Inhibition of Platelet-Derived Growth Factor Receptor α by MEDI-575 Reduces Tumor Growth and Stromal Fibroblast Content in a Model of Non-Small Cell Lung Cancer

Naomi Laing, Brenda McDermott, Shenghua Wen, David Yang, Deborah Lawson, Mike Collins, Corinne Reimer, Peter A. Hall, Harriet Andersén, Michael Snaith, Xin Wang, Vahe Bedian, Zhu A. Cao, and David Blakey

ABSTRACT

Platelet-derived growth factor receptor α (PDGFRα) is a receptor tyrosine kinase that promotes cell survival and is expressed in both the tumor and the stromal components of human cancers. We have developed a fully human monoclonal antibody, MEDI-575, that selectively binds to human PDGFRα with high affinity, with no observable affinity for murine PDGFRα. To more fully characterize the role of PDGFRα in the regulation of tumor stroma, we evaluated the in vivo antitumor effects of MEDI-575 in tumor-bearing severe combined immunodeficient (SCID) mice and in genetically altered SCID mice expressing human PDGFRα in place of murine PDGFRα. We used the Calu-6 non-small cell lung cancer model because it lacks an in vitro proliferative response to PDGFRα activation. Antitumor activity was observed when the study was performed in mice expressing the human receptor, but no activity was observed in the mice expressing the murine receptor. Immunohistologic analysis of the tumors from mice expressing human PDGFRα showed a highly significant reduction in stromal fibroblast content and only minor changes in tumor proliferative index in tumors exposed to MEDI-575 compared with the results seen in vehicle-treated tumors or in tumors from mice expressing murine PDGFRα. Additional in vitro studies indicated that exposure of primary cancer-associated fibroblasts to MEDI-575 can directly affect proliferation and key signaling pathways in these cells. These results highlight the potential for observing antitumor activity with MEDI-575 through modulation of the stromal component of tumors and confirm that the PDGFRα pathway can play a role in maintaining a tumor microenvironment conducive to tumor growth.

Introduction

Platelet-derived growth factor (PDGF) receptors are class III receptor tyrosine kinases that activate downstream signaling cascades that promote cellular survival and growth. aberrant activation of platelet-derived growth factor receptor α (PDGFRα) has been observed in several human tumor types derived mainly from mesenchymal tissues, such as sarcomas, gastrointestinal stromal tumors, and glioblastomas. In these tumor types, increased signaling through tumor cell-expressed PDGFRα has been associated with overexpression, amplification, or mutation of the receptor (Clarke and Dirks, 2003; Joensuu et al., 2005; Lasota and Miettinen, 2006). In contrast, overexpression or mutation of PDGFRα in tumor tissues of epithelial origin is less common but has been observed (Matei et al., 2006; Rikova et al., 2007; McDermott et al., 2009).

Homodimeric ligands that specifically activate PDGFRα (i.e., PDGF-AA and PDGF-CC) are potent chemoattractants and mitogens for normal cells of mesenchymal origin. During development, paracrine signaling through PDGFRα is critical for development of the lungs, skin, and the gastrointestinal tract (Hoch and Soriano, 2003). In these tissues, PDGFRα-expressing mesenchymal cells migrate to sites of future epithelial folding and are required for such processes. In the mouse, both PDGF-A and PDGF-Rα are required for postnatal alveolar septation in the lung and proper villus formation in the gastrointestinal tract, processes involving cross-talk between epithelial cells and PDGFRα-expressing mesenchymal cells (Klinghoffer et al., 2002; Hoch and Soriano, 2003). In addition to facilitating proper development of normal epithelial tissues, cross-talk between epithelial tumor cells and mesenchymal stromal cells can play a role in maintaining

ABBREVIATIONS: CAF, cancer-associated fibroblast; DMEM, Dulbecco’s modified Eagle’s medium; ELISA, enzyme-linked immunosorbent assay; ES cells, embryonic stem cells; huPDGFRα, human PDGFRα; huPDGFRα/SCID, SCID mice expressing human PDGFRα transgenically; mAb, monoclonal antibody; PCR, polymerase chain reaction; PDGF, platelet-derive growth factor; PDGFRα, platelet-derived growth factor receptor α; SCID, severe combined immunodeficient; αSMA, smooth muscle actin; sPDGFRα, soluble extracellular domain of human PDGFRα; VEGF, vascular endothelial growth factor.
a tumor microenvironment that is conducive to increased tumor growth and angiogenesis. In this model, epithelial tumor cells secrete multiple factors that promote the recruitment and activation of host mesenchymal stromal cells, including fibroblasts (Bhowmick et al., 2004; De Wever et al., 2008). These stromal fibroblasts can in turn promote tumor growth and invasion by secreting multiple growth factors and chemokines that act in a paracrine or autocrine manner on multiple tumor components.

