Antiangiogenic versus cytotoxic therapeutic approaches in a mouse model of pancreatic cancer: An experimental study with a multitarget tyrosine kinase inhibitor (sunitinib), gemcitabine and radiotherapy

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Abstract. This work evaluated SU11248 (sunitinib) as a potential therapeutic agent, alone or in combination with the cytotoxic agent gemcitabine or radiotherapy in a murine model of pancreatic cancer. Panc02 cells were injected subcutaneously into HsdOla/MF1 mice (n=222). Treatment was administered during 1 week: sunitinib (SUN), gemcitabine (GEM), radiotherapy (RT), RT+SUN and GEM+SUN. Mice were sacrificed 14 days after treatment. The effect on microvessel density (MVD) was measured by CD31 staining. Apoptosis (sFAS, cleaved caspase-3) and proangiogenic proteins (VEGF, PlGF, EGF) were measured with ELISA and immunohistochemistry. At day 14, tumors in all groups increased significantly despite treatment. Only after RT/SUN treatment tumor growth slowed down, although the accretion was still significant (P=0.033). Highest levels of apoptosis were seen in GEM/SUN, RT/SUN and RT treated mice (respectively P<0.001, P<0.01 and P<0.05 compared to placebo). MVD was lowest in RT/SUN treated mice [compared to placebo (P<0.05), GEM (P<0.05) and GEM/SUN (P<0.01)]. Highest VEGF levels were seen after RT and RT/SUN treatment [vs. placebo (P<0.001) and vs. other treatments (P<0.01 for all comparisons)]. GEM and SUN in monotherapy lead to an up-regulation of PlGF and EGF, respectively. In conclusion, the combination treatments RT/SUN and GEM/SUN result in a more potent antiangiogenic and antitumor effect when compared to either treatment alone. Multitargeted angiogenesis inhibitor therapy with sunitinib combined with either radiotherapy or gemcitabine may be a novel approach for human pancreatic cancer.

Introduction

Pancreatic cancer (PC) is one of the most lethal cancers in humans, with a 5-year survival rate of only 5% (1). Aggressive local growth and early metastases are hallmarks of this disease, distinguishing this tumor type from other adenocarcinomas. Complete resection in an early stage is the only option for cure. Despite potentially curative resection for pancreatic adenocarcinoma, local relapse (liver, intra-abdominal) occurs in 50-85% of patients and results in a 5-year survival of <20% (2-7). The frequency and pattern of recurrence make postoperative chemotherapy and/or radiation an important consideration. The available data from randomized clinical trials of adjuvant therapy suggest that chemoradiotherapy has no obvious advantage compared with chemotherapy alone (8). Chemotherapy with gemcitabine is effective and probably offers the best benefit-risk ratio of all currently available adjuvant treatment options (9-13). Patients with locally advanced disease have a dismal long-term prognosis due to its resistance to chemotherapy and radiotherapy (14). For irreversible tumors radiation is given in combination with 5-FU chemotherapy. Recent evidence suggests that concurrent gemcitabine and radiation yields similar outcomes (15). In metastatic PC gemcitabine was accepted as a first-line therapy based on the results of a single phase III study comparing gemcitabine with bolus 5-FU (16). However, gemcitabine has limited antitumor efficacy, with an objective response rate of only 10%. Although the development of alternative gemcitabine schedules regimens are still ongoing, there is an urgent need for novel targets and strategies (17).

Targeted therapies like bevacizumab [vascular endothelial growth factor (VEGF) inhibitor] and cetuximab [epidermal growth factor (EGF) receptor (R) inhibitor] are not active in monotherapy in advanced PC, neither when added to gem-
citabine (18,19). Recently, erlotinib (EGFR inhibitor) combined with gemcitabine has shown improvement on overall survival and obtained approval in USA and Europe (20). The redundancy of angiogenic pathways or the capacity of the tumor to switch to another pathway in response to treatment, might explain the lack of efficacy of a therapeutic strategy that targets a single angiogenic pathway (21). However, as recently shown in rectal cancer, targeted therapies may play a role as radiosensitizers (22). A recent phase I dose-escalation study in patients with locally advanced pancreatic cancer evaluated the toxicity of capcitabine and bevacizumab combined with radiotherapy (23). Acute toxicity was minimal and easily managed by reducing the dose of capcitabine without interruption or attenuation of either bevacizumab or radiation dose. Another trimodal therapy with gemcitabine, cetuximab and radiotherapy was evaluated in a phase II study in locally advanced pancreatic cancer. Preliminary results showed that 58% of patients were amenable for secondary potentially curative resection (24). Regarding the benefits of targeted therapy in the combined modality treatment using either radiotherapy or chemotherapy, it has become clear that this is an important novel strategy for the treatment of pancreatic cancer. These results seem to be very promising but need to be confirmed.

