Cellular Physiology and Biochemistry

Cell Physiol Biochem 2020;54:707-718
DOI: 10.33594/000000250
Published online: 29 July 2020
Accepted: 10 July 2020

Original Paper

Enhancement of Soft Tissue Sarcoma Response to Gemcitabine through Timed Administration of a Short-Acting Anti-Angiogenic Agent

Jin Cheng[a] John Fuller[a] Regina Feldman[a] William Tap[b] Takashi Owa[c] Zvi Fuks[d] Richard Kolesnick[a]

[a]Laboratory of Signal Transduction, Memorial Sloan Kettering Cancer Center, New York, NY, USA, [b]Sarcoma Medical Oncology Service, Memorial Sloan Kettering Cancer Center, New York, NY, USA, [c]Oncology Business Group, Eisai Co Ltd, Koishikawa, Bunkyo-Ku, Tokyo, Japan, [d]Department of Radiation Oncology, Memorial Sloan Kettering Cancer Center, New York, NY, USA

Key Words
Chemotherapy • Endothelial cells • Anti-angiogenic • ASMase • Ceramide

Abstract
Background/Aims: Despite enormous effort, anti-angiogenic drugs have not lived up to the promise of globally-enhancing anti-cancer therapies. Clinically, anti-angiogenic drugs have been used to persistently suppress vascular endothelial growth factor (VEGF) in order to "normalize" dysfunctional neo-angiogenic microvasculature and prevent recruitment of endothelial progenitors. Recently, we showed that a 1h pre-treatment with anti-angiogenic drugs prior to ultra-high single dose radiotherapy and specific chemotherapies transiently de-represses acid sphingomyelinase (ASMase), leading to enhanced cancer therapy-induced, ceramide-mediated vascular injury and tumor response. Here we formally decipher parameters of chemotherapy induction of endothelial sphingolipid signaling events and define principles for optimizing anti-angiogenic chemosensitization. ***Methods:*** These studies examine the anti-metabolite chemotherapeutic gemcitabine in soft tissue sarcoma (STS), a clinically-relevant combination. ***Results:*** Initial studies address the theoretic problem that anti-angiogenic drugs such as bevacizumab, an IgG with a 3-week half-life, have the potential for accumulating during the 3-week chemotherapeutic cycles currently standard-of-care for STS treatment. We show that anti-angiogenic ASMase-dependent enhancement of the response of MCA/129 fibrosarcomas in sv129/BL6 mice to gemcitabine progressively diminishes as the level of the VEGFR2 inhibitor DC101, an IgG, accumulates, suggesting a short-acting anti-angiogenic drug

Richard Kolesnick, MD

Laboratory of Signal Transduction, Memorial Sloan-Kettering Cancer Center
1275 York Avenue, New York, NY 10065 (USA)
Tel. +1-646-888-2174, Fax +1-646-422-0281, E-Mail r-kolesnick@ski.mskcc.org

might be preferable in multi-cycle chemotherapeutic regimens. Further, we show lenvatinib, a VEGFR2 tyrosine kinase inhibitor with a short half-life, to be superior to DC101, enhancing gemcitabine-induced endothelial cell apoptosis and tumor response in a multi-cycle treatment schedule. ***Conclusion:*** We posit that a single delivery of a short-acting anti-angiogenic agent at 1h preceding each dose of gemcitabine and other chemotherapies may be more efficacious for repeated sensitization of the ASMase pathway in multi-cycle chemotherapy regimens than current treatment strategies.

Introduction

Soft Tissue Sarcoma (STS) comprises nearly 70 histologies arising from soft tissue or bone. They represent an estimated number of ~12,000 cases annually in the U.S. with 39% overall mortality [1]. For stage IV STS, palliative chemotherapy remains standard treatment, though this group is highly resistant to chemotherapy and radiation, with 10-30% response rates using combinations of doxorubicin, ifosfamide, and dacarbazine. A recent phase II trial showed gemcitabine/docetaxel superior to gemcitabine alone with improved progression free (3.0 to 6.2 months) and overall survival (11.5 to 17.9 months) [2].

STS tends to be highly vascular, and high VEGF levels are found in STS patients [3-5]. In this context, pre-clinical studies demonstrate that inhibition of angiogenic pathways or disruption of established vasculature can attenuate growth of sarcomas. However, when used as monotherapy in the clinical setting, targeted anti-angiogenic agents have yielded modest progression free survival but no overall survival benefit. Pre-clinical and early clinical data further suggest that addition of anti-angiogenic agents to conventional chemotherapy may lead to more effective therapy, which constitutes an active area of research in STS [6, 7].

Our data indicate that in select settings, activation of acid sphingomyelinase (ASMase)/ceramide signaling in tumor endothelial cells by radiation and certain chemotherapies synergizes with direct tumor cell damage to impact outcome [8-10]. Mechanistically, activation of ASMase, which is preferentially concentrated in endothelial cells [11-13], leads within minutes to formation of plasma membrane ceramide-rich platforms (CRPs), macrodomains that organize apoptotic signaling programs [14]. In preparation for the current studies, we showed that these ASMase/ceramide signaling events are obligate for gemcitabine-induced apoptotic death of bovine aortic endothelial cells and human coronary artery endothelial cells in primary culture [15]. Further support for our concepts derives from studies showing xenografts of all histologies, when implanted in *asmase-/-* host mice become resistant to various chemotherapies, including paclitaxel [10], etoposide [10], and in unpublished studies gemcitabine [16], and to high single dose radiotherapy [8, 9], reversible by adenoviral *asmase* gene delivery exclusive to tumor microvasculature [14]. Critically, we discovered that VEGF is the principal inhibitor of endothelial ASMase, and that anti-angiogenic drugs de-repress ASMase, amplifying tumor responses to anti-cancer therapies, but only under specific conditions [10, 17]. We found irrespective of $t_{1/2}$ or anti-angiogenic class, these drugs enhance endothelial apoptosis and tumor response only if scheduled at 1-2h preceding anti-cancer therapies, as ASMase can be de-repressed for only 1-2h [10].

