

VU Research Portal

Radiotherapy and angiogenesis inhibition: From bench to bedside

Kleibeuker, E.A.

2016

document version

Publisher's PDF, also known as Version of record

[Link to publication in VU Research Portal](#)

citation for published version (APA)

Kleibeuker, E. A. (2016). *Radiotherapy and angiogenesis inhibition: From bench to bedside*.

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

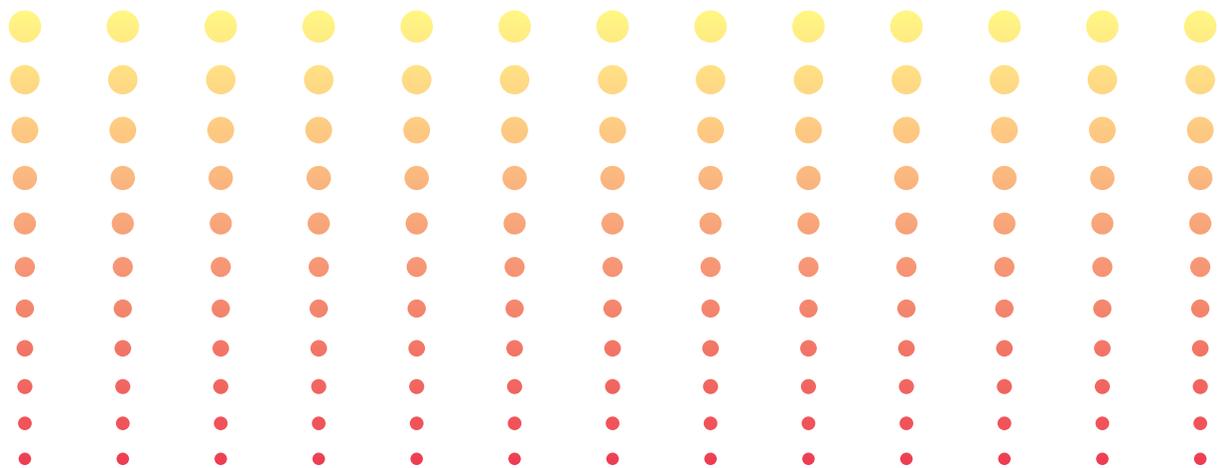
E-mail address:

vuresearchportal.ub@vu.nl

Chapter 9

General discussion

Challenges and opportunities



Introduction

Radiation therapy (RTx) has been an important anti-cancer treatment modality for over 100 years. Technical advances have led to more accurate targeting and dosing of the ionizing radiation which has improved the anti-tumor effect and reduced unwanted side effects in normal tissue. Increasing insight in the cellular mechanisms underlying the response to RTx has provided opportunities for the development of radiosensitizing drugs. For example, the main anti-tumor effect of RTx is the induction of an excess of DNA-damage, resulting in cell death or senescence. Consequently, the combination of RTx with drugs that inhibit DNA-repair has been recognized as a promising combination therapy (1-3). Indeed, pre-clinical studies demonstrate improved tumor growth inhibition when these treatment modalities are combined (3-7). Research has demonstrated that RTx not only causes DNA-damage, but also affects processes in the tumor micro-environment, such as the immune response and the formation of new blood vessels, i.e. angiogenesis (8-11). Regarding the combination of RTx with angiogenesis inhibition, pre-clinical research has demonstrated a potential benefit (12-15). However, since the mechanisms underlying this effect are still elusive, little is known about the optimal dose scheduling for the combination therapy. Together with rare but severe side effects observed in patients, lack of mechanistic insight hampers the clinical implementation (16-18). Therefore, the research described in this thesis was aimed to 1) Study the effects of RTx on tumor angiogenesis and 2) Investigate the efficacy of different treatment schedules of the combination treatment of angiogenesis inhibition and RTx. Here, we discuss the novel insights obtained during our research. These insights are put in broader perspective with the current literature. In addition, we present the opportunities and future challenges of combining RTx with anti-angiogenic drugs.