Sunitinib malate (SU11248) is a highly potent, selective inhibitor of certain receptor tyrosine kinases, including VEGFRs types 1-3 and platelet derived growth factor receptors (PDGFRs) α and β (25). This drug has gained approval in advanced renal cell carcinoma and imatinib-resistant gastrointestinal stromal tumors, where it has shown an objective response rate and improved progression-free survival (26,27). In animal models, sunitinib exhibits potent antitumor activity in glioblastoma, breast and lung tumors (28,29). In human PC, sunitinib exhibited antitumor activity evaluated before therapy (day 0), after 1 week of therapy (day 7) and after an additional week without therapy (day 14). Drugs and treatments. Treatment was administered during 1 week, starting 10-14 days after tumor inoculation. The first day of treatment was assigned ‘day 1’. Drug doses were used at a concentration that alone, or in combination with other drugs, did not cause drug-related deaths or weight loss of >15%. Control mice received placebo (saline 0.9%) intraperitoneally (i.p.) 90 μl, 3 times/week (days 1, 4, 7). Gemcitabine (GEM, Gemzar, Eli-Lilly) was administered at a dose of 60 mg/kg body weight (BW) (1.8 mg in 90 μl). i.p. 3 times/week (days 1, 4, 7). Sunitinib (SUN, Sutent, Pfizer) was administered i.p. at a dose of 40 mg/kg BW (1.4 mg in 200 μl) i.p. per day (SUN, days 1-7). Radiotherapy (RT) was delivered in 5 fractions of 5 Gy on days 1-5, to a cumulative dose of 25 Gy. Mice received sedation during RT [i.p. injection of mixture of 100 mg/kg BW ketamine (Ketalar, Pfizer) and 10 mg/kg BW xylazine (Rompun, Bayer)]. Mice were placed in the prone position and a radiation field was simulated around the tumor on the tumor-bearing leg. Tissue-equivalent silicone bolus of 1 cm was placed on the tumor site, to prevent the build-up effect under the skin. Photon irradiation with 1 direct field at a source-skin distance of 1 meter was performed with a 5-MV linear accelerator (Elekta, Crawley, UK). When combined regimens were given [radiotherapy+sunitinib, RT/SUN], [gemcitabine+sunitinib, GEM/SUN] the same doses and schemes as in monotherapy were combined.

Growth of the subcutaneous tumors was evaluated using a vernier caliper. Results are shown as tumor surface area (defined as tumor length (max horizontal diameter) x tumor width (max vertical diameter), in cm²). Tumor growth was evaluated before therapy (day 0), after 1 week of therapy (day 7) and after an additional week without therapy (day 14). Mice were sacrificed at day 15.

Immunohistochemical determination of CD31 and cleaved caspase-3. Paraffin-embedded tissues were used for immunohistochemical analysis of CD31 and cleaved caspase-3. Sections (4 μm) were mounted on SuperFrost® microscope slides (Menzel-Glaser, Braunschweig, Germany). Sections were deparaffinised in xylene, followed by treatment with a graded series of alcohol and rehydrated.

For CD31 staining, sections were treated with trypsin (Sigma-Aldrich, Bornem, Belgium) for 7 min at 37°C and washed with TBS (Tris-buffered saline). All samples were incubated with hydrogen peroxide in methanol for 20 min to block endogenous peroxidase and washed with TBS. Sections were incubated overnight on room temperature with rat anti-mouse CD31 (BD-Pharma, Erembodegem, Belgium, 1/150), then incubated with peroxidase-labeled IgG (Dako Cytomation, CA, USA) and followed by amplification with the TSA Biotin System (PerkinElmer, Waltham, MA, USA). Sections were rinsed with distilled water, counterstained with Harris’ haematoxylin for 5 sec and mounted with universal mount. Control samples exposed to secondary antibody

Materials and methods

Cell culture. Panc02 cells (a kind gift from Dr A. Märten, University of Heidelberg, Germany) were cultured in RPMI-1640 medium (Invitrogen Corporation, Gibco, Merelbeke, Belgium) supplemented with 10% fetal calf serum (Invitrogen Corporation), 4 mM L-glutamine, 50 μg/ml penicillin and and 50 μg/ml streptomycin (Invitrogen Corporation) and incubated at 37°C in a humidified atmosphere of 5% CO2 in air. When in exponential growth phase, the cells were harvested using trypsin/EDTA (Invitrogen Corporation), washed, suspended in phosphate-buffered saline (PBS) and counted.