The PDGF family of ligands, and PDGFA and PDGF-C in particular, are key stromal support factors produced by some epithelial tumor cells. Forced overexpression of PDGF-C by xenografted melanoma cells was recently shown to increase tumor growth, which is associated with increased recruitment of PDGFRα-expressing fibroblasts into tumor stroma (Anderberg et al., 2009). Conversely, knockdown of tumor-cell-expressed PDGFA or neutralization of PDGF function has been shown to reduce the growth of xenograft tumors that have little or no PDGF receptor expression on the tumor cells (Shikada et al., 2005; Tejada et al., 2006; Crawford et al., 2009; Reinmuth et al., 2009). Studies focused on the β receptor of the PDGF family (PDGFRβ) have provided evidence supporting the role for PDGFRβ signaling in the function of pericytes and interstitial fluid pressure in tumors (Furuhashi et al., 2004; Ostman, 2004; Shen et al., 2009). These studies highlight the potential role of stromal PDGFRα signaling in promoting the growth of epithelial tumors.

To directly probe the role of stromal PDGFRα function in supporting tumor growth, we generated a fully human antibody that neutralizes human PDGFRα, MEDI-575, and tested the antitumor effects of this antibody in the Calu-6 xenograft model of non-small cell lung cancer. This model was chosen because the tumor cells secrete PDGF-AA but do not exhibit a proliferative response to this ligand. In addition, Calu-6 cells elicit a stromal response when grown in vivo, resulting in tumor xenografts containing host stromal cells. In the current study, MEDI-575 was characterized as a selective inhibitor of human PDGFRα function with no detectable activity at the corresponding murine receptor or human platelet-derived growth receptor β (PDGFRβ). The antitumor activity of MEDI-575 was evaluated in host mice expressing either murine or human PDGFRα to probe the specific role of host stromal PDGFRα in the growth of Calu-6 xenografts. Antitumor activity in this model was observed in the mice expressing human PDGFRα but was not observed in the mice expressing murine PDGFRα, suggesting that modulation of the host PDGFRα was critical for effecting reduced tumor growth. We further characterized the effects of MEDI-575 exposure on cancer-associated fibroblasts (CAFs) in vitro and in vivo, and we provide evidence to suggest that specific inhibition of PDGFRα function can reduce tumor growth through modulation of stromal fibroblasts in this model of lung cancer.

Materials and Methods

Generation of MEDI-575. Fully human monoclonal antibodies against PDGFRα were developed by immunizing mice [Xenomouse strain XM3C-1 (IgG4κ); Amgen, Inc., Thousand Oaks, CA] with the extracellular domain of PDGFRα (εPDGFRα; R&D Systems, Minneapolis, MN) along with either TiterMax Gold (TiterMax USA, Norcross, GA) or aluminum phosphate gel adjuvant and ImmuneEasy Mouse Adjuvant (Qiagen, Germantown, MD) twice a week for 7 weeks. Lymph nodes were harvested, and a B-cell suspension from these nodes was fused with myeloma cells (American Type Culture Collection CRL-1580) using standard procedures. After clonal selection and expansion, the heavy and light chain variable domains of the lead IgG4 hybridoma line were sequenced, revealing three framework mutations (at amino acid positions 20, 49, and 107 using the Kabat numbering scheme) in the κ light chain that used the VkO13 and Jκ5 human germline genes. Isotype switching was achieved by subcloning the variable domains into vectors with IgG2 isotype constant domains (Marks and Bradbury, 2004). Germlining was performed by side-directed mutagenesis of the nongermline residues in the framework region of the light chain (Lo, 2004). MEDI-575 was produced using the 293F system (Invitrogen, Carlsbad, CA) and was purified using protein A affinity chromatography.

Cell Line Authentication. The authentication of all cell lines used in this study was performed using CellCheck services (IDEXX-RADIL, Columbia, MO). For human cells, cell lines were verified using nine different short terminal repeat loci, whereas the NIH-3T3 line was verified with a panel of murine-specific microsatellite markers and comparison with the original baseline profiles established for the cell line.

Statistical Analysis of Data. The graphs (Figs. 1–6; Supplemental Fig. 4) are derived from the average of multiple observations (cell data in triplicate at a minimum; in vivo antitumor data using groups of 10 to 12 mice; image quantitation, described later) with error bars representing standard deviation from the mean. Student’s t test was used to evaluate the mean from the two different groups, and P ≤ 0.05 was considered statistically significant.

