CDKN2A/p16 Loss Implicates CDK4 as a Therapeutic Target in Imatinib-Resistant Dermatofibrosarcoma Protuberans

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Abstract

Dermatofibrosarcoma protuberans (DFSP) is an aggressive PDGFB-dependent cutaneous sarcoma characterized by infiltrative growth and frequent local recurrences. Some DFSP progress to a higher-grade fibrosarcomatous form, with rapid growth and increased risk of metastasis. Imatinib provides clinical benefit in approximately 50% of patients with unresectable or metastatic DFSP. However, efficacious medical therapies have not been developed for imatinib-resistant DFSP. We established a model of imatinib-resistant DFSP and evaluated CDK4/6 inhibition as a genomically credentialed targeted therapy. DFSP105, an imatinib-resistant human cell line, was established from a fibrosarcomatous DFSP (FS-DFSP), and was studied by SNP arrays and sequencing to identify targetable genomic alterations. Findings were validated in vitro and in vivo, and confirmed in a series including 12 DFSP and 6 FS-DFSP. SNP analysis of DFSP105 revealed a homozygous deletion encompassing CDKN2A and CDKN2B. The resultant p16 loss implicated CDK4/6 as a potential therapeutic target in DFSP. We further demonstrated CDKN2A homozygous deletion in 1 of 12 conventional DFSP and 2 of 6 FS-DFSP, whereas p16 expression was lost in 4 of 18 DFSP. In vitro treatment of DFSP105 with two structurally distinct selective CDK4/6 inhibitors, PD-0332991 and LEE011, led to inhibition of Rb1 phosphorylation and inhibition of proliferation (G1/s 160 nmol/L and 276 nmol/L, respectively). In vivo treatment of DFSP105 with PD-0332991 (150 mg/kg) inhibited xenograft growth in mice, in comparison with imatinib-treated or untreated tumors. In conclusion, CDKN2A deletion can contribute to DFSP progression. CDK4/6 inhibition is a preclinically effective treatment against p16-negative, imatinib-resistant FS-DFSP, and should be evaluated as a therapeutic strategy in patients with unresectable or metastatic imatinib-resistant DFSP.

Introduction

Dermatofibrosarcoma protuberans (DFSP) is a cutaneous spindle cell sarcoma of presumed fibroblastic lineage characterized by an infiltrative growth pattern and high rate of local recurrence (1). Biologically, DFSP is characterized by a genomic rearrangement involving chromosomes 17 and 22, often in a supernumerary ring chromosome, and less often as the result of the reciprocal balanced translocation t(17;22)(q22;q13) (2–5). Such rearrangements result in the genetic fusion of COL1A1 and PDGFB (6), placing PDGFB under the control of the constitutively active COL1A1 promoter, and leading to overexpression of PDGFB (platelet-derived growth factor beta) and sustained PDGF receptor signaling due to an activating autocrine loop (7). Tyrosine kinase inhibitors, such as imatinib, disrupt this autocrine loop by blocking PDGFR receptor activity (8). An estimated 10% to 20% of DFSP undergo transformation to a higher-grade form, designated fibrosarcomatous DFSP (FS-DFSP; ref. 9–12), which is characterized by rapid growth and increased risk for metastasis (9, 13–16). The biologic mechanisms causing transformation from conventional DFSP to FS-DFSP are not well understood (17). Therefore, biomarkers that predict tumor progression might be helpful in assessing prognosis and generating new therapeutic leads in patients with DFSP.

Sensitivity to imatinib has been observed both in patients with advanced localized DFSP and metastatic FS-DFSP (18). However, imatinib response rates barely surpass 50% (12, 18–22). Many patients do not respond to imatinib (23), and even among those patients who do respond, secondary resistance often emerges rapidly (22, 24). Biologic mechanisms of resistance to imatinib are not well characterized in DFSP, and there are no therapies available for patients with imatinib-resistant tumors, other than aggressive surgical resection that can cause significant morbidity and is an ineffective means for controlling disseminated disease.

