq 2000 Sequencing System (Illumina) were performed by Macrogen, Inc. Cellular RNA samples were prepared for sequencing using a TruSeq RNA Sample Preparation kit (Illumina) to generate an mRNA library, and 100 bases were sequenced from both ends of the library. RSEM software was used to align reads against RefSeq transcripts and calculate expression values for each gene (24). Fold changes in expression levels were calculated to identify downregulated genes (<80% expression) and upregulated genes (>120% expression), relative to Rat-2 mock cells and other cell lines. We also puriﬁed and sequenced total RNA from Rat-2_F3-B cells treated for 24 hours with either 0.1% DMSO or 1 μmol/L CH5183284/Debio 1347. Fold changes were calculated by normalizing gene expression levels in CH5183284/Debio 1347–treated cells to DMSO control cells, identifying suppressed (<50% expression) or induced genes (>200% expression), relative to DMSO controls.

Phosphorylation levels and dimerization activities of FGFR3 constructs

cDNAs encoding wild-type (WT) FGFR3, F3-B, F3-B-ABAR, BAIAP2L1, F3-B lacking the SH domain (aa 342–401; F3-B-ASH), F3-B lacking the SH and BAR domains (F3-B-ABAR/ASH), and kinase dead (K508M) F3-B (F3-B-KD) were inserted into the entire kinase domain of FGFR3. To clarify the role of BAR domain in the FGFR3–BAIAP2L1 fusion kinase, we compared the tumorigenic and dimerization activities of FGFR3–BAIAP2L1 and FGFR3–BAIAP2L1 lacking the BAR domain.

In vitro and in vivo efficacy studies

Cell lines were seeded in 96-well plates (Sumilon CellLight Spheroid 96L1 in medium containing final CH5183284/Debio 1347 concentrations of 0.003 to 20,000 nmol/L and incubated at 37°C for 4 days. Subsequently, cell viabilities were measured using the CellTiter-Glo Luminescent Cell Viability Assay Kit. For in vivo efficacy studies, cells were implanted into mice as described above. After tumors reached approximately 200 to 300 mm³, animals were randomized into groups (4–5/group) and received oral CH5183284/Debio 1347 administration once per day.

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the pCXND3 vector (Kaketsuken) and used to transfect 293 cells. At 72 hours after transfection, cells were lysed in Cell Lysis Buffer (Cell Signaling Technology). Lysates were then studied by Western blot analysis. In separate experiments, 293 cells were transfected with the FLAG-tagged or Myc-tagged expression construct alone or in combination, using the FuGene HD reagent (Promega). At 3 days after transfection, cells were lysed and immunoprecipitation was performed with Anti-FLAG M2 Affinity Gel (Sigma-Aldrich). Precipitates were washed 10 times with Cell Lysis Buffer and eluted at 95°C for 5 minutes with Reducing Reagent for SDS-PAGE (Life Technologies).

Results

Identification of patients harboring FGFR3 rearrangements

Although the FGFR3–BAIAP2L1 fusion gene was identified in a cell line (17), it has not yet been discovered in clinical tumor samples. Therefore, we established PCR and break-apart FISH assays to screen clinical samples for the FGFR3–BAIAP2L1 gene fusion. We detected the FGFR3–BAIAP2L1 fusion by PCR in 1 of 28 patients with lung adenocarcinoma, 1 of 28 patients with squamous cell lung carcinoma, and 2 of 46 patients with bladder cancer (Table 1) and confirmed the junction sequences by Sanger sequencing (Fig. 1A). The clinicopathologic characteristics are available (Supplementary Table S1), and so far, there was no clear association with FGFR fusions. Although the proximity of FGFR3 and TACC3 in the human genome is too close to discern FGFR3–TACC3 gene fusions in break-apart assays (approximately 70 kb; Supplementary Fig. S1), we could detect split FGFR3 signals in SW780 cells harboring the FGFR3–BAIAP2L1 rearrangement [t(4:7); Fig. 1B]. Thus, we performed a prevalence study for FGFR3–BAIAP2L1 rearrangements in bladder cancer specimens and found that 2 of 89 patients with bladder cancer harbored this rearrangement (Fig. 1C).

