Preclinical Development

The CXCR2 Antagonist, SCH-527123, Shows Antitumor Activity and Sensitizes Cells to Oxaliplatin in Preclinical Colon Cancer Models

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Abstract

Colorectal cancer is the second most common cause of cancer-related death in the United States. Recent studies showed that interleukin-8 (IL-8) and its receptors (CXCR1 and CXCR2) are significantly upregulated in both the tumor and its microenvironment, and act as key regulators of proliferation, angiogenesis, and metastasis. Our previous study showed that IL-8 overexpression in colorectal cancer cells triggers the upregulation of the CXCR2-mediated proliferative pathway. The aim of this study was to investigate whether the CXCR2 antagonist, SCH-527123, inhibits colorectal cancer proliferation and if it can sensitize colorectal cancer cells to oxaliplatin both in vitro and in vivo. SCH-527123 showed concentration-dependent antiproliferative effects in HCT116, Caco2, and their respective IL-8-overexpressing variants colorectal cancer cell lines. Moreover, SCH-527123 was able to suppress CXCR2-mediated signal transduction as shown through decreased phosphorylation of the NF-kB/mitogen-activated protein kinase (MAPK)/AKT pathway. These findings corresponded with decreased cell migration and invasion, while increased apoptosis in colorectal cancer cell lines. In vivo results verified that SCH-527123 treatment decreased tumor growth and microvessel density when compared with vehicle-treated tumors. Importantly, these preclinical studies showed that the combination of SCH-527123 and oxaliplatin resulted in a greater decrease in cell proliferation, tumor growth, apoptosis, and angiogenesis that was superior to single-agent treatment. Taken together, these findings suggest that targeting CXCR2 may block tumor proliferation, migration, invasion, and angiogenesis. In addition, CXCR2 blockade may further sensitize colorectal cancer to oxaliplatin treatment. Mol Cancer Ther; 11(6); 1353–64. ©2012 AACR.

Introduction

Colorectal cancer is the leading cause of death from gastrointestinal malignancies, resulting in approximately 51,690 deaths in 2012 (1). Since 2005, no new chemotherapeutic agents have been approved by the Food and Drug Administration for treatment of patients with metastatic colorectal cancer, resulting in a significant need to develop more effective targeted drugs aiming at both the tumor and its microenvironment. Recent studies have identified that certain chemokines and their receptors act as key regulators of colorectal cancer progression and may be important targets for novel drug development strategies (2).

Interleukin-8 (IL-8), a member of the neutrophil-specific C-X-C subfamily of chemokines, acts on endothelial cells via binding onto either CXCR1 or CXCR2 to promote invasion, proliferation, and angiogenesis (2–5). IL-8 expression is upregulated by hypoxia, cytokines, and other environmental stresses, which are mediated by transcription factors, including NF-kB and AP-1 (6, 7). The upregulation of CXCR2 has also been correlated with promotion of tumorigenesis and angiogenesis in lung, melanoma, and ovarian cancers (8–10). Previous studies by our group and others have shown that IL-8 and its receptor CXCR2 are significantly upregulated in the tumor and its microenvironment in colorectal cancer (11–13). These studies showed that expression levels of IL-8 and CXCR2 were associated with tumor proliferation, progression, and sensitivity of oxaliplatin-based therapy in colorectal cancer cell line models and genetic variants in IL-8 and CXCR2 both predict tumor recurrence and oxaliplatin efficacy in patients (11, 14, 15). It is well known that oxaliplatin-based chemotherapy is a standard of care agent used most
commonly in combination with 5-fluorouracil in patients with colorectal cancer (16). Overexpression of IL-8 level in colorectal cancer cells decreased sensitivity to the cytotoxic effects of oxaliplatin and contributed to oxaliplatin chemoresistance (11). Therefore, targeting IL-8 or CXCR2, in addition to having a direct antiangiogenic and antitumor effect, may also increase chemosensitivity to oxaliplatin.