For the studies reported herein, we established an imatinib-resistant FS-DFSP cell line, in which we identified a localized homozygous deletion of the CDKN2A locus, associated with loss of p16 expression, which suggested tumor dependency on CDK4/6. We then screened a series of conventional DFSP and FS-DFSP, showing that CDKN2A deletion and p16 loss are recurrent...
aberrations in DFSP. Targeting CDK4/6 with specific pharmacologic inhibitors decreased DFSP proliferation in vitro and tumor growth in vivo, thereby validating a novel therapeutic strategy in DFSP.

Materials and Methods

Tumor samples

Formalin-fixed, paraffin-embedded tumor samples from 13 conventional and 9 FS-DFSP were obtained from the pathology archives at Brigham and Women’s Hospital (Table 1). Two sequential samples, obtained 3 months to 3 years apart, were available for patients DFSP-12, FS-DFSP-03, FS-DFSP-05, and FS-DFSP-06. Hematoxylin and eosin (H&E)-stained slides were reviewed by two experienced surgical pathologists, confirming the diagnosis of DFSP or FS-DFSP in all cases. Histologically, DFSP is a dermal storiform proliferation of bland spindle cells set in a collagenous matrix without significant cytologic atypia, characteristically extending into subcutaneous fat in a honeycomb pattern. FS-DFSP shows a fascicular arrangement with a herringbone appearance, more cellularity than conventional DFSP, mild nuclear atypia and hyperchromasia, and high mitotic activity (10–20 mitoses per 10 hpf). One of the conventional DFSP had a giant-cell fibroblastoma histomorphology. Where available, snap frozen tissue was collected after surgical resection at Brigham and Women’s Hospital. All samples were collected with Institutional Review Board approval.

Index case and cell lines

We established the spontaneously immortal human DFSP cell line, DFSP105, from a right breast axillary tail FS-DFSP metastasis in a 53-year-old female. The DFSP had been diagnosed 5 years earlier, at which time the patient presented with FS-DFSP on the left lower extremity. Tissue samples were obtained from the DFSP, and from one recurrent conventional DFSP at 7 years and one recurrent FS-DFSP 5 years after initial diagnosis. DFSP105 cells were maintained in Iscove’s Modified Dulbecco’s Medium supplemented with 15% FBS, penicillin/streptomycin, and 1% (v/v) l-glutamine. The cell line was periodically validated by detection of PDGFR rearrangement by FISH. All of the experiments in this report were performed on passage number 10 to 30. Formalin-fixed, paraffin-embedded tissue from this patient’s DFSP was included in the study as case FS-DFSP-05. GIST48 and GIST882, previously published gastrointestinal stromal tumor cell lines developed in our laboratory, were used as controls for in vitro assays (25).

Table 1. Clinicopathologic features of a series of 18 cases (22 samples) of DFSP, including p16/CDKN2A status