Lenvatinib is a small-molecule tyrosine kinase inhibitor (TKI) that inhibits vascular endothelial growth factor receptor (VEGFR1-3), fibroblast growth factor receptor (FGFR14), platelet-derived growth factor receptor a (PDGFRa), stem cell factor (KIT) receptor and rearranged during transfection (RET). A difference between lenvatinib and other TKIs with anti-angiogenic properties is its potency with regard to inhibition of FGFR-1, offering a potential opportunity to block one of the well-known mechanisms of resistance to VEGF/VEGFR inhibitors. In this context, evidence indicates that lenvatinib has a direct anti-oncogenic effect to control tumor cell proliferation by inhibiting these oncogenes [18-20]. Lenvatinib is approved by FDA in differentiated thyroid cancer, hepatocellular carcinoma, and renal cell carcinoma as single agent or in combination with chemotherapy. In addition, it has shown promise in several other tumor types including colon, pancreas, NSCLC, mela-

noma, ovary, and epidermoid [21-23]. The current study is designed to test if an anti-angiogenic drug with a short half-life, in this case lenvatinib, is better suited for repeated cycles of chemosensitization of ASMase/ceramide signaling vs. agents engineered for tonic VEGF suppression. We demonstrate that single doses of lenvatinib delivered 1h prior to chemotherapy at 5-10x the normal daily dose are superior to the long-acting VEGFR2 antagonist DC101 in sensitizing gemcitabine-induced endothelial apoptosis and tumor response in sarcoma xenografts, suggesting lenvatinib (or another short acting anti-angiogenic drug) would need to be reformulated to access our sphingolipid biology for therapeutic gain.

Materials and Methods

Drug formulation and administration

Lenvatinib mesylate (E7080, Eisai Co., Ltd.) was provided as a lyophilized powder and resuspended at 5mg/ml in sterile water for administration by oral gavage. DC101 (BE0060, BioCell) was provided at 8.56mg/ml in PBS for administration by intravenous injection. Gemcitabine injection (Zydus Hospira, Ltd) was provided at 38mg/ml in 0.9% NaCl for administration by intraperitoneal injection.

Dose limiting toxicity study

Lenvatinib was delivered to sv129/BL6 mice via oral gavage at 4 doses: 50, 100, 150, and 200mg/kg given once. Lenvatinib was resuspended as directed at 5mg/ml in sterile water in a light protected vial and mixed for 4 hours at 4°C. Mice were observed carefully for the first 2 hours post treatment for acute toxicity and followed for 72 hours based on the $t_{1/2}$ = 27-29 hours. Three mice were used at each dose.

In vivo experiments

Male, 6-8 weeks old sv129/BL6 mice were purchased from The Jackson Laboratory. Mice were housed at the Research Animal Resource Center (RARC) of Memorial Sloan-Kettering Cancer Center. This facility is approved by the American Association for Accreditation of Laboratory Animal Care and is maintained in accordance with the regulation and standards of the U.S. Department of Agriculture and the Department of Health and Human Services, NIH.

MCA/129 fibrosarcoma cells were maintained in DMEM high glucose supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 µg /ml streptomycin at 37°C in a humidified 5% CO_2 chamber. For experiments, $1x10^6$ cells were gently resuspended into 100 µl PBS and injected subcutaneously into the right flank of mice, as described [8]. Once tumors reached a size of 80-100 mm^3, mice were treated every 4 days with 1600 µg/25 gm mouse intravenously or 100mg lenvatinib/kg by oral gavage at 1h preceding intraperitoneal 240mg gemcitabine/kg. Tumor volumes, based on caliper measurements, were calculated daily according to the formula: V=3 x 4.178 x L x W x D/L + W + D [24]. Complete response was defined as no measurable tumor.

Quantification of apoptosis

Endothelial cell apoptosis was quantified *in vivo* in tumor specimens following double staining with TUNEL, to detect apoptotic cells, and the endothelial cell surface marker MECA-32, to identify tumor endothelium [8]. Briefly, mice were sacrificed at 4h after gemcitabine by CO_2 and tumors were fixed in 4% paraformaldehyde, embedded in paraffin, and 5-µm sections were sequentially stained with TUNEL assay and monoclonal antibody MECA-32. Apoptotic endothelial cells display a red-brown TUNEL positive nuclear signal surrounded by a dark blue plasma membrane signal indicative of MECA-32 staining. A minimum of 2000 endothelial cells were evaluated per point.

Statistics

Statistical analysis was performed using GraphPad Prism 7.0. Values are expressed as 95% confidence limits. For endothelial apoptosis experiments, a two-sided Chi Square test was employed to evaluate significance. For tumor growth studies, twosided Fisher's exact t-test was used compare complete response rates. We considered p values <0.05 to be significant.

Results

Current standard of care clinical regimen for STS at MSKCC

Fig. 1 shows a typical regimen for treatment of STS at Memorial Sloan Kettering Cancer Center (MSKCC) delivering gemcitabine on Days 1 and 8 in combination with the taxane docetaxel on Day 8 of each 3-week cycle. A Phase II clinical trial conducted at MSKCC reported no therapeutic benefit of adding bevacizumab (Avastin) to this regimen on Day 1 of each 3-week cycle [25]. This trial design has the theoretic disadvantage of progressive increase in circulating bevacizumab levels as patients remain for extended periods on the trial as the $t_{1/2}$ of bevacizumab, an IgG, is also 3 weeks [26]. Based on this consideration, here we test the hypothesis that an anti-angiogenic with a short half-life might be better suited for repeated cycles of chemosensitization of ASMase signaling compared to agents engineered for long-term VEGF suppression.

A tight pre-treatment window defines anti-angiogenic chemosensitization

Our previously published pre-clinical studies indicate a strict time constraint for anti-angiogenic drug de-repression of ASMase-driven radiosensitization and chemosensitization using antagonistic antibodies to VEGF or VEGFR2 [10, 17, 27] in fibrosarcoma and melanoma allografts. The current studies represent the first to examine impact of this pre-treatment window using gemcitabine, which is a standard of care in sarcoma. For these investigations, sv129/BL6 mice harboring murine flank MCA/129 fibrosarcomas were treated with a maximally-effective dose of anti-VEGFR2 IgG DC101 (1600 μg/25 gm mouse) at 15, 30, 45, 60, 90, 120, 150, 180 min preceding gemcitabine. At 4h post-chemotherapy, the time of maximal endothelial cell apoptosis [10], tumors were harvested and double-stained for endothelial cells by MECA-32 immunohistochemistry and for apoptosis by TUNEL. Apoptotic endothelial cells display a blue perimeter and a brown nucleus using this procedure. Chemosensitization of endothelial cell apoptosis in MCA/129 fibrosarcomas occurred only when anti-VEGFR2 DC101 was provided at -90 min to -45 min relative to gemcitabine, with minimal tumor endothelial apoptosis sensitization at all other times (Fig. 2), consistent with our published data indicating restricted timing of anti-angiogenic drug delivery relative to paclitaxel and etoposide in order to sensitize ASMase-mediated endothelial injury and enhance human HCT-116 colon cancer xenograft complete response and growth delay [10]. This study defines a restrictive temporal relationship between anti-angiogenic drug and gemcitabine delivery designed to optimally engage sphingolipid-based chemosensitization.