The effect of radiation therapy on tumor angiogenesis

To better understand the effects of RTx on angiogenesis we used the chicken chorioallantoic membrane (CAM) assays as an *in vivo* model. We observed that mature and larger vessels were more resistant to single dose RTx as compared to the immature and smaller vessels. It has been described before that the established vasculature is more resistant to RTx. This can be explained by the low proliferation rate of endothelial cells (i.e. cells that align the blood vessel) of these vessels (19;20). However, the proliferation rate of endothelial cells in the tumor is up to 20 times higher than in normal tissue, due to the constant stimulation of pro-angiogenic factors (20). Their proliferation rate is however still a 3 fold lower than of the cancer cells, making them less sensitive to RTx (21). This differential sensitivity of endothelial and cancer cells could be beneficial to the anti-tumor effect of RTx (22). If blood vessels are less sensitive to RTx the oxygenation of the tumor is maintained during therapy which is important for tumor radiosensitivity. Thus, the well oxygenated remaining cancer cells are more sensitive to the next fraction of RTx treatment. Although this principle is recognized as one of the Rs of radiation

therapy (reoxygenation), the beneficial effect only applies to fractionated RTx. In fact, it could be argued that reoxygenation counteracts single dose RTx because tumor cells that survive the RTx may proliferate more rapidly in a well oxygenated environment. This provides an opportunity to improve the efficacy of single dose RTx with an anti-angiogenic drug. Indeed, in chapter 5 we describe that sunitinib given after single dose RTx enhanced the anti-tumor effect.

Since the most frequently applied RTx schedule in the curative setting is fractionated RTx we also studied the effect of fractionated RTx on angiogenesis and tumor perfusion (Chapter 6). Using a human xenograft model in nude mice we found that fractionated RTx enhances the tumor perfusion 2 weeks after start of treatment, and reduced tumor hypoxia. While it has been described that these observations occur after completion of fractionated RTx (23;24), our data indicate that it already occurs during the course of RTx. As discussed above, the enhanced perfusion could sensitize the cancer cells to subsequent doses of RTx. However, we also observed cancer cell repopulation in the center of the tumor, which was mainly necrotic in the non-irradiated tumor. Cancer cell repopulation has been recognized as an important cause of treatment failure (25). Our results demonstrate that application of sunitinib after start of fractionated RTx reduced the tumor perfusion and prevented the increase in viable tissue in the centre of the tumor (chapter 6). These data suggest that it is beneficial to use anti-angiogenic drugs to interfere with enhanced tumor perfusion during fractionated RTx.

Regarding the mechanism(s) underlying the increased tumor perfusion, we found that fractionated RTx induces cancer cells to produce multiple pro-angiogenic factors, both *in vitro* and *in vivo* (Chapter 6) (26;27). While we also observed an induction of these growth factors after single dose RTx, the induction was much smaller than after fractionated RTx. The mechanism underlying this difference remains elusive. An explanation could be that the relatively low dose irradiation during fractionated treatment triggers activation of survival pathways in the cancer cell that result in enhanced expression of pro-angiogenic factors. For example, we demonstrate that cancer cells have the capacity to activate type I IFN response after fractionated RTx, without the interference of immune cells (Chapter 8). This activation appears to be induced via the STING signaling pathway, similar as described in immune cells. While previous studies indicate that stimulation of the IFN pathway inhibits tumor growth, other studies describe that certain IFN-inducible genes are involved in radioresistance of a tumor (28-30). Interestingly, it has been described that activation of the STING pathway also results in activation of NF- κ B signaling (31). NF- κ B is a pro-survival factor and also known to induce the expression of pro-angiogenic factors, including vascular endothelial growth factor (VEGF), thereby inducing angiogenesis (32-34). In concordance, blockade of NF- κ B reduces the expression of VEGF and consequently tumor angiogenesis (33;35). The induction of type I IFN response could thus be related to the enhanced tumor perfusion during fractionated RTx.

While at the end of fractionated RTx the accumulated high dose will have killed most cancer cells, we also demonstrate *in vitro* that after 10x 2 Gy, the surviving cancer cells become more resistant to RTx, reaching a plateau after 20 fractions (chapter 8). The surviving cells express high levels of pro-angiogenic factors and IFN related genes that could induce tissue

revascularization and tumor regrowth. An approach to counteract such a response would be the application of higher dose fractions, i.e. hypofractionation. The rationale behind regular fractionated RTx is to allow repair of normal tissue damage, while damage of tumor cells accumulate over several fractions. Since technical advances in radiation therapy allow more precise targeting of the tumor tissue while sparing normal tissue, hypofractionation has demonstrated promising effects for several tumor types (36-38). However, the concern for normal tissue damage still exists (36) and it would therefore be valuable to study how to enhance the anti-tumor effect of fractionated RTx with anti-angiogenic drugs.