<table>
<thead>
<tr>
<th>Case</th>
<th>Patient ID</th>
<th>Diagnosis</th>
<th>Sex</th>
<th>Age</th>
<th>Location</th>
<th>PDGFB FISH</th>
<th>p16 expression</th>
<th>CDKN2A FISH</th>
<th>RB1 expression</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>DFSP-01</td>
<td>GCF</td>
<td>M/26</td>
<td></td>
<td>Groin</td>
<td>+ (2–5)</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>DFSP-02</td>
<td>DFSP</td>
<td>M/23</td>
<td></td>
<td>Buttock</td>
<td>+ (1–2)</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>DFSP-03</td>
<td>DFSP</td>
<td>M/34</td>
<td></td>
<td>Forehead</td>
<td>+ (2–5)</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>DFSP-04</td>
<td>DFSP</td>
<td>F/51</td>
<td></td>
<td>Buttock</td>
<td>+ (1–2)</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>DFSP-05</td>
<td>DFSP</td>
<td>F/56</td>
<td></td>
<td>Neck</td>
<td>+ (2–5)</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>DFSP-06</td>
<td>DFSP</td>
<td>F/33</td>
<td></td>
<td>R anterior thigh</td>
<td>+ (1–2)</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>DFSP-07</td>
<td>DFSP</td>
<td>M/27</td>
<td></td>
<td>Forehead</td>
<td>+ (3–5)</td>
<td>–</td>
<td>+</td>
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<tr>
<td>8</td>
<td>DFSP-08</td>
<td>DFSP</td>
<td>F/45</td>
<td></td>
<td>Neck/shoulder</td>
<td>+ (3–5)</td>
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<td>+</td>
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<td>9</td>
<td>DFSP-09</td>
<td>DFSP</td>
<td>M/38</td>
<td></td>
<td>Groin</td>
<td>+ (2–4)</td>
<td>+</td>
<td>+</td>
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<tr>
<td>10</td>
<td>DFSP-10</td>
<td>DFSP</td>
<td>M/47</td>
<td></td>
<td>Thigh</td>
<td>+ (2–5)</td>
<td>+</td>
<td>+</td>
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<td>11</td>
<td>DFSP-11</td>
<td>DFSP</td>
<td>F/42</td>
<td></td>
<td>Abdominal wall</td>
<td>+ (1–2)</td>
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<td>+</td>
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<td>12</td>
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<td>Hypercellular DFSP</td>
<td>M/55</td>
<td></td>
<td>L anterior chest wall</td>
<td>+ (1–2)</td>
<td>–</td>
<td>Homozygous deletion</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>DFSP-13</td>
<td>Hypercellular DFSP</td>
<td>M/55</td>
<td></td>
<td>L anterior chest wall</td>
<td>+ (1–2)</td>
<td>–</td>
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<td>F/75</td>
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<td>FS-DFSP</td>
<td>F/36</td>
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<td>Abdominal wall</td>
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<tr>
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<td>FS-DFSP</td>
<td>M/31</td>
<td></td>
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<td>+</td>
<td>–</td>
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</tr>
<tr>
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<td>FS-DFSP</td>
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<td></td>
<td>R lung metastasis</td>
<td>+ (1–2)</td>
<td>–</td>
<td>Homozygous deletion</td>
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<td>FS-DFSP</td>
<td>M/49</td>
<td></td>
<td>Shoulder</td>
<td>+ (3–5)</td>
<td>+</td>
<td>–</td>
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<tr>
<td>19</td>
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<td>FS-DFSP</td>
<td>M/43</td>
<td></td>
<td>Back</td>
<td>+ (3–5)</td>
<td>+</td>
<td>–</td>
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<td>FS-DFSP</td>
<td>M/44</td>
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<td>F/52</td>
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<tr>
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<td>FS-DFSP</td>
<td>F/53</td>
<td></td>
<td>R breast metastasis</td>
<td>+ (3–5)</td>
<td>–</td>
<td>Homozygous deletion</td>
<td></td>
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</tbody>
</table>

Abbreviation: N/A, not available.

*GCF, giant cell fibroblastoma

*M, male; F, female.

*L, left; R, right.

*Positive/negative (number of copies).

*This case corresponds to the surgical resection from which the cell line DFSP105 was established.

Mol Cancer Ther; 14(6) June 2015 1347
were used for evaluation of the CDKN2A locus. At least 50 nuclei were evaluated for each case; those with greater than 30% of nuclei showing chromosome 9 centromere signals but lacking a CDKN2A hybridization signal were considered to be positive for CDKN2A deletion.

SNP analysis
High molecular weight genomic DNA was isolated from DFSP105 using the QIAamp DNA Mini Kit (QIAGEN). DNA was digested with NspI, and linkers were ligated to the restriction fragments to permit PCR amplification. The PCR products were purified and fragmented by treatment with DNase I, then labeled and hybridized to an Affymetrix 250K SNP array. Signal positions and intensities were analyzed using dChip software. Array intensity was normalized to the array with median intensity. Median smoothing was used to infer copy number.

Massively parallel targeted sequencing
We sequenced a preselected set of 275 cancer-associated genes (OncoPanel), which emphasizes clinically actionable genes, including genes with roles in PDGFR signaling (such as PDGFRB and members of the PI3K/AKT/MTOR, RAS/RAF/ERK, and PKC signaling pathways) and other cancer-related genes, as previously published (27). Sample preparation, size selection, capture to biotinylated baits, paired-end sequencing on HiSeq 2000 (Illumina), and analytical pipeline have also been described previously (27).