Recently, our group focused on inhibitors of the IL-8/CXCR2 pathway, which will have the potential not only to have antitumor activity, but to increase the efficacy of already available cytotoxic and targeted drugs, such as oxaliplatin, for patients with colorectal cancer by targeting the tumor and its microenvironment. SCH-527123, as a novel and selective antagonist of the CXCR2, has shown efficacy in the treatment of inflammatory diseases (17). Moreover, Singh and colleagues showed that SCH-527123 treatment inhibited human melanoma cancer growth and colorectal cancer liver metastases by decreasing tumor cell proliferation, angiogenesis, and enhancing the apoptosis of malignant cells (18, 19).

In this study, we show that treatment with SCH-527123 alone and in combination with oxaliplatin is effective in synergistically inhibiting proliferation, angiogenesis, and enhancing chemosensitivity in colorectal cancer cells and xenografts. Taken together, these findings suggest that CXCR2 antagonists, such as SCH-527123, may be important therapeutic candidates in treating colorectal cancer through attenuating the IL-8/CXCR2 signaling cascade, which influences disease progression and modulates the response to oxaliplatin sensitivity.

Materials and Methods

Compounds and reagents

SCH-527123 was obtained from Schering Plough and synthesized in Petasis group. Compound purity was verified by nuclear magnetic resonance and liquid chromatography/mass spectrometry. Oxaliplatin was purchased from Sigma-Aldrich. CellTiter96 Aqueous One Solution was purchased from Promega. Recombinant human IL-8 (rhIL-8) was purchased from R&D systems. Protease inhibitor cocktail was purchased from Roche Molecular Biochemical.

Cell lines

The human HCT116 and Caco2 colorectal cancer cells were purchased from American Type Culture Collection in August 2008 (no authentication was done by the authors). HCT116 and Caco2 IL-8–overexpressing isogenic cell lines (E2 and IIe) were generated as previously described (11). HCT116 and Caco2 cell lines were maintained in McCoy’s 5A and minimum essential medium, respectively, and supplemented with 10% fetal bovine serum, 5% penicillin/streptomycin, sodium pyruvate and l-glutamine (Mediatech). IL-8 overexpression cells were maintained as described above with the addition of 5 μg/mL blasticidin (Invitrogen). Cells were maintained in an incubator at 37°C with 5% CO2. The cell lines were routinely tested to confirm that they were Microplasma free using Mycoalert Mycoplasma Detection Kit (Lonza).

ELISA

The quantification of IL-8 protein was determined using the Quantikine IL-8 ELISA Kit (R&D systems) according to manufacturers’ instructions. More information is described in Supplementary Methods and Materials.

Terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling assay

Cells were plated in adherent conditions in 6-well plates at 50,000 cells per well. After treatment, cells were fixed and stained for the presence of apoptotic cells by the in situ apoptosis detection kit (Trevigen). Nuclei were counterstained with DAPI in red.

Growth inhibition assay and drug combination analysis

Growth inhibition was measured as previously described (11, 20, 21). The combination effect was determined by the combination index (CI) analysis methods of Chou and Talalay (22) using CalcuSyn software (Biosoft), which quantifies the degree of synergy between 2 agents that both induce a linear pharmacologic response. Fraction affected (FA) was calculated from the percentage growth inhibition: FA = (100% growth inhibition)/100. CI values: less than 1, synergism; 1 to 1.2, additive, and more than 1.2, antagonism. Results are representative of 3 independent experiments conducted in duplicate.

Clonogenicity assay

The clonogenicity assay were conducted as previously described (20). Cells were subsequently treated with SCH-527123 and oxaliplatin either alone or in combination for a period of 72 hours. Cells were washed and incubated in drug-free media for 3 weeks to allow colony formation. All experiments were carried out in triplicate. Drug-treated samples were compared directly with untreated controls set at 100%.

siRNA analysis

siRNA analysis was conducted as previously described (11). siRNAs against CXCR2 were purchased from Ambion. Two different siRNA oligonucleotides [#14777/siRNA#1 and #4067/siRNA#2] were tested, and siRNA#2 was used in the described knockdown experiments. Knockdown was validated by mRNA analysis at 72-hour posttransfection. siRNA-treated cells were normalized to negative control siRNA (NC siRNA) for quantitative PCR (qPCR) and growth inhibition analyses.

Quantitative reverse transcriptase PCR

Total RNA was extracted with QIAGEN Kit (QIAGEN) according to the manufacturer’s instructions. IL-8/CXCR2/ERCC1 primers were used for reverse transcriptase PCR (RT-PCR), and target genes were normalized to β-actin and quantified using the 2^(-ΔΔCt) method (23).