Long acting anti-angiogenic drugs render refractoriness to subsequent antiangiogenic sensitization

Long-acting anti-angiogenic antibodies pose a tactical problem in protocols that use 3week treatment cycles, as the half-life of IgGs is also 3 weeks, resulting in progressive accumulation of antibody during a multi-cycle clinical regimen. To address this theoretic problem

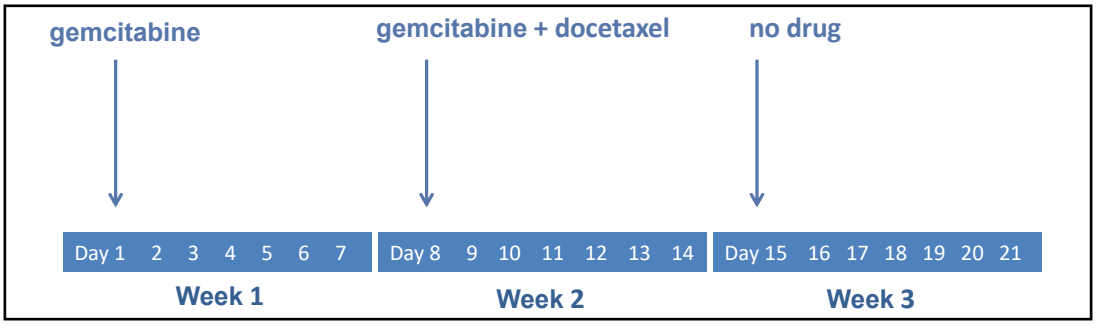

Fig. 1. Scheme depicting the strategy for treatment of advanced sarcoma at MSKCC. Patients are treated with repeated 3-week cycles comprised of gemcitabine (900mg/m^2) on Day 1, gemcitabine (900mg/m^2) + docetaxel (75mg/m^2) on Day 8, and a drug holiday for the third week.

directly, we examined whether having a half or a full dose of anti-VEGFR2 DC101 IgG on board at the time of delivery of full dose DC101 at 1h preceding gemcitabine might impact sphingolipid-based chemosensitization. Note that while the $t_{1/2}$ of IgGs such as DC101 is 21 days in humans, it is 4 days in mice. Conceptually, having a half dose of DC101 on board represents the clinical situation at the time of the 2nd timed dose of a long acting anti-angiogenic immunoglobulin in a 3-week multicycle clinical regimen, while having nearly a full dose of long acting anti-angiogenic drug on board represents the situation at the time of the 4^{th}-5^{th} cycle. For these studies, tumor response was evaluated by caliper measurement and mice harboring tumors were treated with either diluent, half dose DC101, or full dose DC101 at 8h preceding gemcitabine followed by a full dose at 1h before gemcitabine. As in the clinic, STS allografts in mice are largely resistant to gemcitabine, and consistent with our prior studies timed delivery of DC101 at 1h preceding gemcitabine is significantly chemosensitizing. However, if a full dose of DC101 is on board at the time of sphingolipid-based delivery of DC101 followed by gemcitabine, there is highly significant attenuation of chemosensitization (Fig. 3, $p<0.01$ -8h & -1h DC101+Gem vs. -1h DC101+Gem), while a half dose is not attenuating. These informative studies define the parameters of the ASMase/ceramide biology of antiangiogenic chemosensitization and suggest that a short-acting anti-angiogenic drug, such as a VEGFR2 TKI might be preferable to optimally engage our sphingolipid biology in multi-cycle 3-week chemotherapeutic regimens.

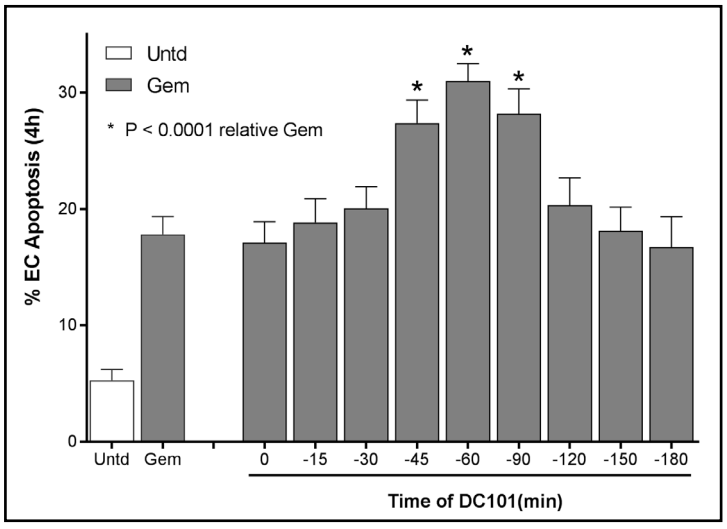

Fig. 2. A 45 min pre-treatment window defines sphingolipid-based anti-angiogenic chemosensitization. $1x10^6$ MCA/129 fibrosarcoma cells were implanted into the right flank of sv129/Bl6JAX mice. When tumors reached an average of $100mm^3$, DC101 (1600 µg/mouse i.v.) was delivered at the indicated times preceding Gemcitabine (Gem; 240 mg/kg i.p.). Mice were sacrificed at 4h after Gem, and 5-µm thicktumor sections were double stained with TUNEL to detect apoptosis and MECA-32 Ab to identify endothelial cells. Data (mean±95% Cl) derive from ~2000 endothelial cells (ECs)/group collated from 3 mice each.