Animals/subcutaneous tumor model. Four-to-six week old male HsdOla/MF1 mice (16-20 gr body weight) were purchased from Harlan Laboratories (Netherlands) and housed in a standard facility at the Department of Laboratory Medicine, Ghent University. All procedures were carried out in accordance to the guidelines and regulations for use and care of animals and approved by our local ethics committee for animal care, Faculty of Medicine, Ghent University.

One million cells were injected in the right hind leg of 222 mice. Each group (n=26-45 mice per group) was randomly assigned to a different treatment regimen.

Drugs and treatments. Treatment was administered during 1 week, starting 10-14 days after tumor inoculation. The first day of treatment was assigned ‘day 1’. Drug doses were used at a concentration that alone, or in combination with other drugs, did not cause drug-related deaths or weight loss of >15%. Control mice received placebo (saline 0.9%) intraperitoneally (i.p.) 90 μl, 3 times/week (days 1, 4, 7). Gemcitabine (GEM, Gemzar, Eli-Lilly) was administered at a dose of 60 mg/kg body weight (BW) (1.8 mg in 90 μl). i.p. 3 times/week (days 1, 4, 7). Sunitinib (SUN, Sutent, Pfizer) was administered i.p. at a dose of 40 mg/kg BW (1.4 mg in 200 μl) i.p. per day (SUN, days 1-7). Radiotherapy (RT) was delivered in 5 fractions of 5 Gy on days 1-5, to a cumulative dose of 25 Gy. Mice received sedation during RT [i.p. injection of mixture of 100 mg/kg BW ketamine (Ketalar, Pfizer) and 10 mg/kg BW xylazine (Rompun, Bayer)]. Mice were placed in the prone position and a radiation field was simulated around the tumor on the tumor-bearing leg. Tissue-equivalent silicone bolus of 1 cm was placed on the tumor site, to prevent the build-up effect under the skin. Photon irradiation with 1 direct field at a source-skin distance of 1 meter was performed with a 5-MV linear accelerator (Elekta, Crawley, UK). When combined regimens were given [radiotherapy+sunitinib, RT/SUN], [gemcitabine+sunitinib, GEM/SUN] the same doses and schemes as in monotherapy were combined.

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alone showed no specific staining. For the quantification of microvessel density (MVD) in sections stained for CD31, the entire tumor section was scanned by 2 different readers (VC, PDM) at low power (magnification, x40) to identify hot spots, which are the areas of highest vascularization. Five hot spots were identified in the peripheral millimeter of the tumor, and 5 hot spots at the centre of the tumor. Individual microvessels were then counted under high power (magnification, x200) to obtain a vessel count in a defined area, and the average vessel count in the 10 hot spots was taken as the MVD.

For cleaved caspase-3 staining, after washing sections, heat induced antigen retrieval was performed for 10 min in citrate buffer (pH 6.0), after which the tissue slides were cooled down for 30 min. The endogenous peroxidase activity was blocked for 5 min with 0.3% hydrogen peroxide. After washing steps, each section was blocked with blocking solution (TBS/0.1% Tween/5% normal goat serum) for 1 h at room temperature. Primary antibody cleaved caspase-3 (Cell Signaling Technology, 1/400 dilution in PBS/0.1%Tween/5% normal goat serum) was than incubated overnight at 4°C. After washing, the tissue sections were incubated for 30 min

Table I. Tumor surface area in different treatment arms.