Western blotting analysis
Frozen tumor samples were diced in ice-cold lysis buffer containing protease inhibitors and homogenized for 3 seconds, 3 to 5 times, on ice. Whole-cell lysates from cell cultures were prepared in lysis buffer. All lysates were cleared by centrifugation at 14,000 rpm for 30 minutes at 4°C, and protein concentrations were determined using a Bradford protein assay (Bio-Rad Laboratories). Electrophoresis, Western blotting, and detection were performed using standard techniques.

Primary antibodies were p16 (Cell Signaling; #4824), phospho-RB1 Ser795 (Cell Signaling; #9301), phospho-RB1 Ser807/811 (Cell Signaling; #9308), RB (Cell Signaling; #9309), CDK4 (Santa Cruz Biotechnology; sc-601), PDGFRB (Santa Cruz Biotechnology; sc-432), phospho-PDGFR (sc-12911), actin (Sigma; A4700), Cyclin A (Novocasta; NCL-CYCLIN A), and Cyclin D1 (Santa Cruz Biotechnology; sc-220044). GIST48, which harbors a homozygous CDKN2A deletion and expresses no p16, and GIST882, which retains wild-type CDKN2A and expresses p16, were used as controls for p16 staining.

Drug response studies
Cell proliferation was measured by BrdUrd incorporation, using a cell proliferation ELISA kit (Roche). Cells were plated at 2,500 cells per well in opaque 96-well flat-bottom plates, and drugs were added with fresh media 24 hours after plating. Cells were incubated in drug for 48 hours, with BrdUrd added to the media for the last 24 hours. Luminescence was detected using a Veritas microplate luminometer (Turner Biosystems). The drugs were imatinib, sunitinib, RAD001 (everolimus) and GDC-0941 (all from LC laboratories); and PD-0332991 (palbociclib) and LEE011 (from ChemieTek). All drugs were dissolved in DMSO. DMSO controls were incorporated in all studies, as solvent-only comparators.

CDK4 knockdowns
 Lentiviral constructs encoding shRNA-specific sequences targeting CDK4 transcripts on the pLKO.puro backbone were selected from the RNAi Consortium (TRC) library (TRCN0000010520: shRNA1; TRCN0000000363: shRNA2; the TRC website is http://www.broadinstitute.org/rnaip/th/). Lentivirus preparations and lentiviral infections were performed as described previously (25). Cells from 6-well plates were lysed for immunoblot analysis after puromycin selection at 10, 14, and 21 days after infection; day 14 is shown. Cell proliferation was measured at day 14 after lentiviral infection, by BrdUrd incorporation over 24 hours under the same conditions as the drug response studies.

Immunohistochemistry
Immunohistochemistry was performed on 4-μm-thick paraffin-embedded tissue sections after pressure cooker antigen retrieval (Biocare Medical; 30 to 40 minutes at 122°C) in citrate buffer (Dako Target Retrieval Solution S1699) using mouse monoclonal antibodies anti-Rb (clone G2-245; BD Biosciences; 1:100 dilution), anti-p16 (clone E6H4; MTM Labs; 1:2 dilution), and anti-CD34 (clone QBEnd10; Dako; 1:400 dilution). Dako Envision+ Mouse (K4007) secondary antibody was used (30-minute incubation at room temperature). The sections were developed using 3,3-diaminobenzidine as substrate and counterstained with Mayer's hematoxylin. Intact expression within the nuclei of endothelial and inflammatory cells served as internal positive controls for p16. Dako mouse IgG1 was used as a negative control. Nuclear staining for RB1 and p16 was scored by two of the authors (G. Eilers and A. Maríno-Enríquez) as negative (loss of expression) when <10% of cells showed nuclear staining.