Western blot

Cells were solubilized in cell lysis buffer containing a protease inhibitor cocktail. For tissue samples, Tissue
**Protein Extraction Reagent** was used for tumor tissue protein extraction. Primary rabbit polyclonal antibodies were purchased from Cell Signaling Technology, and rabbit anti–β-tubulin antibodies were purchased from Santa Cruz Biotechnology. Quantitative analysis of Western blots was conducted by Image J.

**In vivo studies**

Xenograft experiments were conducted in male C57Bl/6 Balb/c mice (Taconic Labs that were 6- to 8-week old. Subcutaneous HCT116 and E2 xenografts were established and allowed to grow until they reached approximately 100 mm³ (day 0). Animals were randomized to treatment groups: vehicle, SCH-527123, oxaliplatin, and combination of SCH-527123 and oxaliplatin (n = 6 per group). SCH-527123 was administered at 50 mg/kg by oral gavage once daily. Oxaliplatin was administered at 7.5 mg/kg by i.p. injection every 4 days.

**Immunohistochemistry and quantitation of microvascular density**

Immunohistochemistry (IHC) was carried out as previously described (11). Formalin-fixed and snap-frozen fragments of tumor specimens were paraffin embedded and sectioned to 4 μm thickness. Primary antibody rat anti-mouse-CD31 (BD Pharmingen) was added. Microvascular density (MVD) was quantified from 4 different tumor samples by counting the total number of CD31-positive vessels across the whole section of tumors for each experimental condition.

**Statistical analysis**

For all analyses, the difference between each cell lines compared and/or treatment groups were evaluated with a 2-tailed t test or ANOVA from GraphPad software. P < 0.05 was considered statistically significant.

**Results**

**IL-8 overexpression modulated CXCR2 mRNA level in colorectal cancer xenografts**

To investigate the gene expression of IL-8 and CXCR2 from xenograft samples of the HCT116 and HCT116-IL-8-overexpressing cells (E2), which were established from s.c. injections in nude mice, qRT-PCR was used and data revealed that IL-8 and CXCR2 mRNA expression were significantly increased at 26-fold (P < 0.005) and 2.5-fold (P < 0.05), respectively, in E2 xenografts when compared with HCT116 xenografts (Fig. 1A). CXCR1 mRNA expression was not significantly different between the HCT116 and E2 xenografts.

**Knockdown of CXCR2 increased sensitivity to oxaliplatin in colorectal cancer cells**

To examine the role of CXCR2 in colorectal cancer cell proliferation, CXCR2 siRNA was used to suppress CXCR2 expression in colorectal cancer cell lines. Two different CXCR2 siRNA oligonucleotides were tested. After 72 hours posttransfection, CXCR2 knockdown was validated by RT-PCR and qRT-PCR, and siRNA#2 was used in subsequent CXCR2 siRNA knockdown experiments (Fig. 1B). qRT-PCR analysis revealed that transfection of CXCR2 siRNA#2 reduced the mRNA expression of CXCR2 by 45% ± 0.039 and 59% ± 0.049 (siRNA#1 and siRNA#2) in HCT116 cells compared with mock-transfected cells (P < 0.005, Fig. 1B). E2 cells transfected with control mock and negative control siRNA had no evidence of CXCR2 knockdown (Fig. 1B). Our previous study showed that IL-8 overexpression in HCT116 cells decreased sensitivity to the cytotoxic effects of oxaliplatin (11). Having confirmed CXCR2 mRNA knockdown, we examined whether decreased CXCR2 mRNA expression in colorectal cell lines can reduce the IL-8–induced chemoresistance to oxaliplatin. The growth profiles of HCT116, E2, Caco2, and Caco2-Ill (Ill) were analyzed after 24 hours treatment with CXCR2 siRNA, and then subsequently treated for 72 hours with increasing concentrations of oxaliplatin by growth inhibition assay (Fig. 1C and D). There was a decrease in the IC50(72 h) of the CXCR2 siRNA/oxaliplatin-treated cells when compared with mock and β-actin control siRNA/oxaliplatin-treated cells. Importantly, IC50(72 h) of CXCR2 siRNA/oxaliplatin-treated cells that overexpressed IL-8 (E2 and Ill) had significant differences than parental siRNA/oxaliplatin-treated cells. These results suggest that the knockdown of CXCR2 increases sensitivity to oxaliplatin in colorectal cancer cells, especially in IL-8–overexpressing colorectal cancer cells.