Oncogenic activity of the FGFR3–BAIAP2L1 fusion kinase in normal rat fibroblast cells

To determine whether the F3-B fusion promotes oncogenic activity, we generated Rat-2 cell line clones stably transduced with an empty lentiviral vector (Rat-2_mock), or lentiviral vectors expressing FGFR3 (Rat-2_FGFR3), F3-B (Rat-2_F3-B), or BAIAP2L1 (Rat-2_BAIAP2L1). Consistent with previous reports (17, 18), we observed a spindle-type cellular morphology in Rat-2_F3-B cells, but not in other transductants (Supplementary Fig. S2A). We also observed ligand-independent FGFR3–BAIAP2L1 phosphorylation in Rat-2_F3-B cells (Supplementary Fig. S2B). Furthermore, Rat-2_F3-B cells showed an anchorage-independent growth in a soft agar assay and in a spheroid formation assay (Fig. 2A and B). FGFR3–BAIAP2L1-expressing 3T3 cells showed similar behaviors in several assays (Supplementary Fig. S2C–S2E). To evaluate the tumorigenic potential of the gene fusion in mice, we inoculated nude mice with Rat-2_mock, Rat-2_FGFR3, Rat-2_F3-B, and Rat-2_BAIAP2L1 cells and measured

Table 1. Prevalence of FGFR3–BAIAP2L1 rearrangements in clinical samples

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>n (%)</th>
</tr>
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<tbody>
<tr>
<td>Lung cancer</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma (n = 28)</td>
<td>1 (3.6)</td>
</tr>
<tr>
<td>Squamous cell (n = 28)</td>
<td>1 (3.6)</td>
</tr>
<tr>
<td>Others (n = 27)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Bladder cancer (n = 46)</td>
<td>2 (4.3)</td>
</tr>
<tr>
<td>Head and neck cancer (n = 17)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Gastroesophageal cancer (n = 18)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

![Figure 1](https://example.com/figure1)

Detection of FGFR3–BAIAP2L1 rearrangements in clinical samples. A, amplified fragments were sequenced by Sanger sequencing. Representative sequence waveforms are shown. The top image shows the waveform of the FGFR3–BAIAP2L1 junction site in a patient with bladder cancer, and the bottom image shows that of WT FGFR3 in the BFTC905 bladder cancer cell line. B, cell lines were analyzed with the break-apart FISH assay. Fluorescein-labeled probes (green) hybridized to the 5’ region of FGFR3 and Texas red–labeled probes (red) hybridized to the 3’ region. Separated signals indicate the presence of the FGFR3–BAIAP2L1 fusion. C, break-apart FISH assay using a bladder cancer tissue microarray. Two positive cases are shown. Scale bars, 1 μm.
tumor sizes at 15 days after inoculation. The Rat-2_F3-B cells formed large tumors (>1,000 mm³), whereas the other cell lines formed small tumors (<100 mm³; Fig. 2C). To demonstrate that kinase activity is essential for the oncogenic activity of FGFR3–BAIAP2L1, we evaluated the antiproliferative activity of CH5183284/Debio 1347, a selective FGFR inhibitor, in spheroid cultures. CH5183284/Debio 1347 showed antiproliferative activity against FGFR3–BAIAP2L1-positive cells only, including the bladder cancer cell line SW780, Rat-2_F3-B cells, and NIH-3T3 cells expressing FGFR3–BAIAP2L1 (3T3_F3-B), but not against NIH-3T3 cells expressing EML4–ALK (3T3_EML4-ALK) or HT1376 cells harboring WT FGFR (Fig. 2D). Other structurally distinct FGFR inhibitors also inhibited cell proliferation of SW780 or Rat-2_F3-B cells (Supplementary Table S2). Furthermore, CH5183284/Debio 1347 induced apoptosis in SW780 and Rat-2_F3-B cells (Supplementary Fig. S3A) and suppressed FRS and ERK phosphorylation (Fig. 2E). CH5183284/Debio 1347 showed in vivo antitumor activity in SW780 and Rat-2_F3-B xenograft models, but not in WT FGFR HT1376 xenograft tumors (Fig. 2F). The downstream signals were suppressed in Rat-2_F3-B xenograft tumors treated by CH5183284/Debio 1347 (Fig. 2G). We also found that siRNAs
targeting the FGFR3, BAIAP2L1, and FGFR3–BAIAP2L1 fusion transcripts showed antiproliferative activities against SW780 cells (Supplementary Fig. S3B and S3C). Collectively, these data demonstrated that the FGFR3–BAIAP2L1 fusion kinase promoted tumor growth in vivo and that its kinase activity was important for oncogenic activity.