Timed delivery of the short-acting anti-angiogenic drug lenvatinib increases gemcitabine-induced endothelial cell apoptosis

To examine the potential for short-acting anti-angiogenic chemosensitization of endothelial apoptosis *in vivo*, lenvatinib (10-200 mg/kg), which has a $t_{1/2}$ in mice of ~5h and in humans of 28h (Eisai Ltd data on file), was delivered by oral gavage to sv129/BL6 mice harboring 80-100 mm^3 MCA/129 murine flank sarcomas at 1h preceding gemcitabine (240 mg/kg). At 4h post treatment, tumors were harvested, fixed and doublelabeled with MECA-32 antibody and TUNEL to identify endothelial cells undergoing apoptosis, respectively. These studies compare a maximal dose of intravenous DC101 (1600 µg/25 gm mouse delivered 1h before gemcitabine) to a range of oral lenvatinib doses (10-200 mg/kg), examining 4-6 mice/group (except for the 75 mg/kg group which used 3 mice) and 10-12 fields/tumor containing a minimum of 2800 endothelial cells (except for 75 mg/kg group which evaluated proportionally less). Fig. 4 presents representative fields from select groups and shows numerous microvascular endothelial cells undergoing apoptotic death upon anti-angiogenic drug pre-treatment. Fig. 5 shows quantitative data indicating that maximal gem-

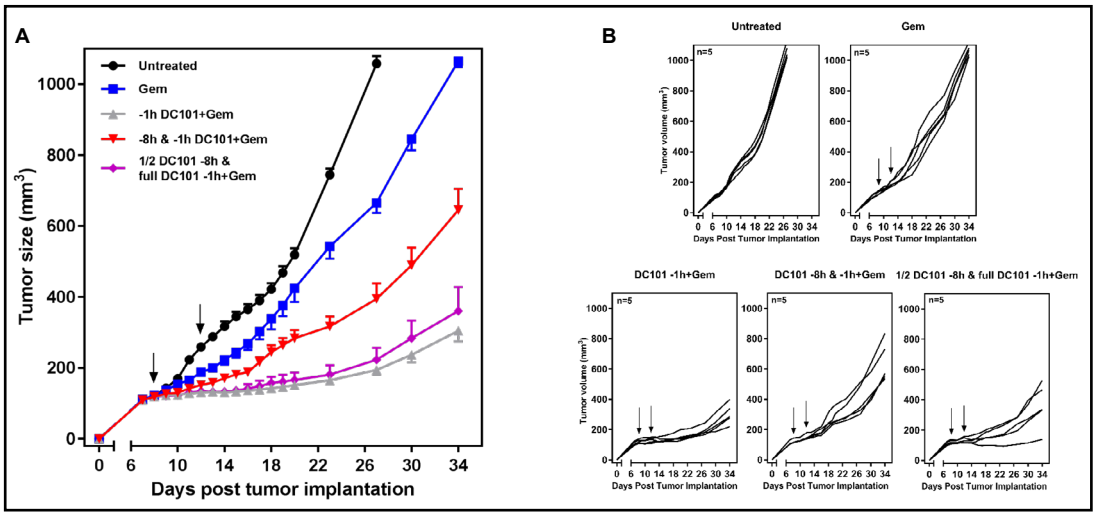

Fig. 3. Evidence that long acting anti-angiogenic drugs render tumors refractory to subsequent anti-angiogenic ASMase-mediated tumor sensitization. A. 1x10^6 MCA/129 fibrosarcoma cells were implanted into the right flank of sv129/BI6JAX mice and tumor volume was measured daily according to the formula of Kim et. al. When tumors reached an average of 100mm^3, mice were treated with gemcitabine 240 mg/ kg i.p. twice at 4 day intervals (black arrows). Some mice received DC101 (1600 µg/mouse = full dose) at 8h and/or 1h before each gemcitabine treatment, as indicated. Data (mean±SEM) are collated from 5 mice/group ($p<0.01$ -8h & -1h DC101+Gem vs. -1h DC101+Gem). B. These data depict the individual tumor response profiles collated in A.

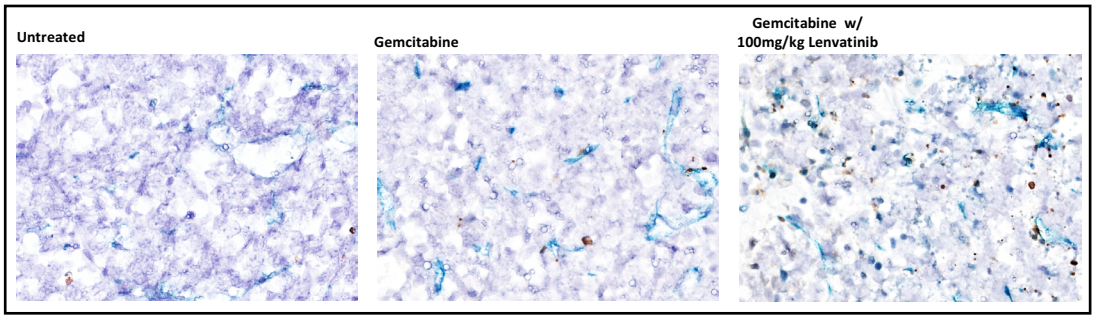

Fig. 4. Lenvatinib increases endothelial cell apoptosis after gemcitabine in vivo. Lenvatinib was administered to sv129/BL6 mice harboring 100mm^3 MCA/129 fibrosarcoma flank tumors and 1h later mice were treated with 240 mg/kg of gemcitabine. After 4h mice were sacrificed, and 5-µm thick tumor sections were double-stained using TUNEL labeling and MECA-32 Ab to identify apoptotic endothelial cells (ECs). Representative fields are shown. Apoptotic endothelial cells exhibit a brown TUNEL-positive nuclear signal surrounded by a dark blue plasma membrane signal for MECA-32.

citabine increases apoptotic endothelial cells from a baseline of 4±1% of the endothelial population to only 11±1% at 4h post treatment, a value previously shown by us to be largely ineffective in inducing tumor response [10]. However, timed delivery of DC101 increases gemcitabine-induced endothelial apoptosis to 26±2% of the population, previously shown by us to induce significantly-enhanced tumor response [10, 27]. Pre-treatment with lenvatinib dosedependently enhanced gemcitabine-induced endothelial apoptosis to a peak of 32±2% of the population, a value statistically greater than that induced by maximal DC101 ($p<0.0001$). These investigations indicate that the short-acting anti-angiogenic drug lenvatinib is at least as effective as the long-acting anti-angiogenic drug DC101 in enhancing gemcitabine-induced endothelial cell apoptosis. Of note, a complementary dose limiting toxicity trial showed gavage of lenvatinib at 50-200 mg/kg was well tolerated, as no signs or symptoms of stress