**SCH-527123 inhibited colorectal cancer proliferation, migration, and invasion**

To investigate the impact of CXCR2 inhibitors, the effect of SCH-527123 was evaluated in colorectal cancer cells. Single-agent efficacy of SCH-527123 was evaluated by the growth inhibition assays in the HCT116, E2, Caco2, and Ill cells. Cells were treated with increasing concentrations of SCH-527123 for 72 hours and showed dose-dependent growth inhibitory activity with IC50(72 h) values ranging from 18 to 40 μmol/L (Fig. 2A). Importantly the IL-8–overexpressing cells showed a higher IC50(72 h) concentration of SCH-527123 than parental cells [HCT116 and E2 (P < 0.005); 28.9 ± 0.02 μmol/L and 39.5 ± 0.01 μmol/L, respectively; Caco2 and Ill (P < 0.005): 18.8 ± 0.03 μmol/L and 25.5 ± 0.02 μmol/L, respectively (Fig. 2A)]. Therefore, we concluded that SCH-527123 decreased growth inhibitory activity in colorectal cancer cell lines. Next, the effect of SCH-527123 on inducing apoptosis was evaluated with a terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) assay in HCT116 and E2 cells. Data showed a significant increase in apoptosis in cells treated with SCH-527123, with a 10% ± 0.5 (P < 0.005; HCT116) and 25% ± 0.8 (P < 0.005; E2) increase in apoptotic cell after 4-day treatment compared with untreated control cells (Fig. 2B). This result suggests that SCH-527123 resulted in a decrease of cell growth followed by induction of significant apoptosis in colorectal cancer
Figure 1. CXCR2 mRNA expression in colorectal cancer HCT116 and E2 xenografts and the influence of CXCR2 knockdown in colorectal cancer on growth of cells treated with oxaliplatin. A, qRT-PCR determination of IL-8/CXCR2 mRNA expression in HCT116 and E2 xenografts. B, HCT116 cells were transfected with mock, β-actin siRNA, and CXCR2 siRNA. Top, qPCR results are representative at 72-hour posttransfection. Bottom, qRT-PCR results are representative of mean ± SD from triplicate samples and presented as mRNA fold change relative to mock-transfected control. C and D, all cell lines were transfected with mock, β-actin siRNA, and CXCR2 siRNA, and then treated with increasing concentrations of oxaliplatin for 72 hours.
Moreover, we found that 25 μmol/L SCH-527123 was sufficient to block IL-8–mediated CXCR2 activation in all cell lines analyzed, in which phosphorylation of downstream kinases (mitogen-activated protein kinase (MAPK)/NF-κB) of CXCR2 was reduced in a concentration-dependent manner (Supplementary Fig. S1).

Because IL-8 functions to promote cellular migration and invasion, we evaluated the effect of SCH-527123 on migration and invasion of colorectal cancer cells. Treatment with SCH-527123 (24 hours) significantly inhibited migration and invasion of HCT116 (24% ± 0.004, P < 0.005; 60% ± 0.003, P < 0.005) and E2 (39% ± 0.01, P < 0.005; 49% ± 0.004, P < 0.005) cells when compared with control treated cells (Fig. 2C). Similar results were observed after the addition of 10 ng/mL RhIL-8 to HCT116 cells, in which following treatment with 25 μmol/L SCH-527123 there was a significant inhibition of migration (59% ± 0.015, P < 0.005) and invasion (57% ± 0.004, P < 0.005; Fig. 2C, Supplementary Fig. S2).