PDGFRα Phosphorylation Assays. Human osteosarcoma MG-63 cells were cultured in serum-free RPMI medium, and then MEDI-575 or buffer was then added to each well followed by PDGF-AA (100 ng/ml; R&D Systems). The primary CAFs, derived from a human male prostate cancer specimen, were obtained from Asterand, Inc. (Detroit, MI). The CAFs were cultured in CAF medium [Dulbecco’s modified Eagle’s medium (DMEM), 1 μg/ml hydrocortisone, 10 ng/ml epidermal growth factor, 5 μg/ml transferrin], followed by the addition of MEDI-575 or buffer, PDGF-AA (100 ng/ml). The level of human phosphorylated PDGFRα (pPDGFRα) in cell lysates was determined by enzyme-linked immunosorbent assay (ELISA) using immobilized goat anti-human PDGFRα antibody (R&D Systems), with antiphosphotyrosine-horseradish peroxidase-conjugated antibody (R&D Systems) for detection.

The murine PDGFRα phosphorylation assay used NIH3T3 cells cultured in serum-free DMEM containing MEDI-575 or control IgG2 antibody, and then PDGF-BB was added (100 ng/ml). After a 10-minute incubation period, the medium was removed, and the phosphorylated receptor was quantitated with ELISA kits for the murine α and β receptors (R&D Systems; Cell Signaling Technology, Danvers, MA, respectively).

Proliferation Assays. All tumor cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA). Tumor cells were cultured in serum-free DMEM medium. For the primary human CAF proliferation assay, CAFs were plated in DMEM medium without serum. For both CAFs and tumor cells, MEDI-575 or buffer was then added followed by PDGF-AA (100 ng/ml) or serum-free medium (control). After 72-hours incubation, the relative proliferation rates were measured with an Alamar blue (Life Technologies, Carlsbad, CA) readout.

Western Blotting of Cell and Tissue Lysates. Cell extracts were run on SDS-PAGE gels, and the proteins were then transferred to a polyvinyl difluoride membrane according to standard procedures. Blots were probed with antibodies to human phosphorylated phospholipase C γ1 (Tyr783), phosphorylated Akt (Ser473 in Akt1 and corresponding residues in Akt2 and Akt3), or phosphorylated mitogen-activated protein kinase (Thr202, Tyr204 in p44 and Thr185, Tyr187 in p42) (all from Cell Signaling Technologies), or a goat anti-human total PDGFRα antibody (R&D Systems) and then with peroxidase-labeled...
secondary antibodies and developed. All blots were directly imaged using an Odyssey Imager (Licor Biosciences, Lincoln, NE).

Generation of Homozygous Severe Combined Immunodeficient Mice Expressing Human PDGFRα Transgenically. For all studies with mice in this report, animals were treated in accordance with local and national animal welfare guidelines and the Institutional Animal Care and Use Committee guidelines. Mice expressing human platelet-derived growth factor receptor α (huPDGFRα) were generated and provided by the AstraZeneca Transgenics Centre, Discovery Sciences, Mölndal, Sweden. The linearized targeting vector was electroporated into R1 mouse embryonic stem (ES) cells (Andras Nagy, Toronto, ON, Canada), and ES cell clones were selected for G418 resistance followed by polymerase chain reaction (PCR) and extensive Southern blot analyses (unpublished data). Correctly targeted ES cell clones were injected into C57BL/6-129 blastocysts (mice obtained from Harlan Laboratories, Indianapolis, IN) to generate chimeras that were subsequently bred to C57BL/6-129 females. Germline transmission was confirmed by PCR genotyping using primers specific for the targeted or wild-type alleles (Supplemental Table 1). An F1 male heterozygous for the desired deletion and insertion was bred with multiple wild-type C57BL/6-129 females. Reverse transcription of liver-derived RNA was performed followed by PCR using primers specific for human (NM006206.4) or mouse (NM001083316.1) PDGFRα to confirm the expression of the human PDGFRα transgene.
(Supplemental Table 1). Breeding of heterozygous mice and severe combined immunodeficient (SCID) mice (Charles River Laboratories, Wilmington, MA) was followed by intercross breeding to generate homozygous human PDGFRα transgenic mice in an immunodeficient background (huPDGFRα/SCID).

In Vivo Tumor Growth Studies and Tissue Harvesting. For all efficacy studies, animals were administered 10 mg/kg MEDI-575, control IgG2, or saline (vehicle) delivered intraperitoneally twice per week. For the U118 study, female CB17 SCID (Charles River Laboratories) mice were implanted subcutaneously in the right flank with 13 × 10^6 U118 human glioblastoma cells combined with matrigel (1:1). For the Calu-6 xenograft studies, SCID mice (Charles River Laboratories) were implanted subcutaneously in the right flank with 2 × 10^6 Calu-6 lung cancer cells. Transgenic mice (huPDGFRα/SCID) were implanted subcutaneously in the right flank with 2 × 10^6 Calu-6 lung cancer cells mixed with matrigel (1:1). Tumor volumes were calculated with caliper measurements using the formula Volume = (Length × Width^2)/2. When tumors reached an average of 300 mm^3 (U118) or 160 mm^3 (Calu-6), the animals were randomized into groups of 10 to 12, and dosing was initiated. Data points represent mean tumor volume ± S.E.M.