Downregulation of tumor-suppressive pathways by the FGFR3–BAIAP2L1 fusion kinase

A previous study reported that the FGFR3–BAIAP2L1 fusion kinase induced ERK and STAT1 phosphorylation (18); however, the mechanisms of tumor growth promotion in vivo by FGFR3–BAIAP2L1 have not been demonstrated. Therefore, we conducted a comprehensive gene expression analysis by NGS using 4 cell lines (Rat-2_mock, Rat-2_FGFR3, Rat-2_F3-B, and Rat-2_BAIAP2L1). These cell lines were cultured under normal growth conditions, and total RNAs were prepared for RNA-Seq with an Illumina HiSeq2000. We identified 1,566 upregulated genes and 1,916 downregulated genes in Rat-2_F3-B cells, compared with expression levels observed in Rat-2_mock cells or Rat-2_FGFR3 cells (Fig. 3A). To select genes that are regulated by FGFR3 kinase, we identified a subset of genes whose differential expression levels were reversed by CH5183284/Debio 1347 treatment (Supplementary Fig. S4). Finally, we selected 210 genes comprising an FGFR3–BAIAP2L1-regulated gene signature (Supplementary Table S3), which was analyzed by Ingenuity Pathway Analysis (IPA) software (Ingenuity Systems) to identify upstream regulators. The results indicated that the RB1, p53, and p16 pathways were suppressed in Rat-2 cells expressing FGFR3–BAIAP2L1, whereas the E2F2 and E2F1 pathways were activated (Table 2). To confirm that these pathways are similarly regulated in vivo, xenograft tumors from mice implanted with Rat-2_mock, Rat-2_FGFR3, Rat-2_F3-B, and Rat-2_BAIAP2L1 cells were analyzed by Western blot (Fig. 3B). As previously reported (18), phospho-ERK and phospho-MEK were upregulated only in Rat-2_F3-B tumors. Cyclin D1 protein levels were elevated because of MAPK pathway activation, whereas phospho-AKT was downregulated. Consistent with the IPA results, p53 and p21 expressions were markedly decreased. Similarly, decreased phosphorylation of RB1 and p27 was observed in Rat-2_F3-B–derived xenograft tumors. Collectively, these results suggested that FGFR3–BAIAP2L1 activated the MAPK pathway and downregulated tumor-suppressive pathways involving RB, p53, and CDKN2A.

Table 2. List of pathways activated or inhibited by FGFR3–BAIAP2L1, as suggested by analysis with IPA software

<table>
<thead>
<tr>
<th>Upstream regulator</th>
<th>Predicted state in Rat-2_F3-B cells</th>
<th>Activation z-score</th>
<th>P value of overlap</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB1</td>
<td>Inhibited</td>
<td>−2.95</td>
<td>9.5E−17</td>
</tr>
<tr>
<td>TP53</td>
<td>Inhibited</td>
<td>−2.05</td>
<td>1.4E−12</td>
</tr>
<tr>
<td>RBL1</td>
<td>Inhibited</td>
<td>−2.75</td>
<td>1.1E−11</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>Inhibited</td>
<td>−2.31</td>
<td>4.9E−10</td>
</tr>
<tr>
<td>NUPR1</td>
<td>Inhibited</td>
<td>−3.21</td>
<td>2.2E−06</td>
</tr>
<tr>
<td>E2F2</td>
<td>Activated</td>
<td>2.00</td>
<td>1.4E−13</td>
</tr>
<tr>
<td>E2F1</td>
<td>Activated</td>
<td>3.52</td>
<td>1.3E−12</td>
</tr>
<tr>
<td>TBX2</td>
<td>Activated</td>
<td>2.83</td>
<td>1.9E−08</td>
</tr>
<tr>
<td>CEBPβ</td>
<td>Activated</td>
<td>2.36</td>
<td>1.9E−05</td>
</tr>
<tr>
<td>STAT3</td>
<td>Activated</td>
<td>2.40</td>
<td>2.7E−04</td>
</tr>
</tbody>
</table>

Figure 3. Analysis of FGFR3-BAIAP2L1 pathways. (A) Venn diagrams showing the number of commonly and differentially upregulated genes (left) and downregulated genes (right) in Rat-2_F3-B, Rat-2_FGFR3, and Rat-2_BAIAP2L1 cells. B, xenograft tumors derived from implantation of Rat-2_mock, Rat-2_FGFR3, Rat-2_F3-B, and Rat-2_BAIAP2L1 cells into mice were lysed and analyzed by Western blot (n = 3).
The FGFR3–BAIAP2L1 fusion kinase BAR domain is essential for oncogenic activity