Xenograft studies
Mice were maintained, injected, and sacrificed in accordance with an approved Institutional Animal Care and Use Committee protocol at Dana Farber Cancer Institute. Athymic nude mice were injected subcutaneously with 2 million DFSP105 cells suspended in Matrigel (BD Biosciences). After the establishment of tumors (~5 weeks after injection), mice were split into imatinib or PD-0332991 treatment arms. Imatinib was dissolved in water and administered daily at 75 mg/kg by gavage. PD-0332991 was dissolved in a buffer consisting of 10% v/v 0.1 mol/L HCl, 10% v/v Cremophor EL (BASE), 20% v/v PEG 300, and 60% v/v acetate buffer, pH 4.6, and administered daily at 150 mg/kg by gavage. No significant weight loss was observed in any of the mice over the experiment period. Tumor volumes were evaluated weekly. After 5 weeks of treatment, mice were given intraperitoneal injections of BrdUrd, and euthanized 2 hours later by CO2 inhalation. Tumors were resected, measured, and photographed, lysates were prepared for Western blot analysis, and tissue was allocated for histologic examination.

Results
DFSP105 has a COL1A1–PDGFB fusion but is resistant to imatinib
FISH using probes flanking the PDGFB locus showed an abnormal hybridization pattern in the FS-DFSP cell line DFSP105 indicative of PDGFB rearrangement and low-level copy-number gain (3–5 extra copies of an unbalanced split FISH signal; Fig. 1A). These findings are consistent with the ring chromosome demonstrated in DFSP105 by giemsa-banding cytogenetic studies and
the clinicopathologic diagnosis of FS-DFSP. In drug sensitivity assays, DFSP105 was resistant to imatinib treatment with a GI₅₀ value (50% growth inhibition dose) greater than 5 μmol/L as measured by ATP-based viability and BrdUrd-based proliferation assays (Fig. 1B). Likewise, DFSP105 proliferation was not inhibited by sunitinib, although both imatinib and sunitinib inhibited PDGFRB phosphorylation in DFSP105 cells (Fig. 1C). DFSP105 growth was only moderately inhibited by the PI3K inhibitor RAD001 (GI₅₀ = 1.905 nmol/L), which was likely present in only a subset of the cells, as well as single nucleotide substitutions in FH (c.655G>A, encoding D219N; predicted to be benign - Polyphen2 score 0.0) and ERCC3 (c.1067C>T, encoding T356I; predicted to be damaging - Polyphen2 score 0.996).

The p16/CDKN2A-CDK4-RB1 pathway is frequently disrupted in FS-DFSP
p16 and RB1 expressions were analyzed by immunohistochemistry in formalin-fixed paraffin-embedded tissue samples from a series of 18 conventional DFSP and FS-DFSP (Table 1). In total, 6 tumors showed loss of either p16 or RB1, in a mutually exclusive manner (2 of 12 conventional DFSP, and 4 of 6 FS-DFSP). Four tumors had loss of p16 expression (2 conventional DFSP and 2 FS-DFSP; Table 1 and Fig. 3). Of the 4 tumors with loss of p16 expression, 3 had homozygous CDKN2A deletion (2 FS-DFSP and 1 conventional DFSP) and 2 had loss of RB1 expression (both, FS-DFSP; Table 1 and Fig. 3). The p16/Cdk4 deletion and also demonstrated a TP53 frameshift mutation (c.582_588TATCCGA-ATA), allelic frequency of approximately 25%, which was likely present in only a subset of the cells, as well as single nucleotide substitutions in FH (c.655G>A, encoding D219N; predicted to be benign - Polyphen2 score 0.0) and ERCC3 (c.1067C>T, encoding T356I; predicted to be damaging - Polyphen2 score 0.996).