Immunohistochemical Analysis. Calu-6 tumor samples were harvested from mice, trimmed into 5 mm^3 pieces, and immediately fixed in either zinc fixative or 10% neutral buffered formalin, then transferred to 70% ethanol, embedded into paraffin blocks, and sectioned (5 μm thickness). Slides were stained using optimized protocols on a Ventana system (Ventana Medical Systems, Tucson, AZ) using various antibodies as follows: a rat anti-mouse CD31 antibody (BD Biosciences, San Jose, CA); a rabbit polyclonal smooth muscle actin (αSMA) antibody (Abcam, Cambridge, MA); a rabbit polyclonal cleaved caspase-3 antibody (Cell Signaling Technologies); a rabbit polyclonal antibody to NG-2 chondroitin sulfate proteoglycan (Chemicon, Temecula, CA); or a murine Ki67 monoclonal antibody (mAb) (Ventana Medical Systems). All slides were stained with dianiminobenzidine (Ventana Medical Systems).

Images were generated using the ImageScope, version 8, imaging system (Aperio, Vista, CA), and the signal density was analyzed using Aperio color deconvolution imaging software (version 8). The viable areas of the regions of the tumors were selected for Aperio analysis using the color deconvolution imaging software. The staining intensities from three entire sections representing different depths from each tumor were quantitated. All images were postprocessed in Photoshopped version 7 (Adobe Systems, San Jose, CA) by employing a custom action applying equal amounts of sharpening and curves to all images before conversion to JPEG format. The intensities of each stain in the vehicle and treated tumors were normalized such that 100% represented the average vehicle staining intensity.

Results

Generation and Characterization of MEDI-575. Xenomouse mice (IgG4 strain) were immunized with sPDGFRα, and hybridomas were generated from the animals that exhibited a positive titer for the presence of sPDGFRα-binding activity. A lead hybridoma line that exhibited potent binding to PDGFRα and did not bind to PDGFRβ was chosen for cloning and sequencing (unpublished data). Human IgG4 antibodies have been shown to be susceptible to exchange of IgG half-molecules (one heavy and one light chain) under in vivo conditions, resulting in bispecific antibodies with altered characteristics (Aalberse and Schuurman, 2002). MEDI-575 was generated by switching the isotype to IgG2, and then germlining to remove three framework mutations. The final antibody (MEDI-575) produced effective inhibition of human PDGFRα phosphorylation induced by PDGF-AA and PDGF-BB in a human osteosarcoma cell line (Fig. 1, A and B, respectively). In similar studies with NIH-3T3 cells, MEDI-575 did not inhibit ligand-induced phosphorylation of murine PDGFRα or PDGFRβ (Fig. 1, C and D). The monovalent affinity of MEDI-575 for human sPDGFRα was determined using surface plasmon resonance technology (Supplemental Methods for Fig. 1), with the results indicating that the equilibrium dissociation constant was 8.6 pM (Supplemental Fig. 1A). Because PDGFRα and PDGFRβ have distinct tissue expression profiles and different tissue-specific functions (Hoch and Soriano, 2003), it was important to understand the potential for MEDI-575 to bind to and modulate human PDGFRα, given that the original hybridoma-generated antibody had shown no interaction with human PDGFRβ. The surface plasmon resonance studies showed no detectable interaction of soluble human PDGFRβ with immobilized MEDI-575 (unpublished data), likely owing to the low homology (32% identity) between the extracellular domains of these two receptors. A fluorescence-activated cell sorter titration analysis (BD Biosciences) of the cells overexpressing human PDGFRα was performed (Supplemental Fig. 1B), which produced a Kₐ value of less than 1 nM.

PDGFRα Signaling in Cancer-Associated Fibroblasts. Fibroblasts are cells of mesenchymal origin that have been shown to express both PDGFR receptors. Because stromal fibroblasts within human tumors can play a role in maintaining a tumor microenvironment that promotes tumor growth and angiogenesis, we evaluated the mitogenic response of cultured primary human CAFs to PDGFAA exposure.

The addition of this ligand to serum-starved CAFs produced nearly a 2-fold increase in proliferation and an over 3-fold increase in phosphorylated PDGFRα over untreated controls (unpublished data), indicating that these cancer stromal cells expressed functional PDGFRα. MEDI-575 effectively reduced the mitogenic effect of PDGFAA in these CAFs (Fig. 1E). In addition, a dose-dependent inhibition of ligand-induced PDGFRα phosphorylation in CAFs was observed (Fig. 1E). It is known that signaling via PDGFRα can activate multiple downstream signaling pathways in cancer cells (Rikova et al., 2007); therefore, we investigated which of these pathways were activated in these CAFs after ligand-induced PDGFRα stimulation. Exposure of serum-starved cells to PDGFAA induced the phosphorylation of phospholipase Cγ, Akt, and mitogen-activated protein kinase (Fig. 1F). In the presence of MEDI-575 there was a clear dose-dependent reduction in the ligand-induced phosphorylation of all of these signaling proteins, while expression of total PDGFRα protein levels were not altered. These data indicate that activation of PDGFRα in CAFs can promote activation of multiple intracellular signaling cascades and proliferation, whereas exposure to MEDI-575 effectively abrogates these effects.