**SCH-527123 in combination with oxaliplatin synergistically suppressed cell growth and survival**

We next investigated the antiproliferative effects of combining SCH-527123 and oxaliplatin. All 4 cell lines were treated with increasing concentrations of SCH-527123 and oxaliplatin alone and in combination for 72 hours, then growth inhibition was measured by an MTS assay. The median effect analysis method (22)
was used in the evaluation of the combination drug effect. The effects of simultaneous treatment with both agents in HCT116 and Caco2 cell lines produced synergistic growth inhibition resulting in synergistic CI value less than 1 for the majority of concentrations tested at 0.5 FA (Fig. 3A and C, left). The combination of SCH-527123 (10–18 μmol/L) and oxaliplatin (0.25–1.5 μmol/L) in E2 and IIIe cell lines also produced synergistic growth inhibition resulting in FA range of 0.6 to 0.8 and synergistic CI value less than 1 (Fig. 3B and D, left). These results displayed that SCH-527123 synergistically increased sensitivity to oxaliplatin in HCT116 and Caco2 and IL-8–overexpressing cells, which was consistent with CXCR2 siRNA results described above.

To determine whether alterations in sensitivity to SCH-527123 and oxaliplatin observed by growth inhibition assay translated to changes in the ability of cells to recover from drug treatment, a clonogenicity assay was conducted. All 4 cell lines were treated with SCH-527123 and oxaliplatin alone and selected combinations for 72 hours followed by outgrow in drug-free medium for 21 days. Combined drug analysis was conducted with increasing concentrations of both agents. As shown in Fig. 3A–D (right) and Supplementary Fig. S2, in all cell lines, SCH-527123 and oxaliplatin alone resulted in a dose-dependent suppression of colony formation. Importantly, the combinations of SCH-527123 and oxaliplatin synergistically suppressed colony formation at all combinations tested in all cell lines (HCT116: 60%, E2: 64%, Caco2: 72%, IIIe: 60%). Therefore, these findings suggest that targeting CXCR2 increases drug sensitivity of colorectal cancer cells to the one of the current colorectal cancer chemotherapies, oxaliplatin.

**SCH-527123 alone and in combination with oxaliplatin suppressed downstream signaling and modulated apoptotic markers in colorectal cancer cell lines**

To evaluate the expression of CXCR2 and IL-8 in our colorectal cancer cell lines treated with SCH-527123 and oxaliplatin alone and combination, qRT-PCR analysis and ELISA assay were conducted. Although there were no differences in the level of IL-8 mRNA expression in all cell lines with treatment (data not shown), IL-8 protein expression was significantly downregulated in HCT116, E2, and IIIe cells with both agents alone and in combination (Fig. 4A). These data suggest that SCH-527123–mediated antagonism may decrease IL-8 protein expression at the posttranslational level.

To evaluate the effect of combination treatment on IL-8/CXCR2 downstream signaling, the activation of NF-kB/AKT/MAPK pathway was tested by Western blot. All 4 cell lines were treated with SCH-527123 and oxaliplatin alone and combination for 24 hours. Phospho-NF-kB/phospho-AKT/phospho-MAPK were significantly suppressed in all cell lines with combination treatment compared with untreated and single agents alone (Fig. 4B). These results suggest that the effects of SCH-527123 alone and in combination with oxaliplatin may be mediated by NF-kB/AKT/MAPK signaling cascade. Furthermore, using immunoblotting assay, we observed NF-kB–targeted antiapoptotic gene BCL-2 expression, proapoptotic protein BAX expression, which acts as an antagonist of BCL-2 and promotes apoptosis by forming a heterodimer (BCL-2/BAX) and therefore losing the proapoptotic effect of BCL-2, and apoptotic protein PARP activation, which is involved in DNA repair (Fig. 4C). After exposure of all cells to SCH-527123 and oxaliplatin alone and combination, levels of BCL-2 protein expression decreased and BAX protein expression increased. HCT116 and E2 cells showed enhanced cleavage of PARP into 116/89 kDa fragments by combination of both agents compared with oxaliplatin alone (Fig. 4C). These findings suggest that SCH-527123 alone and in combination with oxaliplatin increased apoptosis in colorectal cancer cells.