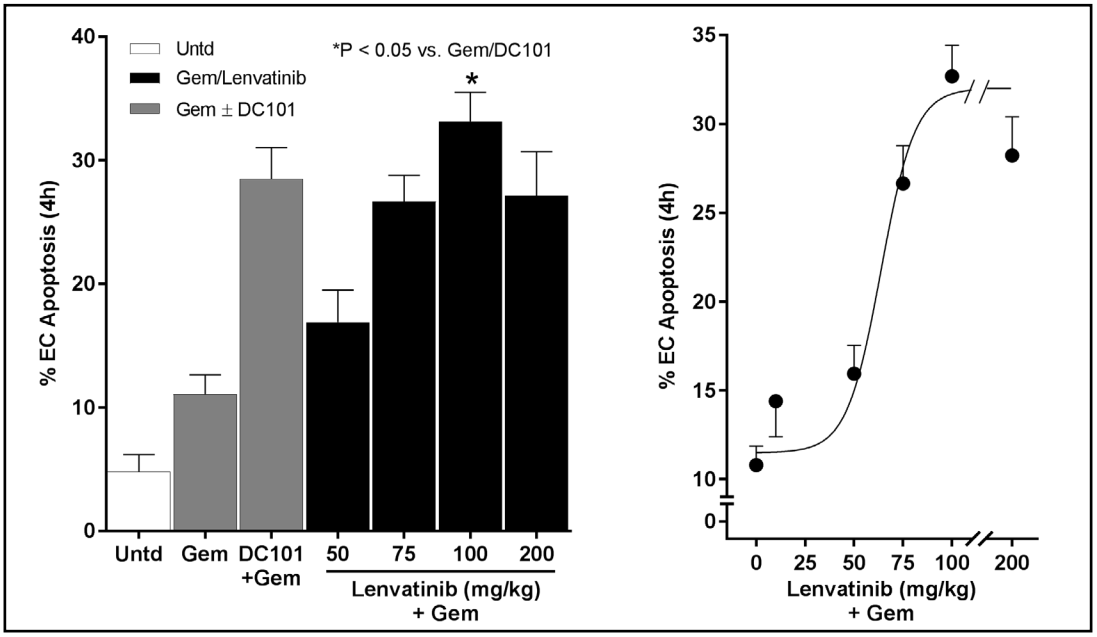

Fig. 5. Lenvatinib is more effective than anti-VEGFR2 DC101 in sensitizing gemcitabine-induced apoptosis. MCA/129 fibrosarcomas were implanted into the flank of sv129BL/6 mice. When tumors reached an average of 100mm^{3,} animals were treated with DC101 or increasing doses of lenvatinib 1h before a single 240 mg/kg dose of gemcitabine. Tumors were harvested at 4h after gemcitabine treatment, the time of maximal endothelial damage, and double-stained with TUNEL for apoptosis and Meca32 for endothelial cells (ECs). Data (mean±95% Cl) are collated from 4-6 mice/group. Results show lenvatinib is more effective than DC101 in enhancing ASMase-dependent endothelial apoptosis.

or toxicity were observed at this range of doses (Supplementary Table S1 – for all supplementary material see www.cellphysiolbiochem.com).

Lenvatinib is more effective than anti-VEGFR2 DC101 in sensitizing gemcitabine-induced tumor response

Fig. 6 presents data from two independent tumor response studies using MCA/129 fibrosarcomas implanted in sv129/BL6 mice. The study was designed to mimic the human clinical trial [25], which delivered standard of care gemcitabine (Days 1 & 8) and docetaxel (Day 8) plus bevacizumab (Day 1), an IgG with a 21-day half-life, in multiple 3-week cycles. As the $t_{1/2}$ of an IgG in mice is 4 days, each cycle for our mouse lenvatinib study compared gemcitabine with or without anti-angiogenic drug (maximal dose DC101 at 1600 μg/25 gm mouse or lenvatinib at 100 mg/kg) delivered 1h before gemcitabine every 4 days. Collated and individual tumor data are shown for each experiment. It is clear that lenvatinib, delivered according to the temporal principles of ASMase/ceramide biology, is the superior drug for chemosensitization of tumor response as compared with DC101 ($p<0.001$).

Discussion

Clinical use of anti-angiogenic drugs is predicated on two non-mutually exclusive concepts that have dictated how this class of drugs has been delivered in human therapy. The concepts are that anti-VEGF agents will: 1) normalize tumor vasculature to improve blood flow/delivery of cytotoxic agents, and 2) inhibit recruitment of endothelial progenitors via the systemic circulation [28, 29]. Timed delivery of anti-angiogenic drugs to transiently de-repress ASMase/ceramide signaling prior to chemotherapy is thus a new approach for use of this class of agents with associated potential benefits and liabilities.