Taken together, these results identified MEDI-575 as a neutralizing mAb with a high affinity for human PDGFRα, with little or no detectable interaction with human PDGFRβ or murine PDGF receptors. This mAb was employed in selected in vivo studies as a specific antagonist of human PDGFRα function.

The Expression and Activation of PDGFRα in a Tumor Cell Panel. To identify a tumor xenograft model that is apparently not dependent on autocrine PDGFRα signaling for tumor growth, we analyzed a panel of tumor cell lines for the expression of PDGFRα protein and a proliferative response to PDGFAA ligand (Fig. 2, A and B). The U118 glioma and MG-63 osteosarcoma lines expressed high levels of PDGFRα,
consistent with the fact that they are derived from mesenchymal tissues that commonly express PDGFRα. High expression of PDGFα was also detected in the NCI-H1703 lung cancer line, consistent with observations from a previous publication (Rikova et al., 2007). The level of PDGFα expressed by the Calu-6 lung adenocarcinoma cell line was detectable but extremely low, whereas the other four cell lines expressed no detectable PDGFRα. The expression of PDGFα tracked with the mitogenetic response to PDGF-AA stimulation under serum-starved culture conditions (Fig. 2, A and B). Importantly, these studies identified the Calu-6 line as one that exhibited no detectable mitogenic response to PDGF-AA, confirming a previous report that these cells did not proliferate in response to PDGF-AA (Tejada et al., 2006). In the same study, it was shown that Calu-6 cells secret PDGF-AA, and that this ligand was the major fibroblast activation factor secreted by these cells when in culture. The Calu-6 model was therefore selected for in vivo studies with MEDI-575 to probe the role of PDGFRα signaling in the stromal support of the growth of xenografted tumors from this cell line.

Because the U118 glioma cell line showed a proliferative response to PDGFRα activation, and previous studies reported that a selective antibody to human PDGFRα showed antitumor activity in xenograft studies with the U118 model (Loizos et al., 2005), we tested MEDI-575 for antitumor activity in this model. Treatment with MEDI-575 produced 101% inhibition of growth (P < 0.0001; Fig. 2C), indicating that MEDI-575 can abrogate PDGFRα signaling in vivo in a model that is directly dependent on signaling through this receptor for tumor growth. Treatment with MEDI-575 did not induce changes in body weight compared with the mice treated with vehicle (Supplemental Fig. 4).

**Characterization of huPDGFRα/SCID Mice and Calu-6 Tumors Grown in Either huPDGFRα/SCID or Nude Mice.** Our studies identified a fully human mAb that antagonized human PDGFRα function and did not bind appreciably to, or modulate the activity of, murine PDGFRα. To evaluate the role of stromal PDGFRα in xenograft tumor growth in mice using MEDI-575, we generated genetically modified SCID mice expressing human PDGFRα in the place of murine PDGFRα (huPDGFRα/SCID) with expression of the human gene under the control of the promoter for murine PDGFRα (Supplemental Fig. 2). Germline transmission was confirmed by PCR genotyping of the F1 offspring (Supplemental Fig. 3), and RT-PCR analysis performed on the offspring from F1 intercrosses confirmed that the homozygous mice expressed only human PDGFRα (Fig. 3A). These homozygous mice were both viable and did not exhibit most of the strong phenotypes associated with null mutations of PDGFRα in mice (Klinghofer et al., 2002; Hoch and Soriano, 2003), indicating that the human allele provided some compensation for the loss of the mouse gene.

Western analyses of selected tissues from the huPDGFRα/SCID mice and from nude mice were performed and the data confirmed that the human PDGFRα protein was expressed in the transgenic mice, while the corresponding murine receptor was expressed in nude mice (Fig. 3B). Western blot analysis of Calu-6 xenograft tumors generated in nude mice and in the huPDGFRα/SCID mice was performed (Fig. 3C). Calu-6 tumors expressed low levels of human PDGFRα when grown in nude mice, which was much lower than in a human U118 glioma xenograft, reflecting the low tumor cell expression of human PDGFRα. Murine PDGFRα was detected in Calu-6 tumors from nude mice, indicating the presence of host PDGFRα-expressing cells within these tumors. These data provided evidence suggesting that Calu-6 tumors show low tumor cell expression of human PDGFRα and contain PDGFRα-expressing stromal cells derived from the host, consistent with the observation that this model contains PDGFRα-positive stromal cells (Tejada et al., 2006).