Optimizing the treatment schedule of angiogenesis inhibition and RTx

In chapter 5 we describe that sunitinib given after single dose RTx enhanced the anti-tumor effect. Interestingly, less effect was reached when RTx was given half way sunitinib treatment. In addition, RTx after sunitinib had no beneficial effect compared to sunitinib alone. The latter appears to conflict with the literature where it has been described that also angiostatic treatment before RTx can potentiate the effect of RTx by inducing vascular normalization (39;40). Normalization of the tumor vasculature improves perfusion and tumor oxygenation (41), thereby enhancing radiosensitivity (42). However, the time window of vascular normalization lasts only for a few days, after which the tumor vasculature regresses and oxygenation decreases (41;43). The absence of a positive effect when applying sunitinib prior to RTx in our study might thus be related to inappropriate timing of both treatment schedules, as also described in another study (44). The importance of proper timing illustrates a potential pitfall for attempts to exploit vascular normalization in the clinical setting. To this point, evidence for the occurrence of a vascular normalization window in tumors in patients is lacking. Besides, given the short time frame of the vascular normalization it will be difficult to schedule the RTx within this window. In addition, too long anti-angiogenic treatment could induce hypoxia and thus counteract the RTx, which has been described in a clinical study (45). We therefore favor the view that anti-angiogenic drugs given before RTx in the clinical setting is not a feasible strategy.

Clinicians have been reluctant to combine RTx with anti-angiogenesis therapy due to observed severe side effect, such as gastro-intestinal perforations or fatal hemorrhages (16;46;47). While these side effects also occur with monotherapy of angiostatic drugs, their severity and frequency seems to enhance in combination with RTx (17;48-50). As expected, in phase I trials that combined sunitinib and RTx, it was demonstrated that a lower dose of sunitinib was also associated with fewer and less severe side effects (51;52). The difference in therapeutic efficacy of the reduced sunitinib dose has never been explored. We now demonstrate *in vivo* that in combination with RTx, reducing the sunitinib dose by 50% results in an equal tumor growth inhibition compared to full dose sunitinib (Chapter 5). In addition, when applied after RTx, low dose sunitinib significantly enhanced the anti-tumor effect (Chapter 6). Our results indicate that this might be related to inhibition of the enhanced perfusion and

expression of pro-angiogenic factors, observed during fractionated RTx. However, beneficial effect of low dose sunitinib was also observed after single dose RTx, although we did not observe enhanced perfusion after single dose RTx. While it has been demonstrated before that single dose RTx does enhance tumor perfusion at a later time point (24;53), there are also other possible mechanisms that could contribute to the enhanced effect of angiostatic therapy after RTx (briefly discussed in chapter 4). This includes enhanced cancer cell apoptosis after RTx through inhibition of the AKT/ERK pathway by sunitinib (54). It has also been shown that sunitinib decreases NF- κ B signaling, which would hamper multiple cell-survival mechanism (55;56). In addition, it has been described *in vivo* that both RTx and angiostatic therapy enhance the anti-tumor immune response (10;57-59). Whether they potentiate each other remains a subject for future research.

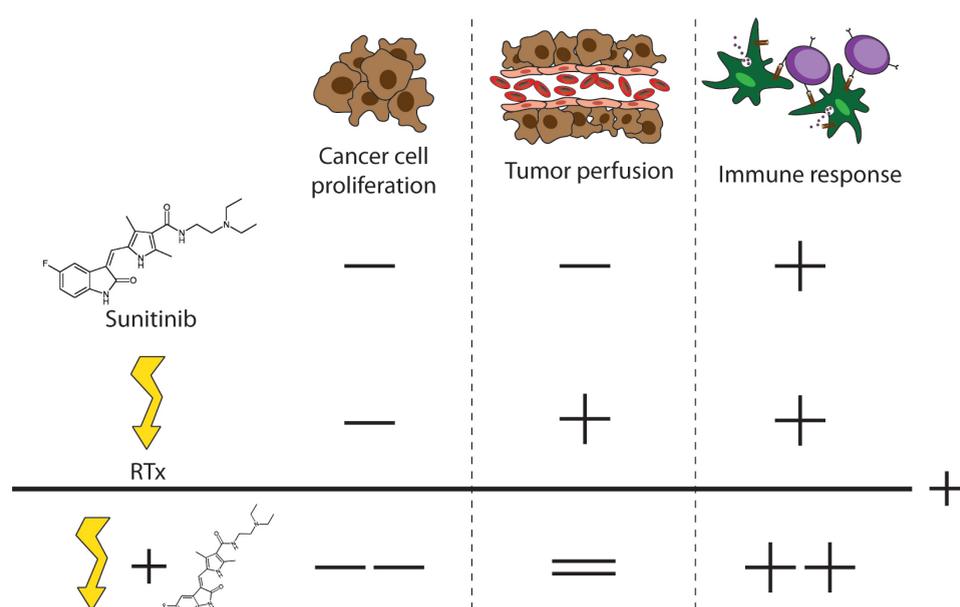


Figure 1. Overview of possible mechanisms leading to beneficial anti-tumor effect with the combination of sunitinib and radiation therapy.