Most partner genes of fusion kinases have dimerization motifs such as coiled-coil domains, and kinase domain dimerization can lead to constitutive kinase domain activation (25). The BAR domain of BAIAP2L1 contains a known dimerization motif, but it is uncertain if the BAR domain facilitates dimerization or promotes oncogenic activity through constitutive kinase activation. To study BAR domain function in FGFR3–BAIAP2L1, we established Rat-2 cells expressing FGFR3–BAIAP2L1 without the BAR domain (Rat-2_F3-B-ΔBAR, Fig. 4A) and compared its oncogenic potential with that observed with Rat-2_F3-B cells. We first measured the spheroid formation capacity of Rat-2_F3-BΔBAR cells relative to those of Rat-2, Rat-2_mock, Rat-2_FGFR3, and Rat-2_F3-B cells. Rat-2_F3-BΔBAR formed 1.4-fold more spheroids than Rat-2_mock cells, whereas Rat-2_F3-B formed 26-fold higher spheroids (Fig. 4B). Next, we evaluated tumorigenesis of Rat-2_F3-B cells in nude mice. Xenograft tumors derived from Rat-2_F3-B cells formed large tumors (average size: 1,600 mm³) by 14 days after tumor implantation (Fig. 4C). However, Rat-2_F3-BΔBAR cells formed tumors that were 10-fold smaller (average size: 89 mm³). We confirmed that the FGFR3 constructs were expressed approximately equally in each cell line (Supplementary Fig. S5). The observations that the spheroid forming activities of Rat-2, F3-B and Rat-2_F3-BΔBAR cells were essentially the same in the presence of FGF1 ligand (Fig. 4D) and that FGF1 induced F3-BΔBAR phosphorylation (Supplementary Fig. S6) both suggested that differences in tumorigenic activity without FGF1 were not due to conformational alterations of the fusion proteins that affected kinase activity, but were solely dependent on BAR domain function. These data demonstrated that the BAR domain of FGFR3–BAIAP2L1 was essential for the observed oncogenic activity.

Contribution of the FGFR3–BAIAP2L1 fusion kinase BAR domain to dimerization activity

To study the requirement of the FGFR3–BAIAP2L1 BAR domain for oncogenic activity, we examined activation statuses with an FGFR3 deletion series in Rat-2 cells. 293 cells were transfected with the empty pCXND3 vector, or pCXND3 constructs encoding FGFR3, F3-B, F3-BΔBAR, F3-B with the SH domain deleted (F3-BΔASH), F3-B with the BAR and SH3 domains deleted (F3-BΔBAR/ΔASH), and kinase dead F3-B (F3-B-KD; Fig. 5A). Next, FGFR3 phosphorylation in transfectants was analyzed in Western blots (Fig. 5B). Compared with FGFR3, F3-B phosphorylation was markedly increased, and no phosphorylation of the kinase dead mutant (K508M) was detected, suggesting that FGFR3 phosphorylation results from autophosphorylation. Among the F3-B deletion mutants studied, FGFR3 phosphorylation in cells expressing the F3-BΔBAR and F3-BΔBAR/ΔSH variants was remarkably diminished. Then, we validated FGFR3 dimerization in 293 cells transfected with FLAG-tagged and/or Myc-tagged FGFR3 fusion variants by immunoprecipitation of FLAG-tagged proteins (Fig. 5C). Although the Myc-tagged F3-B was coimmunoprecipitated with FLAG-tagged F3-B, we only detected a minor portion of coimmunoprecipitated Myc-tagged F3-BΔBAR with FLAG-tagged F3-B.
FGFR3–BAIAP2L1. These data indicated that the FGFR3–BAIAP2L1 protein dimerizes through the BAR domain to facilitate constitutive FGFR3 kinase domain activation.