<table>
<thead>
<tr>
<th>Tumors (n=195)</th>
<th>Treatment</th>
<th>Placebo (n=45)</th>
<th>GEM (n=34)</th>
<th>GEM/SUN (n=26)</th>
<th>SUN (n=32)</th>
<th>RT (n=29)</th>
<th>RT/SUN (n=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area (cm²)</td>
<td>Day 0</td>
<td>0.88</td>
<td>0.79</td>
<td>0.81</td>
<td>0.96</td>
<td>1.29</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>Day 7</td>
<td>1.22</td>
<td>1.02</td>
<td>1.01</td>
<td>1.47</td>
<td>1.42</td>
<td>1.09</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>1.76</td>
<td>1.36</td>
<td>1.52</td>
<td>2.08</td>
<td>1.63</td>
<td>1.10</td>
</tr>
<tr>
<td>Day 0 vs Day 14</td>
<td>P-value</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.005</td>
<td>0.033</td>
</tr>
</tbody>
</table>

GEM, gemcitabine; GEM/SUN, gemcitabine/sunitinib; SUN, sunitinib; RT, radiotherapy; RT/SUN, radiotherapy/sunitinib. P-values report differences in tumor surface area at day 0 compared with day 14.
at room temperature with a labelled polymer-HRP anti-rabbit secondary antibody (DakoCytomation). The colour reaction was developed using the chromogen 3,3-diaminobenzidine+ (DAB) (DakoCytomation) for 10 min. After washing, the tissue sections were counterstained with Mayer's haematoxylin. TBS/0.1% Tween/5% normal goat serum instead of the primary antibody was used as negative control on each slide in order to exclude false positive responses from non-specific binding of the secondary antibody. For the quantification of degree of apoptosis, 2 different readers (VC, PDM) first 'scanned' the entire tumor section at low power (magnification, x40) to obtain a global view per tumor (qualitative assessment). The number of apoptotic bodies was then counted in at least ten high power fields (magnification, x200) per tumor slice to obtain an individual score for degree of apoptosis (quantitative assessment). Per treatment group a mean score was calculated and this value was used for further analysis.

Prior to staining the specimens, an isotype control was performed to estimate the non-specific binding of target primary antibodies to cell surface antigens.

Enzyme-linked immunoabsorbent assay (ELISA) for sFas, VEGF, EGF, placental growth factor (PlGF). Protein lysates from fresh frozen tissues (n=113) were extracted with RIPA buffer (2.5 mM Tris, 50 mM NaCl, 0.5% NP40, 0.5% deoxycholate, 0.1% SDS, 0.555 g/ml β-glycerophosphate, 1 mM DTT, 20 μl mini EDTA-free protease inhibitor cocktail). Total protein was determined with a Biorad DC protein assay kit (Biorad, Nazareth, Belgium) and absorbance was measured on a plate reader with a 630-nm long-pass filter.

VEGF-A, EGF, PlGF and sFas levels were determined by commercially available kits from R&D Systems (Abingdon, UK) according to the manufacturer's instructions.

Statistics. All statistics were calculated using SPSS for Windows, version 15.0. Kolmogorov-Smirnov test was used to check on normality of data distribution. Differences between groups were assessed using analysis of variance (ANOVA and post-hoc LSD comparisons) in case of normal distributed data. Kruskal-Wallis test was used to compare groups in case of departure from normality. Correction for multiple comparisons (Bonferroni) was applied. Significance was defined as P<0.05.
Results

Treatment with placebo, gemcitabine (GEM), gemcitabine+sunitinib (GEM/SUN), sunitinib (SUN), radiotherapy (RT), radiotherapy + sunitinib (RT/SUN): Effect of different treatment strategies on tumor growth. One million Panc02 cells were injected in the right hind leg of 222 mice. Tumors became clearly visible after a median of 7 days after inoculation (range 6-17 days). Mice were randomly stratified into different treatment groups and underwent i.p. therapy during 1 week. Mice were sacrificed 14 days after the start of therapy. No significant differences in body weight were found between treatment and control groups, and no toxic reactions occurred.

Tumor growth curve. The tumor growth curve is shown in Fig. 1. Although randomized, tumors in the RT group were larger at day 0 than in other groups (P<0.001, see Table I). At day 14, tumors in all groups had grown significantly despite treatment. Only in RT/SUN treated mice tumor growth was retarded, however, the enlargement was still significant (P=0.033).

Apoptosis: Expression of sFas and cleaved caspase-3 in different treatment groups sFAS (Fig. 2A). Highest levels of sFas were seen in GEM/SUN, RT/SUN and RT treated mice. This was significantly higher than in the placebo group (P<0.001, P<0.01 and P<0.05 respectively, ANOVA, LSD post hoc comparison). sFAS levels in GEM/SUN mice were significantly higher than in SUN treated mice (P=0.012). No difference was seen between placebo mice and GEM or SUN treated mice (P>0.05).