**Antitumor Activity in the Calu-6 Model with MEDI-575 Required Host Expression of Human PDGFRα.** To probe the role of PDGFRα in stromal support for tumor growth, we compared the effects of MEDI-575 on the growth of Calu-6 tumors hosted in two strains of mice expressing murine PDGFRα (nude and SCID mice) to that observed in mice expressing human PDGFRα (huPDGFRα/SCID mice). Due to the observed specificity of MEDI-575 for human PDGFRα, modulation of PDGFRα function in the host tissues was expected to occur after treating the huPDGFRα/SCID mice, but not the nude or SCID mice, with this antibody.

**Fig. 2.** PDGFRα expression and signaling in human CAFs and tumor cell lines and in vivo antitumor activity in the U118 model. (A) Expression of PDGFRα protein in human tumor cell lines. The lines that were tested were Calu-6 (1), U118 (2), MG-63 (3), NCI-H1703 (4), IGROV-1 (5), Colo205 (6), MDA-MB231 (7), and MCF-7 (8). Western analysis for Hsp60 level was performed as a protein loading control. (B) The effects of PDGF-AA exposure on the proliferation of serum-starved tumor cell lines. Data are reported as the fluorescent units from the proliferation readout for the serum-starved cells with and without ligand addition. (C) The effects of dosing tumor-bearing mice with MEDI-575 on the growth of U118 glioma xenografts. MEDI-575 or vehicle was administered starting on day 21 for the duration of the study.
Dosing with MEDI-575 resulted in reduced growth of Calu-6 tumors when hosted in the huPDGFRα/SCID mice (Fig. 4A; 66% inhibition, \( P = 0.0102 \)) while no significant activity was observed when the same tumors were hosted in either SCID mice or nude mice (Fig. 4 B and C; \( P > 0.5 \) for both studies). Administration of MEDI-575 produced no noteworthy body-weight loss for the duration of the studies in the mice expressing human PDGFRα (Supplemental Fig. 4). The lack of activity in the SCID and nude mice when MEDI-575 would be expected to modulate only tumor cell-derived PDGFRα is consistent with the in vitro data (Fig. 2B) indicating that Calu-6 cells do not exhibit a proliferative response to PDGFRα activation.

The antitumor activity observed when the tumors were hosted in mice expressing human PDGFRα raised the possibility that the effects of MEDI-575 were mediated through modulation of the host-derived tumor stroma. We therefore investigated the histologic changes associated with the reduced tumor growth after exposure to this antibody and compared the histology
with the tumors from the SCID mice where antitumor efficacy was not observed.

**Immunohistochemical Analysis Revealed Modulation of Stromal Cells by MEDI-575.** Calu-6 tumors from huPDGFRα/SCID mice were stained for αSMA to visualize the stromal myofibroblasts present in the tumors. Histologic changes were observed in tumors harvested after 7 days of exposure to MEDI-575 or vehicle (Fig. 5A). In the tumors from vehicle-treated mice, positive staining was noted throughout the body of the xenograft, characterized by random foci of stellate to spindle-shaped myofibroblast cells making close contact with tumor cells and other myofibroblasts (Fig. 5A, left panel). Tumors from mice treated with MEDI-575 showed an overall decrease in the density of αSMA staining (Fig. 5A, top panels). Quantitative threshold image analysis of the αSMA staining indicated a significant reduction in the total staining for αSMA in the treated tumors (Fig. 5B).

The vascular content, proliferative index, and apoptosis levels in treated tumors were also determined via immunohistochemical analyses after 7 days of exposure to MEDI-575 or vehicle (Fig. 5). A moderate but statistically significant reduction in vascular content, as determined by CD31