In order to investigate the contribution of the immune response to the beneficial effect of the combination therapy, a feasible and immune competent *in vivo* tumor model should be chosen. In the research described in this thesis, we have used two *in vivo* tumor models, 1) tumors grafted on the CAM (Chapters 3 and 5) and 2) tumor xenografts in balb/c nude mice (Chapters 6 and 8). Both tumor models lack a fully competent immune system. The chicken embryos develop their immune system around embryonic development day (EDD) 14, which allows tumor grafting during early embryogenesis. Tumor graft rejection starts from EDD19 (60). Balb/c nude mice are thymic deficient, and lack therefore a sufficient T-lymphocyte response (61), which prevents the rejections of tumor xenografts. Consequently, these tumor models do not allow proper analysis of the involvement of the immune system. The use of immune-competent mice with a syngeneic murine tumor model would be useful to gain more insight in the role of the immune system in the combination therapy. The use of such tumor models would also be valuable to get more insight in the pro- or anti-tumor effect of the interferon (IFN) response induced by fractionated RTx (Chapter 8).

Combination therapy in the clinical setting

Our preclinical studies indicated that dose reductions of angiostatic drugs do not affect therapeutic efficacy when combined with RTx (chapter 5). Applying low dose angiostatic drugs will cause less severe side effects, making it safer to combine the drug with RTx. These results justify the design of a clinical trial exploring the efficacy of dose reductions during combination therapy. However, the effects of fractionated RTx on the tumor vasculature and pro-angiogenic factor expression in patients are not well studied (62). We have therefore initiated a clinical pilot study to determine the angiogenic alterations in the tumor during fractionated RTx, in patients with esophageal cancer patients that receive both neo-adjuvant chemotherapy and RTx. A better understanding of the treatment effects on tumor angiogenesis is essential in order to design feasible combination treatment schedules for following clinical trials.

While we stated above that anti-angiogenic treatment should be given after the start of RTx, it will still be difficult to implement this schedule for the right patient groups. Often, patients receive fractionated RTx before surgery. A surgical procedure is a contraindication for simultaneously applying angiostatic drugs since this would also inhibit physiological angiogenesis, which could affect wound healing after surgery. Thus, anti-angiogenic treatment after fractionated RTx is not suitable when it is given before surgery. In addition, patients often receive chemotherapy during fractionated RTx. This combination could affect the pro-angiogenic response. Our current clinical pilot study will give insight in the influence of chemoradiation on the pro-angiogenic response. But until then, it will be challenging to translate the current findings regarding the optimal combination schedule to patient receiving chemoradiation. More suitable patients would be those who receive adjuvant fractionated RTx after surgery, for example certain breast cancer patients. In addition, we demonstrate that anti-angiogenic drugs also enhance the therapeutic efficacy of single dose RTx. This has a major implication for patients that receive single dose RTx in the palliative setting, for example for pain relief of metastases. Since the RTx is then often applied as monotherapy, it will be easier to implement the combination with anti-angiogenic drugs.

While we have counteracted the increased tumor perfusion with sunitinib, many other anti-angiogenic drugs are available or currently being developed. Several combination therapies have been discussed in chapter 1, including RTx with bevacizumab or sorafenib. Bevacizumab is a monoclonal anti-body against VEGF (63). Its target mechanism is thus more specific than the multi-tyrosine kinase receptor inhibitors sunitinib or sorafenib. While we have studied the effect of sunitinib on tumor perfusion, it is likely that sunitinib also targets other cellular mechanisms, such as proliferation and apoptosis, thereby improving the efficacy of RTx. Bevacizumab may affect fewer mechanisms that could benefit the RTx. Due to their different mechanisms of action, dose schedules for the combination treatment with RTx should be carefully studied for each anti-angiogenic drug separately. In addition, carefully selecting the right tumor type for the right patient group should be another focus. While the pre-clinical studies suggest that

RTx enhances the tumor sensitivity for the anti-angiogenic drug, pre-existing sensitivity for the drug likely benefits the anti-tumor effect of the combination therapy.