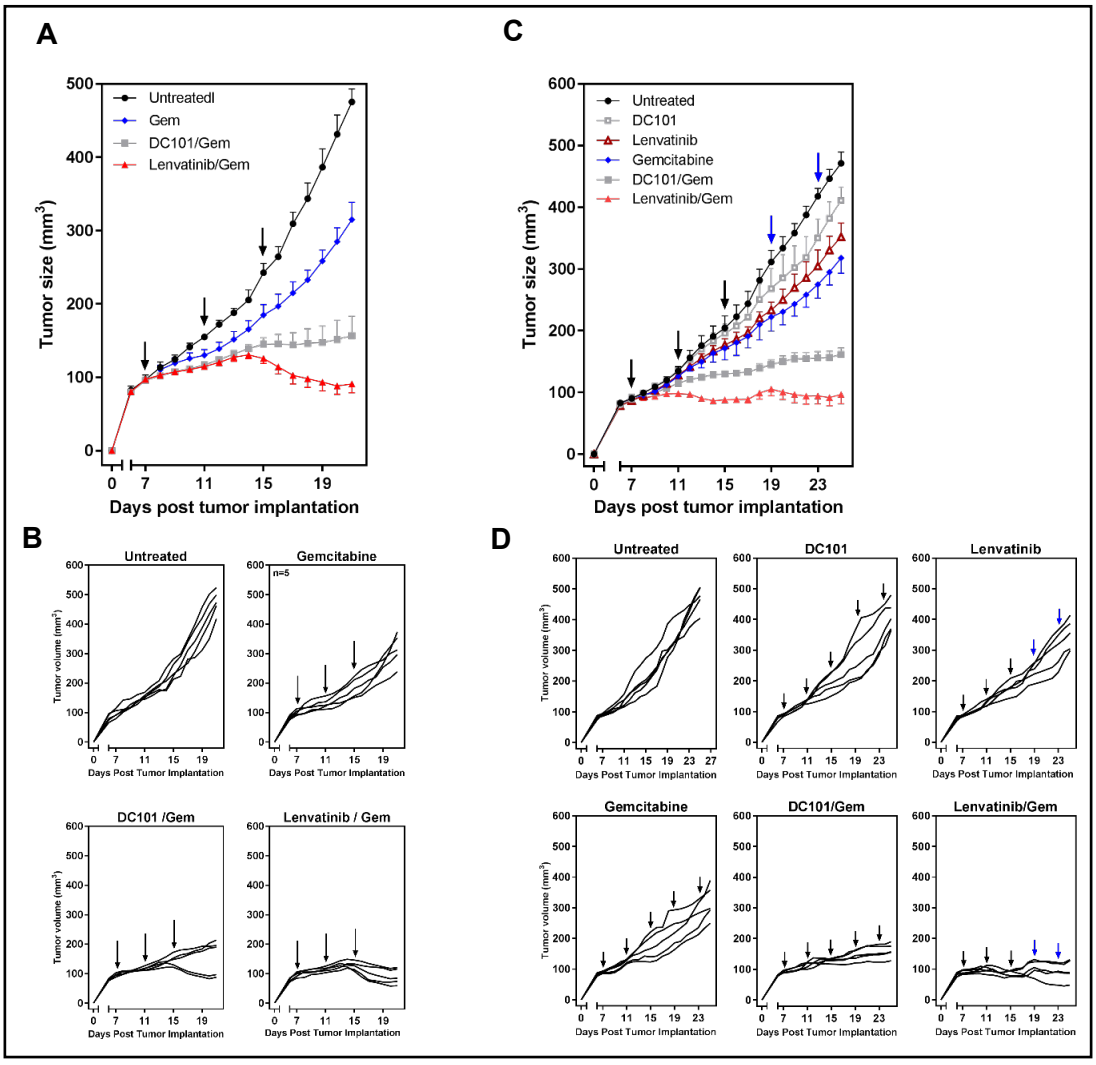

Fig. 6. Lenvatinib is more effective than anti-VEGFR2 DC101 in sensitizing gemcitabine-induced tumor response. MCA/129 fibrosarcomas tumors were implanted into the flank of a sv129BL/6 mice. When tumors reached an average of 80mm^3, animals were treated every 4 days with 1600 µg/mouse DC101 or 100 mg/kg lenvatinib at 1h before 240 mg/kg of gemcitabine. In each experiment, 5 mice were used per group. Tumors were measured daily through the course of the experiment. Arrows designate treatment days. Black arrows indicate lenvatinib was prepared at time of first treatment, blue arrows indicate that lenvatinib was prepared the day of treatment. A. Collated tumor growth for Experiment #1 shows a significant difference ($p<0.001$) between treatment groups of lenvatinib+gemcitabine vs. gemcitabine alone. Black arrows designate treatment days. B. Individual tumor response for Experiment #1. C. Collated tumor growth for Experiment #2 shows a significant difference ($p<0.001$) between lenvatinib+gemcitabine and all other treatment groups. D. Individual tumor response for Experiment #2. Note in the lenvatinib +gemcitabine group, the slight regrowth from day 15 to 19, is suppressed when fresh lenvatinib was prepared.

TKIs as a class were designed for daily use to continuously suppress VEGF. Resulting sustained anti-angiogenesis is associated with bleeding problems, which at times can be severe [30, 31]. While there is no evidence that a single dose of TKI, even at doses beyond the daily dose, results in excessive toxicity, even a single dose of a long acting anti-angiogenic immunoglobulin can, at times, enhance bleeding [32, 33], suggesting a substantive advantage of delivery of a TKI timed to chemotherapy. A potential disadvantage of use of lenvatinib is that the dose required for optimal ASMase-based chemosensitization is significantly higher than

the allometrically scaled daily dose. In this context, lenvatinib delivered dose-dependent enhancement of ASMase-mediated vascular biologic effects, optimal at 100 mg/kg lenvatinib, a dose equivalent to 8-10x the isoeffective human daily dose (10 mg/kg in mice = 20 mg/day in humans, which is within the upper range of the human daily dose) (personal communication, Dr. Takashi Owa). Further, this is approximately 6-8 times the maximally-tolerated dose of 25mg/day determined using sustained exposure [34-36]. Whether use of other TKIs timed to ASMase/ceramide signaling to enhance chemotherapy will similarly require doses exceeding the daily maximally-tolerated dose will require additional investigation.

A second less important disadvantage of using lenvatinib is the inconvenience of the currently-available pill dosage. Hence rather than having patients take 5-10 pills at one time to attain ASMase-dependent chemosensitization, it probably would be most efficacious if the TKI were reformulated for single high dosing, assuming that this strategy is safe and demonstrates clinical effectiveness.

While lenvatinib enhancement of gemcitabine-induced endothelial cell damage is delineated in the current studies, the mechanism of such chemosensitization of tumor effect remains under investigation. Preliminary data indicate that this ASMase/ceramide effect involves enhancement of gemcitabine uptake into both endothelial and tumor cells. In this context, the role of ceramide signaling in facilitated diffusion of nucleosides and nucleoside drugs is an active area of investigation in our laboratory.

Another importance of the current findings is that they add to the ongoing body of evidence that the ceramide signaling pathway is pharmacologically tractable. Gilenya, a sphingosine 1-phosphate receptor antagonist, an effective inhibitor of T cell egress from lymph nodes, is approved for treatment of relapsing remitting multiple sclerosis [37, 38], while eliglustat, a glucosyl ceramide synthase competitive inhibitor is approved for treatment of Gaucher's Disease [39-41]. Other strategies to enhance or inhibit ASMase/ceramide signaling are under development including ceramide-nanoliposomes for treatment of cancer [42-44], a dihydroceramide desaturase 1 (DES1) inhibitor for inhibition of diabetic lipodystrophy [45], and anti-ceramide antibodies for treatment of the Radiation GI Syndrome [46].

Here we address which class of anti-angiogenic drugs is preferable to optimize sphingolipid-based signaling. Based on the pharmacokinetics (PK) of long-acting antiangiogenics, and the dynamics of ASMase de-repression, we posit that an antiangiogenic agent with a short half-life may be more efficacious for repeated sensitization of the ASMase pathway in multi-cycle chemotherapy regimens, as prolonged VEGF inhibition using antibodies with half-lives of weeks violates the precise time-window for chemosensitization, rendering ASMase refractory to subsequent rounds of anti-VEGF derepression, likely sustained until decay of the anti-angiogenic effect re-sets ASMase sensitivity.