**SCH-527123 alone or in combination with oxaliplatin enhanced antiproliferative activity in tumor xenografts**

To explore the enhanced antiproliferative effects of SCH-527123 alone or in combination with oxaliplatin on tumor growth in vivo, and to assess the potential for SCH-527123 to compromise oxaliplatin activity, nude mice implanted with HCT116 or E2 cells were exposed to both single agents and their combination. SCH-527123 was administered at 50 mg/kg/d, in which the dose was determined by the in vitro IC₅₀ values and previously published studies. Oxaliplatin was administered at 7.5 mg/kg oxaliplatin every 4 days. Following 21 days of treatment, average tumor volume (TV) for the vehicle treated control, oxaliplatin, and SCH-527123 single-agent treatment groups were 831 ± 133 mm³, 382 ± 69 mm³, and 386 ± 64 mm³, respectively, in the HCT116 xenografts. Interestingly, in the E2 xenografts, SCH-527123 alone decreased the TV with an average of 229 ± 56 mm³ and was more potent than oxaliplatin alone with a TV of 295 ± 61 mm³ in which vehicle-treated control tumors had TV of 391 ± 121 mm³. Importantly, the combination displayed a significant decrease in TV [HCT116: 340 ± 63 mm³ (P < 0.05) and E2: 123 ± 26 mm³ (P < 0.05)], compared with either single agent alone from day 13 through the end of the study (Fig. 5A). Moreover, combination treatment did not cause any significant difference in body weight compared with vehicle-treated control (P = 0.67, Fig. 5B).

On day 21, the tumors were excised and evaluated for the mRNA expression of CXCR2 and IL-8 following treatment with either single agent alone or in combination by qRT-PCR. qRT-PCR analysis showed that the combination of SCH-527123 and oxaliplatin resulted in a significant reduction of CXCR2 and IL-8 mRNA in HCT116 (P < 0.05) and E2 (P < 0.005) xenografts when compared with vehicle-treated controls (Fig. 5C). Measurement of circulating IL-8 levels in serum which were collected at the time of necropsy showed a dramatic decrease in both
Figure 3. SCH-527123 combined with oxaliplatin synergistically suppresses colorectal cancer cell proliferation and survival. A–D, left, growth inhibition assay. A–D, right, clonogenicity assay. All cell lines were treated with increasing concentrations of SCH-527123 and oxaliplatin alone and in combination for 72 hours. Data are presented as histograms of the mean percentage of colony formation compared with untreated controls (100%) ± SD. The combined drug effects were analyzed with the CI with FA values for combinations.
The combination of SCH-527123 and oxaliplatin synergistically suppresses downstream signaling and angiogenic activity in xenograft model

We examined the activity of NF-κB/AKT/MAPK by Western blot in each of the xenografts after SCH-527123 and oxaliplatin treatments at the end of the study. In HCT116 and E2 xenografts, SCH-527123 alone and in combination with oxaliplatin resulted in a significant decrease in the protein activation of phospho–NF-κB/ phospho-AKT/phospho-MAPK (Fig. 6A). These in vivo data are consistent with the in vitro data and confirm that SCH-527123 inhibits the NF-κB/AKT/MAPK signaling cascades in both models.

To establish whether the decreased tumorigenicity and growth of tumors was associated with decreased angiogenesis, IHC was used to measure the expression of CD31 (mouse endothelial cell specific) to evaluate MVD in the tumor specimens. MVD in the HCT116 xenografts was decreased following treatment with SCH-527123 [28% ± 0.04 (P = 0.02)] and oxaliplatin [10% ± 0.05 (P = 0.06)] when compared with vehicle-treated xenografts. Importantly, when used in combination, MVD were significantly decreased by 41% ± 0.05 (P = 0.006) in HCT116 xenografts. Similarly, in the E2 xenografts, MVD was decreased by SCH-527123 by 44% ± 1.08 (P < 0.05), oxaliplatin by 32% ± 0.58 (P < 0.005), and their combination by 67% ± 0.6 (P < 0.005) when compared with vehicle-treated xenografts (Fig. 6B and C). These results show that SCH-527123 in combination with oxaliplatin resulted in a significant inhibition of angiogenic activity as determined by CD31 IHC expression.