Besides directly targeting the VEGF-pathway with an anti-angiostatic drug, the research described in this thesis also gives a rationale to target other signaling pathways. Inhibition of the IFN response could for example lead to radiosensitization of the cancer cells. On the other hand, as discussed above, it might also inhibit the anti-tumor immune response, thereby hampering the anti-tumor efficacy. A better target may therefore be NF- κ B activity, potentially reducing cancer cell proliferation and tumor perfusion via several down-stream pathways. However, a clinically approved inhibitory drug for this pathway is not yet available, which makes fast translation to the clinical setting difficult.

Taken together, the research described in this thesis has provided novel insights in the understanding of the effects of RTx on angiogenesis. We demonstrate that:

- 1) RTx enhances the tumor sensitivity to the anti-angiogenic drug
- 2) The effectiveness of the combination therapy is not hampered with a lower dose of the anti-angiogenic drug
- 3) The combination therapy is effective with both single dose and fractionated RTx
- 4) The STING/IFN signaling axis plays an important role in the response to fractionated RTx.

All these findings provide opportunities for designing optimal treatment schedules for the combination therapy. However, several important challenges remain in order to successfully implement this combination therapy in the clinical setting. These include:

- 1) Selecting the right patients
- 2) Designing drug-specific treatment schedules to maximize the therapeutic effect
- 3) Determining the right dose of the anti-angiogenic drug
- 4) Further exploration of the mechanisms of action other than the interaction on tumor perfusion for the combination therapy.

Overall, the combination of RTx with angiostatic drugs deserves further pre-clinical and clinical investigation in order to improve the efficacy of RTx as this will benefit many cancer patients.