---

**Fig. 5.** Evidence for tumor stromal modulation after 7 days of exposure to MEDI-575: immunohistochemical staining of Calu-6 tumors harvested from the huPDGFRα/SCID mice after dosing mice with MEDI-575 or vehicle. Mice were dosed with vehicle or 10 mg/kg MEDI-575 on days 1 and 4, and the tumors were harvested on day 7. (A) Representative images of sections stained with antibodies to detect αSMA (stromal myofibroblasts, pericytes), CD31 (vasculature), Ki67 (proliferation), and cleaved caspase-3 (cc-3; apoptosis). (B) Threshold image quantitation of tumor staining reported as a percentage of the vehicle control values. The *P* values generated from Student’s *t* test comparing the treated and control groups were αSMA 0.005, CD31 0.020, Ki67 0.65, and cleaved caspase-3 0.005. The staining intensity for the vehicle group was set at 100% for quantitative comparison.
Fig. 6. Stromal modulation after 21 days of exposure to MEDI-575 in tumors from huPDGFRα mice. Mice were dosed twice weekly with vehicle or MEDI-575, and the tumors were harvested for analysis after 21 days (huPDGFRα/SCID mice) or 17 days (SCID mice). (A) Representative images of tumors from huPDGFRα/SCID mice stained with antibodies to NG-2 (chondroitin sulfate proteoglycan 4), αSMA, and Ki67. Representative images of tumors from SCID mice stained with αSMA are shown in the lowest panels. (B) Threshold image quantitation of the stained tumor sections. The staining intensity for the vehicle groups was set at 100% for quantitative comparison. There was a statistically significant difference between tumors from huPDGFRα/SCID mice treated with vehicle.
staining density, was associated with MEDI-575 exposure. There were no apparent differences between the groups in their various proliferative indices as determined by Ki67 staining, supporting the notion that inhibition of PDGFRα function in the Calu-6 model does not directly affect tumor cell proliferation. However an increase (40%) in the staining for cleaved caspase-3 was observed after dosing with MEDI-575, suggesting that some cells within the tumor became apoptotic after exposure to this antibody. These results show that inhibition of PDGFRα with MEDI-575 produced a significant reduction in αSMA content and a smaller reduction (∼30%) in vessel density in the tumors 7 days after the start of administration of MEDI-575. Importantly, these observed changes preceded major tumor growth delay, as there was little modulation in tumor size 7 days after the initiation of dosing (Fig. 4A).

To determine whether the stromal modulation that was observed in tumors after 7 days of exposure to MEDI-575 persisted during the 21-day time period associated with tumor growth delay, an immunohistochemical analysis was performed on tumors after 21 days of twice-weekly dosing with this antibody. In this study, tumors were stained with two different markers, NG-2 and αSMA, that can stain both myofibroblasts and pericytes, respectively. Tumors from the vehicle-treated group showed a similar pattern of stromal staining for these two markers, indicating the continued presence of stromal cells within vehicle-treated tumors during the study (Fig. 6A, left panels). After 21 days of dosing tumor-bearing huPDGFRα/SCID mice with MEDI-575, the tumors showed a decrease in both NG-2 and αSMA staining, characterized by a major reduction in the number and density of positively stained stromal cells (Fig. 6A, right panels) and a statistically significant decrease in staining intensity for these two markers (Fig. 6B).

Consistent with the observations on day 7, the staining for Ki67 was not altered in tumors from the huPDGFRα mice after 21 days of exposure to MEDI-575 (Fig. 6B). Furthermore, analysis of Calu-6 tumors from standard SCID mice showed that treatment with MEDI-575 was not associated with significantly reduced αSMA staining after 17 days of exposure (Fig. 6B; \( P = 0.104 \)), in contrast with the marked effects on SMA in tumors from the huPDGFRα SCID mice on both days 7 and 21. Taken together, the studies performed on tumors after both 7 and 21 days of exposure to MEDI-575 indicated that tumor growth reduction was associated with reduced stromal myofibroblast content, but no observable changes to the total proliferative index of the tumor were observed.

In contrast to the moderate changes in vasculature content observed in tumors harvested 7 days after MEDI-575 dosing, the quantitative analysis of the CD31 staining showed no difference between the tumors treated with vehicle or MEDI-575 after 21 days of exposure to this mAb (unpublished data). In addition, analysis of the intratumoral content of vascular endothelial growth factor (VEGF) in these same tumors via ELISA showed no significant difference between the two groups (unpublished data). Therefore, there was no sustained effect of MEDI-575 on the tumor vasculature density or VEGF levels in this study.

Discussion

Herein, we report the characterization of MEDI-575, a potent and selective antibody to human PDGFRα, and we provide evidence that targeting stromal fibroblasts with MEDI-575 can reduce tumor growth. Previous studies with another anti-human PDGFRα antibody, IMC-3G3, indicated that targeting human PDGFRα can reduce the growth of human glioma and sarcoma xenografts (Loizos et al., 2005); both tumor types are of mesenchymal origin and likely derive a strong growth signal from autocrine PDGFRα signaling. We have confirmed these findings in the U118 Glioma models where robust growth inhibition with MEDI-575 was achieved, indicating that MEDI-575 might be a promising antibody for treating tumors driven by autocrine PDGFRα signaling. Outside the direct tumor growth drive provided by autocrine PDGFRα signaling, little is known about the additional mechanisms by which PDGFRα signaling may promote a microenvironment conducive to tumor growth. Multiple studies have highlighted the importance of various PDGF ligands in the recruitment of tumor stroma and subsequent effects on tumor growth, but the role of the specific PDGF receptors in these studies is unclear. In a xenograft model of breast carcinoma, it was shown that expression of dominant-negative PDGF abolishes desmoplasia, which is a host fibroblast-mediated response that can promote tumor growth (Shao et al., 2000). In a similar fashion, expression of dominant-negative PDGF reduced the in vivo growth of A549 lung xenograft tumors, which was associated with reduced content of stromal fibroblasts and pericyte coverage of the tumor vessels (Shikada et al., 2005). Because the dominant-negative PDGFs used in both of these studies reduced the function of both PDGF-A and PDGF-B, and because these ligands as homodimers or heterodimers can, in turn, activate PDGFRα and PDGFRβ, these studies did not directly address which PDGF receptor was critical for the effects observed.