Discussion

The role of IL-8 and CXCR2 in tumor development and progression has been well documented in a wide range of cancer types (8, 27–31). Our previous data and others have shown that the IL-8/CXCR2 pathway plays a key role in mediating colorectal cancer development (11, 14, 15, 32). In follow-up to our previously published data in colorectal cancer, this study focused on the impact of inhibition of CXCR2 on the proliferation, survival, invasion, and migration in colorectal cancer in vitro and in vivo models. To the best of our knowledge, this study shows for the first time that the CXCR2 antagonist, SCH-527123, has significant antitumor activity in colorectal cancer preclinical models and can further sensitize colon cancer cells to oxaliplatin-based treatment. Furthermore, our key findings showed that the antitumor activity of SCH-527123 resulted from inhibition of cancer cell growth, motility, and angiogenesis through the NF-κB/AKT/MAPK signaling pathways, and this signaling could be further attenuated when SCH-527123 was coadministered with oxaliplatin.

Previous data provide evidence that IL-8 is constitutively expressed in mCRC and primarily associated with the proliferation, metastasis, and the induction of angiogenesis (11, 33). Important roles of its receptors,
Figure 5. Antitumor activity of SCH-527123 combined with oxaliplatin in HCT116 and E2 xenografts. A, examination of tumor growth in xenografts of colorectal cancer cells. The graphs indicate the mean tumor growth rates ± SEM of each group. Statistical significance was determined by ANOVA with Graphpad Software, P < 0.05. B, mouse body weight represented as the percentage initial body weight at day 21 compared with day 1. C, qRT-PCR was used to determine the level of IL-8, CXCR2, and ERCC1 mRNA in HCT116 and E2 xenografts with treatment. D, serum IL-8 production was measured by ELISA, and the results were normalized to whole blood volume and presented as the mean ± SD. The results are representative of a minimum of 3 independent experiments.
CXCR1 and CXCR2, have also been defined in colorectal cancer progression (34, 35). Our previous study showed that knockdown IL-8 expression can inhibit colorectal cancer cell growth and metastasis. Conversely, IL-8 overexpression can increase colorectal cancer cell metastatic and angiogenic potential, as well as increasing chemoresistance to oxaliplatin (11). However, neutralizing antibodies against other chemokines [such as IL-6 (36), IL-5 (37)] also show similar results. Importantly, CXCR2 has been shown to interact and bind diverse ligands in addition to CXCR1, therefore, resulting in the targeting of IL-8/CXCR2 signaling showing a more effective and broader inhibition in colorectal cancer development and progression.

Several small-molecule inhibitors targeting IL8/CXCR2 signaling have been developed to suppress inflammatory diseases (38, 39). A recent study has shown the potential of SCH-527123 in inhibiting human colon liver metastases using in vivo models (18). In our in vitro study, we showed that SCH-527123 treatment inhibited cell proliferation and induced apoptosis in both HCT116 and Caco2 parental and IL-8–overexpressing colorectal cancer cells. The in vitro findings were validated in an in vivo study in which mice were given SCH-527123 orally and exhibited a reduction in TV when compared with the vehicle-treated control group, which further supported the antiproliferative effect of SCH-527123. SCH-527123 also showed a decrease in tumor vascular density when compared with the vehicle-treated control group. These findings indicated the targeting CXCR2 with antagonists such as SCH-527123 may be a promising therapeutic for colorectal cancer.

In prostate cancer cells, it was reported that IL-8 signaling contributes to the intrinsic resistance of the cancer cells to undergo apoptosis in response to either