References

- (1) Barakat K, Gajewski M, Tuszynski JA. DNA repair inhibitors: the next major step to improve cancer therapy. *Curr Top Med Chem* 2012;12(12):1376-90.
- (2) Helleday T, Petermann E, Lundin C, Hodgson B, Sharma RA. DNA repair pathways as targets for cancer therapy. *Nat Rev Cancer* 2008 Mar;8(3):193-204.
- (3) Thoms J, Bristow RG. DNA repair targeting and radiotherapy: a focus on the therapeutic ratio. *Semin Radiat Oncol* 2010 Oct;20(4):217-22.
- (4) Wu L, Shao L, Li M, Zheng J, Wang J, Feng W, et al. BMS-345541 sensitizes MCF-7 breast cancer cells to ionizing radiation by selective inhibition of homologous recombinational repair of DNA double-strand breaks. *Radiat Res* 2013 Feb;179(2):160-70.
- (5) Stachelek GC, Peterson-Roth E, Liu Y, Fernandez RJ, III, Pike LR, Qian JM, et al. YU238259 is a Novel Inhibitor of Homology-dependent DNA Repair that Exhibits Synthetic Lethality and Radiosensitization in Repair-deficient Tumors. *Mol Cancer Res* 2015 Jun 26.
- (6) Lamb R, Fiorillo M, Chadwick A, Ozsvari B, Reeves KJ, Smith DL, et al. Doxycycline down-regulates DNA-PK and radiosensitizes tumor initiating cells: Implications for more effective radiation therapy. *Oncotarget* 2015 Jun 10;6(16):14005-25.
- (7) Jang NY, Kim DH, Cho BJ, Choi EJ, Lee JS, Wu HG, et al. Radiosensitization with combined use of olaparib and PI-103 in triple-negative breast cancer. *BMC Cancer* 2015;15:89.
- (8) Sofia Vala I, Martins LR, Imaizumi N, Nunes RJ, Rino J, Kuonen F, et al. Low doses of ionizing radiation promote tumor growth and metastasis by enhancing angiogenesis. *PLoS One* 2010;5(6):e11222.
- (9) Sonveaux P, Brouet A, Havaux X, Gregoire V, Dessy C, Balligand JL, et al. Irradiation-induced angiogenesis through the up-regulation of the nitric oxide pathway: implications for tumor radiotherapy. *Cancer Res* 2003 Mar 1;63(5):1012-9.
- (10) Shahabi V, Postow MA, Tuck D, Wolchok JD. Immune-priming of the tumor microenvironment by radiotherapy: rationale for combination with immunotherapy to improve anticancer efficacy. *Am J Clin Oncol* 2015 Feb;38(1):90-7.
- (11) Dewan MZ, Galloway AE, Kawashima N, Dewynngaert JK, Babb JS, Formenti SC, et al. Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody. *Clin Cancer Res* 2009 Sep 1;15(17):5379-88.
- (12) Kleibeuker EA, Griffioen AW, Verheul HM, Slotman BJ, Thijssen VL. Combining angiogenesis inhibition and radiotherapy: A double-edged sword. *Drug Resist Updat* 2012 Jun;15(3):173-82.
- (13) Kleibeuker EA, Ten Hooven MA, Castricum KC, Honeywell R, Griffioen AW, Verheul HM, et al. Optimal treatment scheduling of ionizing radiation and sunitinib improves the antitumor activity and allows dose reduction. *Cancer Med* 2015 Jul;4(7):1003-15.
- (14) Hoang T, Huang S, Armstrong E, Eickhoff JC, Harari PM. Enhancement of radiation response with bevacizumab. *J Exp Clin Cancer Res* 2012 Apr 26;31(1):37.
- (15) Wild AT, Gandhi N, Chettiar ST, Aziz K, Gajula RP, Williams RD, et al. Concurrent versus sequential sorafenib therapy in combination with radiation for hepatocellular carcinoma. *PLoS One* 2013;8(6):e65726.
- (16) Peters NA, Richel DJ, Verhoeff JJ, Stalpers LJ. Bowel perforation after radiotherapy in a patient receiving sorafenib. *J Clin Oncol* 2008 May 10;26(14):2405-6.
- (17) Spigel DR, Hainsworth JD, Yardley DA, Raefsky E, Patton J, Peacock N, et al. Tracheoesophageal fistula formation in patients with lung cancer treated with chemoradiation and bevacizumab. *J Clin Oncol* 2010 Jan 1;28(1):43-8.
- (18) Lind JS, Senan S, Smit EF. Pulmonary toxicity after bevacizumab and concurrent thoracic radiotherapy observed in a phase I study for inoperable stage III non-small-cell lung cancer. *J Clin Oncol* 2012 Mar 10;30(8):e104-e108.
- (19) Hirst DG, Denekamp J, Hobson B. Proliferation studies of the endothelial and smooth muscle cells of the mouse mesentery after irradiation. *Cell Tissue Kinet* 1980 Jan;13(1):91-104.
- (20) Denekamp J. Endothelial cell proliferation as a novel approach to targeting tumour therapy. *Br J Cancer* 1982 Jan;45(1):136-9.
- (21) Tannock IF. Population kinetics of carcinoma cells, capillary endothelial cells, and fibroblasts in a transplanted mouse mammary tumor. *Cancer Res* 1970 Oct;30(10):2470-6.