Cleaved caspase-3 (Fig. 2B). Highest degrees of apoptosis were seen in GEM/SUN, RT and RT/SUN treated mice (no significant difference between these 3 groups). The degree of apoptosis in GEM/SUN, RT and RT/SUN was significantly higher compared to placebo (P<0.01) and GEM (P<0.05). Lower grade of apoptosis was detected in the GEM, SUN and placebo group (no significant difference between these 3 groups).

Microvessel density (MVD). Lowest MVD was seen in RT/SUN treated mice, which was significantly lower than in the placebo group (P<0.05), GEM (P<0.05) and GEM/SUN group (P<0.01, Fig. 3). Differences between other groups were not significant after Bonferroni correction. Examples of CD31 staining for different treatment arms are given in Fig. 4.

Expression of VEGF, PlGF and EGF in different treatment groups (Table II, Fig. 5).

VEGF. Lowest levels of VEGF were found in placebo, SUN, GEM and GEM/SUN groups (no significant difference between these 4 groups). Highest VEGF levels were seen in RT and RT/SUN mice, which was significantly higher compared to placebo (P<0.001) and other treatment groups (P<0.01 for all comparisons, ANOVA, LSD post hoc comparison). VEGF level was not significantly different between RT and RT/SUN groups.

PlGF. Lowest levels of PlGF were found in placebo, RT and RT/SUN treated mice (no significant difference between these 3 groups). Highest levels of PlGF were observed after GEM and SUN treatment. PlGF level after GEM therapy was
significantly higher compared to placebo (P<0.01), RT (P<0.01) and RT/SUN group (P<0.001). The difference between SUN treated mice and other groups did not reach statistical significance after Bonferroni correction. Intermediate levels of PlGF were found in GEM/SUN treated mice, but the difference with other groups was also not statistically significant.

EGF. Highest levels of EGF were found after treatment with SUN. These values were significantly higher than in the placebo-group (P<0.05). Other comparisons did not reach statistical significance after Bonferroni correction.

Discussing
Local recurrences and distal metastasis continue to impact survival in pancreatic cancer. Combining conventional cytotoxic drugs with novel targeted agents that specifically interfere with key pathways controlling cancer cell survival has gained wide interest in recent years. Therefore the aim of our study was to compare treatment effects of gemcitabine (GEM), sunitinib (SUN) and radiotherapy (RT) in a preclinical model of pancreatic adenocarcinoma.

It has already been shown in the past that administration of SUN leads to inhibition of VEGFR-, PDGFR-, KIT-phosphorylation (25,28,35-37). Direct measures of VEGF- and PDGF-receptor inhibition are difficult in clinical settings. In vivo, tumors are exposed to a complex mixture of growth factors, stimulating multiple signaling pathways, including those not inhibited by the administered therapy. All these growth factors can stimulate both tumor cells and endothelial cells in an autocrine/paracrine way (38). In the present study, the presence of proangiogenic proteins was quantified by ELISA: VEGF-A and PlGF as ligands for VEGFR and EGF as a ligand for EGFR. Recently, SUN was used in bevacizumab-refractory renal cell carcinoma and VEGF-A, VEGF-C,
soluble VEGF receptor [sVEGFR]-3 and PlGF were measured as biomarkers (39). Mean plasma VEGF-A and PlGF levels significantly increased whereas VEGF-C and sVEGFR-3 levels decreased with SUN treatment. Lower baseline levels of sVEGFR-3 and VEGF-C were associated with longer progression-free survival and objective response rate. These baseline levels may have potential utility as biomarkers of clinical efficacy in this setting.

Immunohistochemical staining for CD31 was used as surrogate marker of tissue hypoxia and effect on vessel density. Cleaved caspase-3 and sFAS were used as markers of apoptosis. As measured by these biomarkers, combined treatments have some advantages over either therapy alone. Highest degrees of apoptosis were seen in GEM/SUN, RT and RT/SUN treated mice. It is possible that apoptosis and cytotoxic damage to tumor cells caused by RT and conventional chemotherapeutics like GEM, might have been exacerbated through inhibition of tumor-supporting stroma and blood vessels by SUN. Antiangiogenic treatments directly target angiogenic endothelial cells (ECs) that line tumor blood vessels and lead to their destruction. In addition, some treatments target non-endothelial cells like tumor cells or smooth muscle cells. The latter two can release pro-survival factors such as VEGF that renders ECs more resistant to cytotoxic treatments. Targeting these non-endothelial cells might therefore increase the sensitivity of ECs to radiotherapy and chemotherapy (40,41). Although, adding radiotherapy to antiangiogenic treatment does not seem logical. A decrease in tumor vessel density induced by antiangiogenic drugs would be expected to reduce tumor perfusion and thereby oxygen delivery (42). However, it has already been shown in the past that antiangiogenic treatment induces destruction of immature and inefficient vessels and promotes maturation of the remaining vessels (43).