Despite enormous effort on the part of the medical community, anti-angiogenic drugs have not lived up to the promise of globally-enhancing anti-cancer therapies. The current studies provide the first support for an alternative use of a short-acting VEGFR2 TKI, such as lenvatinib, to solve the half-life problem associated with long-acting anti-angiogenic drugs, such as bevacizumab, in sensitizing, via ASMase, multi-cycle chemotherapy regimens required for treatment of human cancer. We believe that human clinical trials testing these concepts in STS are warranted.

Conclusion

A single dose of a short-acting VEGFR2 TKI, delivered at 1h preceding each dose of gemcitabine and other chemotherapies, may be more efficacious than a long-acting immunoglobulin for repeated ASMase/ceramide-mediated sensitization of endothelial apoptosis and tumor response in multi-cycle chemotherapy regimens.

Acknowledgements

This work was supported by Mr. William H. Goodwin and Mrs. Alice Goodwin and the Commonwealth Foundation for Cancer Research and The Center for Experimental Therapeutics at Memorial Sloan Kettering Cancer.

Author contributions

JC, JF and RF performed experiments and analyzed data; JC and RK wrote the manuscript; WT and TO provided scientific expertise; ZF and RK provided overall scientific direction and supervision.

Funding

This research was funded in part by Grant 11950073 from the NCI Exploratory/Developmental Research Grant Program (NCI OMNIBUS R21) and a Sponsored Research Agreement between MSKCC and Ceramedix Holding, LLC. Lenvatinib and an Investigator-initiated Research Award were provided by Eisai. The sponsors had no role in the study design, analysis, or interpretation.

Disclosure Statement

Patents unrelated to this work: RK (US7195775B1, US7850984B2, US10052387B2, US8562993B2, US9592238B2, US20150216971A1, and US20170335014A1, US20170333413A1, US20180015183A1, US10414533B2, US10450385B2), ZF (US 10413533B2, US20170333413A1, and US20180015183A1).

Richard Kolesnick and Zvi Fuks are co-funders of Ceramedix Holding L.L.C. The remaining authors have declared that no conflicts of interest exist.

References

1 Siegel R, Ma J, Zou Z, Jemal A: Cancer statistics, 2014. CA Cancer J Clin 2014;64:9-29.
2 Maki RG, Wathen JK, Patel SR, Priebat DA, Okuno SH, Samuels B, et al.: Randomized phase II study of gemcitabine and docetaxel compared with gemcitabine alone in patients with metastatic soft tissue sarcomas: results of sarcoma alliance for research through collaboration study 002 [corrected]. J Clin Oncol 2007;25:2755-2763.
3 Feldman AL, Pak H, Yang JC, Alexander HR, Jr., Libutti SK: Serum endostatin levels are elevated in patients with soft tissue sarcoma. Cancer 2001;91:1525-1529.
4 Graeven U, Andre N, Achilles E, Zornig C, Schmiegel W: Serum levels of vascular endothelial growth factor and basic fibroblast growth factor in patients with soft-tissue sarcoma. J Cancer Res Clin Oncol 1999;125:577-581.
5 Yoon SS, Segal NH, Olshen AB, Brennan MF, Singer S: Circulating angiogenic factor levels correlate with extent of disease and risk of recurrence in patients with soft tissue sarcoma. Ann Oncol 2004;15:1261-1266.
6 Jain RK: Antiangiogenesis strategies revisited: from starving tumors to alleviating hypoxia. Cancer Cell 2014;26:605-622.
7 Wong PP, Bodrug N, Hodivala-Dilke KM: Exploring Novel Methods for Modulating Tumor Blood Vessels in Cancer Treatment. Curr Biol 2016;26:R1161-R1166.
8 Garcia-Barros M, Paris F, Cordon-Cardo C, Lyden D, Rafii S, Haimovitz-Friedman A, et al.: Tumor response to radiotherapy regulated by endothelial cell apoptosis. Science 2003;300:1155-1159.
9 Fuks Z, Kolesnick R: Engaging the vascular component of the tumor response. Cancer Cell 2005;8:89-91.
10 Marathe S, Schissel SL, Yellin MJ, Beatini N, Mintzer R, Williams KJ, et al.: Human vascular endothelial cells are a rich and regulatable source of secretory sphingomyelinase. Implications for early atherogenesis and ceramide-mediated cell signaling. J Biol Chem 1998;273:4081-4088.