![Figure 6. SCH-527123 in combination with oxaliplatin significantly suppressed NF-κB/Akt/MAPK downstream signaling and angiogenic activity in HCT116 and E2 xenografts. A, cell lysates of each tissue were immunoblotted with anti-phospho-NF-κB-p65/Akt/p44/42MAPK or antitotal NF-κB–p65/Akt/p44/42MAPK antibodies; β-tubulin as a loading control and normalization protein for quantitation. B and C, immunohistochemistry and quantitation of MVD (CD31) from tumor tissue slides. In HCT116 and E2 tumor specimens, rat anti-mouse CD31 antibodies were added to tissue sections. Data shown represent the mean ± SD (*, P < 0.05; **, P < 0.005).](image)
environmental or chemical stress (40). One of the key investigations in this study was to evaluate the antiproliferative effects of combining the CXCR2 antagonist with cytotoxic chemotherapy. Oxaliplatin is a platinum-based DNA-damaging chemotherapy that shows clinical benefit for patients with high-risk stage II and III colorectal cancer, as well as patients with advanced disease (41, 42). However, cancer cells are frequently resistant to oxaliplatin (43). Our study showed CXCR2 to be a key mediator of IL-8-mediated chemoresistance to oxaliplatin and using CXCR2siRNA to knockdown its expression in colorectal cancer cells sensitized them to oxaliplatin. These results led to the hypothesis that targeting IL-8/CXCR2 in combination with oxaliplatin may increase sensitivity to oxaliplatin, providing enhanced efficacy and eventually benefit in colorectal cancer treatment. Our novel observation showed that the combination of SCH-527123 and oxaliplatin resulted in synergistic suppression of colorectal cancer cell proliferation and survival with additive to synergistic effects. These synergistic antitumor properties were also observed in the IL-8-overexpressing cell lines, which were more insensitive to single-agent SCH-527123 treatment. Our in vitro data confirmed the in vitro findings and showed that the combination of SCH-527123 and oxaliplatin significantly inhibits tumor growth when compared with single agents alone. ERCC1 mRNA levels have been shown to be predictive of oxaliplatin cytotoxicity in the HCT116 cell line (25) and a useful marker in predicting response to oxaliplatin-based treatment for colorectal cancer patients (44). High ERCC1 mRNA expression has been shown to be associated with resistance to oxaliplatin (26). Supplementary Fig. S3 showed, in an oxaliplatin-resistant cell line (HCT116-OR, in which resistance was induced by long-term culture in the presence of low-dose oxaliplatin), or in E2 cell line, that both cell lines showed an increased level of ERCC1 mRNA expression. Our in vivo data showed that treatment with both agents in combination dramatically lowered ERCC1 mRNA expression, which suggests that SCH-527123 may be associated with increased sensitivity to oxaliplatin through modulation of the DNA nucleotide excision repair pathway.

To elucidate the mechanism of action of SCH-527123, we analyzed the activation of the downstream pathway of IL-8/CXCR2. IL-8/CXCR2 has previously been shown to signal through AKT and MAPK pathways (45, 46). Wilson group showed that IL-8/CXCR2 signaling confers resistance to oxaliplatin through NF-κB activity, which is an important determinant of cancer cell sensitivity to oxaliplatin (40). In this study, our findings suggest that SCH-527123 decreases NF-κB activity through IL-8/CXCR2 signaling, which is in turn responsible for enhancing sensitivity to oxaliplatin. Moreover, our study showed that SCH-527123 in combination with oxaliplatin significantly increased apoptotic signaling in colorectal cancer cells. Therefore, we propose that inhibition of CXCR2/IL-8 signaling increases oxaliplatin sensitivity that is mediated partially by attenuating NF-κB activity and also inducing apoptosis. However, further investigations are warranted to elucidate the mechanism of oxaliplatin sensitivity mediated through NF-κB activity and apoptosis.

In conclusion, our studies provide significant evidence that the CXCR2 antagonist, SCH-527123 shows antitumor effects and increases sensitivity to oxaliplatin therapy in both in vitro and in vivo colorectal cancer models. The antitumor activity of SCH-527123 observed in colorectal cancer cells was shown to be due to decreased cell proliferation, migration, invasion, and increased apoptosis. In addition, it was shown that combination of SCH-527123 and oxaliplatin increased sensitivity to oxaliplatin in colorectal cancer cells in vitro and in vivo. Moreover, the combination of SCH-527123 and oxaliplatin synergistically inhibited in vivo tumor growth and vascularity. Based on these preclinical results, CXCR2 may represent a novel therapeutic target in colorectal cancer, that when targeted in combination with the DNA-damaging agent, oxaliplatin will increase chemosensitivity.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Grant Support
This study was supported by the NIH grant 5 P01CA14089-27S1, the Kroba/Casner Family Foundation, Dhont Family, and Wunderlio Foundation.

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Received November 29, 2011; revised February 15, 2012; accepted February 19, 2012; published OnlineFirst March 5, 2012.

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Mol Cancer Ther; 11(6) June 2012

Molecular Cancer Therapeutics
The CXCR2 Antagonist, SCH-527123, Shows Antitumor Activity and Sensitizes Cells to Oxaliplatin in Preclinical Colon Cancer Models

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*Mol Cancer Ther* 2012;11:1353-1364. Published OnlineFirst March 5, 2012.

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