Discussion

Although a comprehensive landscape of molecular alterations associated with cancer was provided by The Cancer Genome Atlas Project and by other groups, FGFR3–BAIAP2L1 rearrangements have not been previously discovered in clinical tumor samples (8, 15, 26, 27). The lack of reports documenting oncogenic FGFR3–BAIAP2L1 rearrangements may relate to the detection methods used. By using an FGFR3–BAIAP2L1-specific PCR assay, we attained higher sensitivity than expected with NGS technology. Currently, the number of novel genes implicated in tumors is still increasing rapidly with increasing numbers of samples studied, as demonstrated by saturation analysis (28). According to this report, creating a comprehensive catalogue of somatic point mutations representing most cancer genes will require analyzing approximately 2,000 samples per tumor type. Therefore, prevalence studies using more sensitive and specific methods and expanded sample sizes should be conducted.

Investigating signaling pathways associated with this novel oncogene provides important information for combination therapies or resistance mechanisms against inhibitors. The signaling pathway of fusion kinases can differ from WT kinases or fusion kinases with different partners, possibly due to different protein interactions. For instance, it was suggested that cancer cells harboring the NPM–ALK fusion depend on STAT3 pathway activation, but cancer cells harboring the EML4–ALK fusion do not (5). Similarly, the FGFR3–BAIAP2L1 fusion induces phosphorylation of the CDK2/Cyclin E complex, which can further induce p27 degradation (Fig. 3B). Inhibition of tumor-suppressive pathways by oncogene fusions was also reported with NPM–ALK (29).

Figure 5. Dimerization activity of FGFR3–BAIAP2L1 lacking the BAR domain. A, schematic diagrams of full-length FGFR3 and FGFR3 variant constructs used in this study. B, comparison of dimerization activities. FGFR3, F3-B, or F3-B-ΔBAR and subsequently lysed. The lysates were immunoprecipitated with anti-FLAG M2 Affinity Gel and detected in Western blots using anti-FLAG or anti-Myc antibody. Total cell lysates were also analyzed by Western blot.
theory of "oncogene-induced senescence" supports the suppressive effect of the FGFR3–BAIAP2L1 fusion kinase (30). Oncogene-induced senescence is a mechanism used by cells to stay in a premalignant stage, wherein cells do not become malignant without an additional genetic alteration(s). This phenomenon has been documented in cells harboring variants of BRAF (31), KRAS (32), and EGFR (33), and involves p53 or RB pathway activation (34). Therefore, both MAPK pathway activation and the escape from senescence through p53 or RB suppression may promote FGFR3–BAIAP2L1-expressing cells to undergo transformation. This suggests that the combined inhibition of suppressive activity and FGFR may result in synergistic antitumor activity against cancers harboring FGFR3–BAIAP2L1.

The FGFR3–BAIAP2L1 fusion kinase is the first fusion kinase utilizing a BAR domain as a dimerization motif. We demonstrated that the BAR domain is essential for aberrant FGFR3 kinase activation and oncogenic activity (Fig. 4). Thus, small-molecule FGFR inhibitors may facilitate targeted therapy for patients harboring the FGFR3–BAIAP2L1 rearrangement (Fig. 2F). An emerging clinical issue is the development of drug resistance, and next-generation inhibitors against the same target are under clinical investigation because these tumors may depend upon the same targets, even after resistance development. Similarly, point mutations in the FGFR kinase domain are implicated in resistance against FGFR kinase inhibitors (35, 36). Therefore, the development of alternative therapeutic antagonists of the same targets is important. Deletion of the BAR domain dramatically impairs dimerization activity (Fig. 5), suggesting that targeting the BAIAP2L1 BAR domain may be a viable therapeutic approach. Interestingly, BAIAP2L1 siRNA did not cause cytotoxicity in RT112/84 or HT1376 cells, which have WT BAIAP2L1 (Supplementary Fig. S3). Therefore, the inhibition of BAIAP2L1 is expected to be safe, although further investigation is necessary.

In summary, we demonstrated that FGFR3–BAIAP2L1 exerts potent tumorigenic activity through ligand-independent and constitutive dimerization via the BAR domain, and a cell line harboring this gene fusion was sensitive to the FGFR inhibitor CH5183284/Debio 1347. We also detected this rearrangement in human clinical bladder and lung cancer specimens. Therefore, treating patients harboring FGFR gene fusions such as FGFR3–BAIAP2L1 with CH5183284/Debio 1347 or other FGFR inhibitors may be a promising approach in the future. CH5183284/Debio 1347 is currently under phase I clinical investigation by Debiopharm International S.A. in patients harboring FGFR genetic alterations (NCT01948297).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: Y. Nakashishi
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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Y. Nakashishi, T. Fuji
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Administrative, technical, or material support: Y. Nakashishi, N. Akiyama
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