- (22) Karnabatidis D, Dimopoulos JC, Siablis D, Papazafiroopoulos D, Kalogeropoulou CP, Nikiforidis G. Quantification of the ionising radiation effect over angiogenesis in the chick embryo and its chorioallantoic membrane by computerised analysis of angiographic images. *Acta Radiol* 2001 May;42(3):333-8.
- (23) Dalrymple SL, Becker RE, Zhou H, DeWeese TL, Isaacs JT. Tasquinimod prevents the angiogenic rebound induced by fractionated radiation resulting in an enhanced therapeutic response of prostate cancer xenografts. *Prostate* 2012 May 1;72(6):638-48.
- (24) Thanik VD, Chang CC, Lerman OZ, Greives MR, Le H, Warren SM, et al. Cutaneous low-dose radiation increases tissue vascularity through upregulation of angiogenic and vasculogenic pathways. *J Vasc Res* 2010;47(6):472-80.
- (25) Kim JJ, Tannock IF. Repopulation of cancer cells during therapy: an important cause of treatment failure. *Nat Rev Cancer* 2005 Jul;5(7):516-25.
- (26) Solberg TD, Nearman J, Mullins J, Li S, Baranowska-Kortylewicz J. Correlation between tumor growth delay and expression of cancer and host VEGF, VEGFR2, and osteopontin in response to radiotherapy. *Int J Radiat Oncol Biol Phys* 2008 Nov 1;72(3):918-26.
- (27) Tsai MH, Cook JA, Chandramouli GV, DeGraff W, Yan H, Zhao S, et al. Gene expression profiling of breast, prostate, and glioma cells following single versus fractionated doses of radiation. *Cancer Res* 2007 Apr 15;67(8):3845-52.
- (28) Khodarev NN, Beckett M, Labay E, Darga T, Roizman B, Weichselbaum RR. STAT1 is overexpressed in tumors selected for radioresistance and confers protection from radiation in transduced sensitive cells. *Proc Natl Acad Sci U S A* 2004 Feb 10;101(6):1714-9.
- (29) Pitroda SP, Wakim BT, Sood RF, Beveridge MG, Beckett MA, MacDermed DM, et al. STAT1-dependent expression of energy metabolic pathways links tumour growth and radioresistance to the Warburg effect. *BMC Med* 2009;7:68.
- (30) Duarte CW, Willey CD, Zhi D, Cui X, Harris JJ, Vaughan LK, et al. Expression signature of IFN/STAT1 signaling genes predicts poor survival outcome in glioblastoma multiforme in a subtype-specific manner. *PLoS One* 2012;7(1):e29653.
- (31) Ishikawa H, Barber GN. STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. *Nature* 2008 Oct 2;455(7213):674-8.
- (32) Huang S, Robinson JB, Deguzman A, Bucana CD, Fidler IJ. Blockade of nuclear factor-kappaB signaling inhibits angiogenesis and tumorigenicity of human ovarian cancer cells by suppressing expression of vascular endothelial growth factor and interleukin 8. *Cancer Res* 2000 Oct 1;60(19):5334-9.
- (33) Xie TX, Xia Z, Zhang N, Gong W, Huang S. Constitutive NF-kappaB activity regulates the expression of VEGF and IL-8 and tumor angiogenesis of human glioblastoma. *Oncol Rep* 2010 Mar;23(3):725-32.
- (34) Aggarwal BB, Vijayalekshmi RV, Sung B. Targeting inflammatory pathways for prevention and therapy of cancer: short-term friend, long-term foe. *Clin Cancer Res* 2009 Jan 15;15(2):425-30.
- (35) Shibata A, Nagaya T, Imai T, Funahashi H, Nakao A, Seo H. Inhibition of NF-kappaB activity decreases the VEGF mRNA expression in MDA-MB-231 breast cancer cells. *Breast Cancer Res Treat* 2002 Jun;73(3):237-43.
- (36) Ray KJ, Sibson NR, Kiltie AE. Treatment of Breast and Prostate Cancer by Hypofractionated Radiotherapy: Potential Risks and Benefits. *Clin Oncol (R Coll Radiol)* 2015 Jul;27(7):420-6.
- (37) Whelan TJ, Kim DH, Sussman J. Clinical experience using hypofractionated radiation schedules in breast cancer. *Semin Radiat Oncol* 2008 Oct;18(4):257-64.
- (38) Freedman GM. Hypofractionated radiation therapy in the treatment of early-stage breast cancer. *Curr Oncol Rep* 2012 Feb;14(1):12-9.
- (39) Matsumoto S, Batra S, Saito K, Yasui H, Choudhuri R, Gadisetti C, et al. Anti-angiogenic agent sunitinib transiently increases tumor oxygenation and suppresses cycling hypoxia. *Cancer Res* 2011 Aug 30.
- (40) Dings RP, Loren M, Heun H, McNiel E, Griffioen AW, Mayo KH, et al. Scheduling of radiation with angiogenesis inhibitors anginex and Avastin improves therapeutic outcome via vessel normalization. *Clin Cancer Res* 2007 Jun 1;13(11):3395-402.
- (41) Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. *Science* 2005 Jan 7;307(5706):58-62.
- (42) Vaupel P. Tumor microenvironmental physiology and its implications for radiation oncology. *Semin Radiat Oncol* 2004 Jul;14(3):198-206.
- (43) Carmeliet P, Jain RK. Principles and mechanisms of vessel normalization for cancer and other angiogenic diseases. *Nat Rev Drug Discov* 2011 Jun;10(6):417-27.