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DFSP105 harbors a homozygous CDKN2A deletion
Copy-number analyses from DFSP105 SNP profiles revealed a localized homozygous deletion at 9p21, encompassing CDKN2A and CDKN2B, which encode p14ARF, p15INK4b, and p16INK4a (Fig. 2A). This was the only homozygous deletion detected in DFSP105, although there were heterozygous deletions at 4q31-32, 5p13-15, 6q14-27, 17p12-13, 18q12-23, 19q13, and 22q13. Low-level copy-number gains were present at 17q and 22q, corresponding to extra copies of the COL1A1-PDGFBR fusion present in ring chromosomes. Additional low-level copy-number gains were present at 5q35, 14q11, 16p11-13, and 16q22. The homozygous CDKN2A deletion was confirmed by FISH, which showed no hybridization signals for the CDKN2A locus, whereas two signals per cell were demonstrated for the chromosome 9 centromeric region (in both DFSP105 and in sections of the corresponding tumor, FS-DFSP-06). The CDKN2A deletion was associated with complete loss of p16 expression, as evaluated by Western blot, whereas RB1 expression was retained (Fig. 2B). Oncopanel sequencing confirmed CDKN2A homozygous deletion and also demonstrated a TP53 frameshift mutation (c.582_588TATCCGA-ATA), allelic frequency of approximately 25%, which was likely present in only a subset of the cells, as well as single nucleotide substitutions in FH (c.655G>A, encoding D219N; predicted to be benign - Polyphen2 score 0.0) and ERCC3 (c.1067C>T, encoding T356I; predicted to be damaging - Polyphen2 score 0.996).
160 nmol/L for PD-0332991 and 276 nmol/L for LEE011 (Fig. 4B). shRNA-mediated CDK4 knockdown resulted in substantial inhibition of DFSP105 cell proliferation (Fig. 4C and D). In vivo efficacy of CDK4/6 inhibition was evaluated in mouse DFSP105 xenografts, comparing PD-0332991 with PDGF receptor inhibition with imatinib, and to untreated tumors. Growth of DFSP105 xenografts was completely suppressed by PD-0332991, with no detectable increase in tumor size over the treatment period (Fig. 5A). PD-0332991 significantly reduced DFSP105 xenograft growth compared with no treatment (P = 0.0011) and imatinib (P = 0.0069), whereas the growth of imatinib-treated tumors was not significantly different from the untreated controls (P = 0.3408). Resected xenografts from mice treated with PD-0332991 showed marked decrease in RB1 phosphorylation (Fig. 5B). Histologically, the PD-0332991–treated xenografts had similar cell density, with reduced proliferative activity by BrdUrd incorporation, compared with xenografts from untreated and imatinib-treated mice (Fig. 5C).

Discussion

There are few effective therapeutic options for unresectable or metastatic DFSP; although disruption of autocrine PDGF receptor signaling by imatinib has been effective in approximately 50% of DFSP patients (12, 18–21), the responses are short-lasting and there are no known biomarkers of response. Patients with conventional DFSP often show a good initial response to imatinib, which may enable more effective surgical resection in a neoadjuvant clinical setting (28). In patients with FS-DFSP, responses to imatinib are often transient, incomplete, and unpredictable (22, 24). Currently, no effective targeted pharmacologic therapies have been described for patients with imatinib-resistant DFSP (29).

To study genetic progression and imatinib resistance in DFSP, we established a human cell line, DFSP105, from a patient with imatinib-resistant metastatic FS-DFSP. DFSP105 cells expressed tyrosine-phosphorylated PDGFRB, consistent with persistent autocrine PDGFB activation from the underlying genomic COL1A1-PDGFB rearrangement. However, DFSP105 appeared to be PDGFR-independent, as evidenced by failure of imatinib and sunitinib to inhibit cell proliferation and viability, even though both drugs inhibited PDGFRB phosphorylation (Fig. 1). Notably, in evaluating potential targets downstream of PDGFRB, we found that DFSP105 proliferation was inhibited potently by mTOR inhibition, whereas antiproliferative response to the PI3K inhibitor GDC-0941 was less dramatic than that reported in other malignancies with oncogenic PI3K activation (30), suggesting that DFSP105 has partial resistance to this agent. Taken together, these observations suggest continued dependence of DFSP105 on downstream PDGFR signaling, with a receptor independent imatinib-resistance mechanism. However, targeted sequencing of 275 cancer-related genes did not reveal mutations in genes that regulate mTOR, and are downstream of PI3K, including AKT family members, PTEN, TSC1, TSC2, and MTOR itself. Hence, the specific molecular mechanism of imatinib resistance in DFSP105 remains to be pinpointed. The mutations detected in the cancer gene screen were a TP53 frameshift mutation which appeared to be a late genetic event (likely present in only a subset
of the cells), an FH mutation predicted to be benign, and an ERCC3 mutation predicted to be deleterious. None of these mutations have been associated convincingly with acquired resistance to tyrosine kinase inhibitors, and mutations in these genes were not identified by whole genome sequencing in another imatinib-resistant DFSP (31). Interestingly, it is unclear why a subset of DFSP manifest primary resistance to imatinib, even though they have the same COL1A1-PDGFB primary mutation found in patients whose DFSP respond initially to imatinib. In sum, we conclude that the mechanisms of imatinib primary and secondary resistance remain to be identified in DFSP, and the evidence in our study justifies a further focus on candidates downstream of PDGFRB, in the PI3K-mTOR pathway.