11 Santana P, Pena LA, Haimovitz-Friedman A, Martin S, Green D, McLoughlin M, et al.: Acid sphingomyelinase-deficient human lymphoblasts and mice are defective in radiation-induced apoptosis. Cell 1996;86:189-199.
12 Tabas I: Secretory sphingomyelinase. Chem Phys Lipids 1999;102:123-130.
13 Stancevic B, Kolesnick R: Ceramide-rich platforms in transmembrane signaling. FEBS Lett 2010;584:1728-1740.
14 van Hell AJ, Haimovitz-Friedman A, Fuks Z, Tap WD, Kolesnick R: Gemcitabine kills proliferating endothelial cells exclusively via acid sphingomyelinase activation. Cell Signal 2017;34:86-91.
15 Jacobi J, Garcia-Barros M, Rao S, Rotolo JA, Thompson C, Mizrachi A, et al.: Targeting acid sphingomyelinase with anti-angiogenic chemotherapy. Cell Signal 2017;29:52-61.
16 Ganju A, Kolesnick R.: manuscript in preparation, 2020.
17 Rao SS, Thompson C, Cheng J, Haimovitz-Friedman A, Powell SN, Fuks Z, et al.: Axitinib sensitization of high Single Dose Radiotherapy. Radiother Oncol 2014;111:88-93.
18 Capozzi M, De Divitiis C, Ottaiano A, von Arx C, Scala S, Tatangelo F, et al.: Lenvatinib, a molecule with versatile application: from preclinical evidence to future development in anti-cancer treatment. Cancer Manag Res 2019;11:3847-3860.
19 Suyama K, Iwase H: Lenvatinib: Lenvatinib: A Promising Molecular Targeted Agent for Multiple Cancers. Cancer Control 2018;25:1073274818789361.
20 Yamamoto Y, Matsui J, Matsushima T, Obaishi H, Miyazaki K, Nakamura K, et al.: Lenvatinib, an angiogenesis inhibitor targeting VEGFR/FGFR, shows broad antitumor activity in human tumor xenograft models associated with microvessel density and pericyte coverage. Vasc Cell 2014;6:18.
21 Jayson GC, Kerbel R, Ellis LM, Harris AL: Antiangiogenic therapy in oncology: current status and future directions. Lancet 2016;388:518-529.
22 Lorusso L, Pieruzzi L, Biagini A, Sabini E, Valerio L, Giani C, et al.: Lenvatinib and other tyrosine kinase inhibitors for the treatment of radioiodine refractory, advanced, and progressive thyroid cancer. Onco Targets Ther 2016;9:6467-6477.
23 Stjepanovic N, Capdevila J: Multikinase inhibitors in the treatment of thyroid cancer: specific role of lenvatinib. Biologics 2014;8:129-139.
24 Kim JH, Alfieri AA, Kim SH, Young CW: Potentiation of radiation effects on two murine tumors by lonidamine. Cancer Res 1986;46:1120-1123.
25 Dickson MA, D'Adamo DR, Keohan ML, D'Angelo SP, Carvajal RD, Gounder MM, et al.: Phase II Trial of Gemcitabine and Docetaxel with Bevacizumab in Soft Tissue Sarcoma. Sarcoma 2015;2015:532478.
26 Kazazi-Hyseni F, Beijnen JH, Schellens JH: Bevacizumab. Oncologist 2010;15:819-825.
27 Truman JP, Garcia-Barros M, Kaag M, Hambardzumyan D, Stancevic B, Chan M, et al.: Endothelial membrane remodeling is obligate for anti-angiogenic radiosensitization during tumor radiosurgery. PLoS One 2010; DOI:10.1371/annotation/6e222ad5-b175-4a00-9d04-4d120568a897.
28 Jain RK: Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science 2005;307:58-62.
29 Shaked Y, Henke E, Roodhart JM, Mancuso P, Langenberg MH, Colleoni M, et al.: Rapid chemotherapy-induced acute endothelial progenitor cell mobilization: implications for antiangiogenic drugs as chemosensitizing agents. Cancer Cell 2008;14:263-273.
30 Je Y, Schutz FA, Choueiri TK: Risk of bleeding with vascular endothelial growth factor receptor tyrosine-kinase inhibitors sunitinib and sorafenib: a systematic review and meta-analysis of clinical trials. Lancet Oncol 2009;10:967-974.
31 Qi WX, Tang LN, Sun YJ, He AN, Lin F, Shen Z, et al.: Incidence and risk of hemorrhagic events with vascular endothelial growth factor receptor tyrosine-kinase inhibitors: an up-to-date meta-analysis of 27 randomized controlled trials. Ann Oncol 2013;24:2943-2952.
32 Chen HX, Cleck JN: Adverse effects of anticancer agents that target the VEGF pathway. Nat Rev Clin Oncol 2009;6:465-477.
33 Zangari M, Fink LM, Elice F, Zhan F, Adcock DM, Tricot GJ: Thrombotic events in patients with cancer receiving antiangiogenesis agents. J Clin Oncol 2009;27:4865-4873.
34 Boss DS, Glen H, Beijnen JH, Keesen M, Morrison R, Tait B, et al.: A phase I study of E7080, a multitargeted tyrosine kinase inhibitor, in patients with advanced solid tumours. Br J Cancer 2012;106:1598-1604.

35 Kurzrock R, Sherman SI, Ball DW, Forastiere AA, Cohen RB, Mehra R, et al.: Activity of XL184 (Cabozantinib), an oral tyrosine kinase inhibitor, in patients with medullary thyroid cancer. J Clin Oncol 2011;29:2660-2666.
36 Yamada K, Yamamoto N, Yamada Y, Nokihara H, Fujiwara Y, Hirata T, et al.: Phase I dose-escalation study and biomarker analysis of E7080 in patients with advanced solid tumors. Clin Cancer Res 2011;17:2528-2537.
37 Calabresi PA, Radue EW, Goodin D, Jeffery D, Rammohan KW, Reder AT, et al.: Safety and efficacy of fingolimod in patients with relapsing-remitting multiple sclerosis (FREEDOMS II): a double-blind, randomised, placebo-controlled, phase 3 trial. Lancet Neurol 2014;13:545-556.
38 Khatri B, Barkhof F, Comi G, Hartung HP, Kappos L, Montalban X, et al.: Comparison of fingolimod with interferon beta-1a in relapsing-remitting multiple sclerosis: a randomised extension of the TRANSFORMS study. Lancet Neurol 2011;10:520-529.
39 Cox TM, Drelichman G, Cravo R, Balwani M, Burrow TA, Martins AM, et al.: Eliglustat compared with imiglucerase in patients with Gaucher's disease type 1 stabilised on enzyme replacement therapy: a phase 3, randomised, open-label, non-inferiority trial. Lancet 2015;385:2355-2362.
40 Lukina E, Watman N, Arreguin EA, Dragosky M, Iastrebner M, Rosenbaum H, et al.: Improvement in hematological, visceral, and skeletal manifestations of Gaucher disease type 1 with oral eliglustat tartrate (Genz-112638) treatment: 2-year results of a phase 2 study. Blood 2010;116:4095-4098.
41 Mistry PK, Lukina E, Ben Turkia H, Amato D, Baris H, Dasouki M, et al.: Effect of oral eliglustat on splenomegaly in patients with Gaucher disease type 1: the ENGAGE randomized clinical trial. JAMA 2015;313:695-706.
42 Barth BM, Cabot MC, Kester M: Ceramide-based therapeutics for the treatment of cancer. Anticancer Agents Med Chem 2011;11:911-919.
43 Kester M, Bassler J, Fox TE, Carter CJ, Davidson JA, Parette MR: Preclinical development of a C6-ceramide NanoLiposome, a novel sphingolipid therapeutic. Biol Chem 2015;396:737-747.
44 Lin L, Liu A, Peng Z, Lin HJ, Li PK, Li C, et al.: STAT3 is necessary for proliferation and survival in colon cancer-initiating cells. Cancer Res 2011;71:7226-7237.
45 Chaurasia B, Tippetts TS, Mayoral Monibas R, Liu J, Li Y, Wang L, et al.: Targeting a ceramide double bond improves insulin resistance and hepatic steatosis. Science 2019;365:386-392.
46 Rotolo J, Stancevic B, Zhang J, Hua G, Fuller J, Yin X, et al.: Anti-ceramide antibody prevents the radiation gastrointestinal syndrome in mice. J Clin Invest 2012;122:1786-1790.