The current studies examined the specific role of paracrine PDGFRα signaling in the stroma using the Calu-6 model of non-small cell lung cancer, which is not responsive to or dependent on autocrine tumor cell PDGFRα activation for growth in culture. Decreased growth of Calu-6 xenograft tumors after intratumoral administration of adenoviral constructs encoding either soluble PDGFRα or PDGFRβ has been reported (Lo, 2004), suggesting some dependency on PDGF signaling for tumor growth in this model. The results reported here indicate that modulation of host PDGFRα by MEDI-575 is required for the antitumor effects observed, as reduced tumor growth was only observed when the host mouse expressed human PDGFRα in the place of the murine receptor. Our results are in agreement with a report that treatment of Calu-6 xenograft-bearing mice with an antagonistic antibody specific for murine PDGFRα results in reduced tumor growth, which suggested that host-specific PDGFRα modulation was the mechanism for antitumor activity (Gerber et al., 2012). In addition, we observed that the antitumor activity of MEDI-575 was associated with reduced tumor stromal fibroblast content, and there was evidence for altered morphology of the intratumoral fibroblasts that remained after exposure to this
mAb. To our knowledge, this is the first direct preclinical evidence indicating that specific modulation of PDGFRα with an antibody can effect the tumor growth reduction that is associated with significant modulation of stromal fibroblasts.

The data presented in the current study fit with the model that epithelial carcinoma cells can secrete soluble factors that attract and/or promote the survival and activation of fibroblasts within the tumor and that the fibroblasts in turn secrete multiple factors that induce epithelial tumor cell proliferation or promote angiogenesis and invasion (Bhowmick et al., 2004; Tejada et al., 2006; De Weyer et al., 2008; Anderberg et al., 2009; Crawford et al., 2009). In our study, MEDI-575 exposure reduced intratumoral stromal fibroblast content that was not associated with a sustained decrease in vessel content. These observations are in agreement with a study on another lung cancer xenograft model where neutralization of both PDGF-A and PDGF-B function reduced tumor growth but did not change the vessel count or VEGF content of the tumors (Reimuth et al., 2009). Although MEDI-575 did not constitutively alter angiogenesis in the model used for our study, these observations may be specific to the model used, because others have reported a link between PDGFRα signaling, VEGF levels, and angiogenesis in lung cancer models (Shikada et al., 2005).

In summary, our study provides direct evidence indicating that targeting PDGFRα with MEDI-575 can reduce tumor growth by modulating stromal fibroblasts in a model of lung cancer where the tumor cells are not directly dependent on autocrine PDGFRα signaling for growth. These results underscore the potential importance of fibroblast-epithelial tumor cell crosstalk in promoting xenograft tumor growth and that disrupting this crosstalk can reduce tumor growth by targeting a mechanism that is distinct from existing cancer therapies such as chemotherapy and antiangiogenic agents.

Acknowledgments

The authors thank the following people for their contributions to this work: Philippe Soriano (Fred Hutchinson Cancer Center) for the constructs required to generate the transgenic mice, Christophe Queva (Fred Hutchinson Cancer Center) for the affinities determinations with Biacore and fluorescence-activated cell sorter, Gadi Gazit-Bornstein (Pfizer), Ianault (Agenon), and Jaspal Kang (Innovative Targeting Solutions) for the immunization and screening efforts to identify the original antibody, Lauren Bateman for immunohistochemistry assistance, and Philipp Steiner, Joseph Shaw, and Theresa LaVallee (MedImmune, LLC) for reviewing the manuscript and providing thoughtful input.

Authorship Contributions

Participated in research design: Laing, McDermott, Hall, Reimer, Andersén, Cao, Blakey. Conducted experiments: McDermott, Wen, Yang, Lawson, Collins, Hall, Andersén, Cao. Contributed new reagents or analytic tools: Andersén, Snaith, Wang, Bedian, Cao. Performed data analysis: Laing, McDermott, Wen, Yang, Collins, Reimer, Hall, Andersén, Wang, Cao. Wrote or contributed to the writing of the manuscript: Laing, McDermott, Andersén, Snaith, Bedian, Blakey, Cao, Hall.

References


Address correspondence to: Naomi Laing, AstraZeneca R&D Boston, 35 Gatehouse Drive, Waltham, MA 02451. E-mail: Naomi.laing@astraZeneca.com