SNP analysis of DFSP105 demonstrated a relatively simple genome, notable for a localized 9p21 homozygous deletion, encompassing CDKN2A and CDKN2B, which encode p14ARF, p15INK4b, and p16INK4a. CDKN2A is one of the most frequently altered genes in cancer, and its protein product, p16, acts to restrict cell cycle progression by inhibiting CDK4/6 activity. CDKN2A deletion results in constitutive CDK4/6 activity, leading to RB1 hyperphosphorylation and dysregulated cell cycle activity (32, 33). Cell cycle control in CDKN2A-null but RB1-positive tumors can be restored by pharmacologic inhibition of CDK4/6 activity. CDKN2A deletion results in constitutive CDK4/6 activity, leading to RB1 hyperphosphorylation and dysregulated cell cycle activity (32, 33). Cell cycle control in CDKN2A-null but RB1-positive tumors can be restored by pharmacologic inhibition of CDK4/6 activity. CDKN2A deletion results in constitutive CDK4/6 activity, leading to RB1 hyperphosphorylation and dysregulated cell cycle activity (32, 33).

In this study, the selective CDK4/6 inhibitors PD-0332991 and LEE011 inhibited DFSP105 cell proliferation with GI50 of 160 mM/L and 276 mM/L, respectively, which are concentrations within the previously reported therapeutic range for other RB1-positive cancer models (34, 37). These interventions not only inhibited DFFP cell proliferation in vitro and tumor growth in vivo, but—in keeping with the expected drug mechanisms—inhibited RB1 phosphorylation and inhibited expression of the proliferation marker cyclin A.

To more generally assess the rationale of CDK4 inhibition in DFSP treatment, we queried a series of conventional DFSP and FS-DFSP to determine the frequency of p16 loss in a series of patient samples ranging from treatment-naïve conventional DFSP to imatinib-resistant, metastatic FS-DFSP. Loss of p16 expression and homozygous CDKN2A deletion were demonstrated in 22% of cases (4 of 18), including 1 of 12 conventional DFSP and 2 of 6 FS-DFSP. The one conventional DFSP with CDKN2A deletion showed hypercellular areas and high mitotic activity, suggesting that CDKN2A deletion may serve as a biomarker of tumor progression from DFSP to FS-DFSP, in addition to identifying DFSP with strong rationale for therapeutic CDK4 inhibition.

In summary, we demonstrate CDKN2A deletions in a subset of FS-DFSP and show that CDK4/6 pharmacologic inhibition in FS-DFSP reduces cell proliferation in vitro and tumor burden in vivo. These data provide a rationale to clinically evaluate CDK4/6 inhibition as a therapeutic strategy in p16-negative FS-DFSP.
Disclosure of Potential Conflicts of Interest

A.J. Wagner reports receiving a commercial research grant from and is a consultant/advisory board member for Novartis. No potential conflicts of interest were disclosed by the other authors.

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Grant Support

This work was supported by grants from the US NIH, including 1P50CA168512 (to J.A. Fletcher and A. Maritio-Enriquez) and 1P50CA127003 (to J.A. Fletcher), and from The Sarcoma Alliance for Research through Collaboration (to A. Maritio-Enriquez).

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Received September 16, 2014; revised March 11, 2015; accepted March 28, 2015; published OnlineFirst April 7, 2015.

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Molecular Cancer Therapeutics

CDKN2A/p16 Loss Implicates CDK4 as a Therapeutic Target in Imatinib-Resistant Dermatofibrosarcoma Protuberans

Grant Eilers, Jeffrey T. Czaplinski, Mark Mayeda, et al.

Mol Cancer Ther 2015;14:1346-1353. Published OnlineFirst April 7, 2015.

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