After ionizing radiation, hypoxia inducible factor-1 (HIF-1) will promote tumor cells to express cytokines (like VEGF) that render protective effects on neighboring ECs (44). Also in our study, we found a substantial release of VEGF after radiation. These cytokines send antiapoptotic signals to tumor vessels, making them resistant to radiation. Blocking these cytokines (like VEGF) can dramatically increase the radiosensitivity of tumor vasculature leading to elevated overall tumor radioresponsiveness (45). Congruent with this theory, the largest reduction in tumor growth and MVD was observed in the RT/SUN group. So these findings suggest that adding SUN to RT leads to an effective pruning of (immature) vessels, rendering this therapy regimen more efficient than RT alone.

Placental growth factor (PlGF), a VEGF homolog, is a pleiotropic cytokine that stimulates EC growth, migration and survival (46). PlGF binds to VEGFR-1 and acts independent of VEGF in ECs and tumor cells, which primarily express VEGFR-1. Genetic studies show that PlGF is redundant for vascular development and maintenance, but contributes to the ‘angiogenic switch’ in disease (47). By up-regulating PlGF, ECs amplify their responsiveness to VEGF during the ‘angiogenic switch’ in many pathological disorders. PlGF levels in plasma and tumors correlate with tumor stage, vascularity, recurrence, metastasis and survival in various tumors (48,49). Of note, PlGF is up-regulated after anti-VEGF therapy and gemcitabine (26,50,51). These findings point to a role for PlGF in rapid restoration of tumor blood supply after treatment and thus, to enhanced likelihood of tumor regrowth. Also in our experiments, PlGF expression was induced by GEM monotherapy. Lowest levels were seen in RT and especially RT/SUN treated mice, suggesting that this rapid restoration of blood supply could have been prohibited in the latter 2 treatment groups. Prevention of this angiogenic response to treatment may require administration of antiangiogenic therapy during, rather than after radiotherapy treatment.

Multitargeted inhibitors like SUN provide the opportunity to attack tumor growth and survival by multiple mechanisms. Considering the fact that an escape mechanism via EGFR-signaling might emerge in vivo, we determined levels of EGF protein in each tumor. EGF protects ECs from apoptosis both by directly acting on the EC compartment as well as via paracrine induction of survival factors (52). We found that EGF levels were elevated after SUN therapy, whereas in all other groups, this level remained at the placebo level. One possible explanation could be in the interaction between HIF-1α and EGFR. Hypoxia and von Hippel-Lindau tumor-suppressor protein control HIF-1α stability and function, hereby regulating transcription of hypoxia-inducible genes such as VEGF and PDGF. EGFR (ErbB1/2) receptor signaling plays an important role in the regulation of HIF-1α-mediated VEGF expression via the Ras/Raf/MEK and PI3K/Akt pathways (53). Therefore, dual inhibition of EGFR signaling and VEGF/PDGF pathways might provide a better therapeutic benefit than either agent alone and could overcome resistance to EGFR-targeted therapies.

Recently, similar findings were reported in vitro and in vivo (28,34). Sunitinib was shown to sensitize ECs to the cytotoxic effects of ionizing radiation. Similar to our findings, combined treatment with RT/SUN delayed tumor growth, by enhanced destruction of tumor vasculature (MVD) in addition to enhanced killing of pancreatic cancer cells. In addition, in the present study we showed that GEM/SUN combination therapy has superior proapoptotic activity. Adding RT or GEM to SUN seems to intensify the antitumor effect of either therapy alone. Our study also suggests that treatment modulates circulating VEGF pathway biomarkers like VEGF, PlGF and EGF. Further studies are needed to define whether these changes may have potential utility as biomarkers of clinical efficacy in this setting.

In conclusion, this study describes a murine model of pancreatic cancer, where combined treatment with RT/SUN and GEM/SUN has advantages over RT, GEM and SUN alone. Multitargeted angiogenesis inhibitor therapy with sunitinib combined with either radiotherapy or gemcitabine may be a novel approach for human pancreatic cancer.

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10.51. references