- (44) Weiss A, Bonvin D, Berndsen RH, Scherrer E, Wong TJ, Dyson PJ, et al. Angiostatic treatment prior to chemor photodynamic therapy improves anti-tumor efficacy. *Sci Rep* 2015;5:8990.
- (45) Lewin J, Khamly KK, Young RJ, Mitchell C, Hicks RJ, Toner GC, et al. A phase Ib/II translational study of sunitinib with neoadjuvant radiotherapy in soft-tissue sarcoma. *Br J Cancer* 2014 Dec 9;111(12):2254-61.
- (46) Crane CH, Ellis LM, Abbruzzese JL, Amos C, Xiong HQ, Ho L, et al. Phase I trial evaluating the safety of bevacizumab with concurrent radiotherapy and capecitabine in locally advanced pancreatic cancer. *J Clin Oncol* 2006 Mar 1;24(7):1145-51.
- (47) Seiwert TY, Haraf DJ, Cohen EE, Stenson K, Witt ME, Dekker A, et al. Phase I study of bevacizumab added to fluorouracil- and hydroxyurea-based concomitant chemoradiotherapy for poor-prognosis head and neck cancer. *J Clin Oncol* 2008 Apr 1;26(10):1732-41.
- (48) Yoon SS, Rivera R, Shah A, Ozden N. GI perforation associated with bevacizumab. *Gastrointest Endosc* 2011 Aug 19.
- (49) Hapani S, Chu D, Wu S. Risk of gastrointestinal perforation in patients with cancer treated with bevacizumab: a meta-analysis. *Lancet Oncol* 2009 Jun;10(6):559-68.
- (50) Walraven M, Witteveen PO, Lolkema MP, van Hillegersberg R, Voest EE, Verheul HM. Antiangiogenic tyrosine kinase inhibition related gastrointestinal perforations: a case report and literature review. *Angiogenesis* 2011 May;14(2):135-41.
- (51) Kao J, Packer S, Vu HL, Schwartz ME, Sung MW, Stock RG, et al. Phase 1 study of concurrent sunitinib and image-guided radiotherapy followed by maintenance sunitinib for patients with oligometastases: acute toxicity and preliminary response. *Cancer* 2009 Aug 1;115(15):3571-80.
- (52) Corn PG, Song DY, Heath E, Maier J, Meyn R, Kuban D, et al. Sunitinib plus androgen deprivation and radiation therapy for patients with localized high-risk prostate cancer: results from a multi-institutional phase 1 study. *Int J Radiat Oncol Biol Phys* 2013 Jul 1;86(3):540-5.
- (53) Hou H, Lariviere JP, Demidenko E, Gladstone D, Swartz H, Khan N. Repeated tumor pO₂ measurements by multi-site EPR oximetry as a prognostic marker for enhanced therapeutic efficacy of fractionated radiotherapy. *Radiother Oncol* 2009 Apr;91(1):126-31.
- (54) Cuneo KC, Geng L, Fu A, Orton D, Hallahan DE, Chakravarthy AB. SU11248 (sunitinib) sensitizes pancreatic cancer to the cytotoxic effects of ionizing radiation. *Int J Radiat Oncol Biol Phys* 2008 Jul 1;71(3):873-9.
- (55) Miller SC, Huang R, Sakamuru S, Shukla SJ, Attene-Ramos MS, Shinn P, et al. Identification of known drugs that act as inhibitors of NF-kappaB signaling and their mechanism of action. *Biochem Pharmacol* 2010 May 1;79(9):1272-80.
- (56) Deorukhkar A, Krishnan S. Targeting inflammatory pathways for tumor radiosensitization. *Biochem Pharmacol* 2010 Dec 15;80(12):1904-14.
- (57) Bose A, Taylor JL, Alber S, Watkins SC, Garcia JA, Rini BI, et al. Sunitinib facilitates the activation and recruitment of therapeutic anti-tumor immunity in concert with specific vaccination. *Int J Cancer* 2011 Nov 1;129(9):2158-70.
- (58) Shrimali RK, Yu Z, Theoret MR, Chinnasamy D, Restifo NP, Rosenberg SA. Antiangiogenic agents can increase lymphocyte infiltration into tumor and enhance the effectiveness of adoptive immunotherapy of cancer. *Cancer Res* 2010 Aug 1;70(15):6171-80.
- (59) Burnette BC, Liang H, Lee Y, Chlewicki L, Khodarev NN, Weichselbaum RR, et al. The efficacy of radiotherapy relies upon induction of type I interferon-dependent innate and adaptive immunity. *Cancer Res* 2011 Apr 1;71(7):2488-96.
- (60) Murphy JB. Studies in tissue specificity: II. The ultimate fate of mammalian tissue implanted in the chick embryo. *J Exp Med* 1914 Feb 1;19(2):181-6.
- (61) Pelleitier M, Montplaisir S. The nude mouse: a model of deficient T-cell function. *Methods Achiev Exp Pathol* 1975;7:149-66.
- (62) Nozue M, Isaka N, Fukao K. Over-expression of vascular endothelial growth factor after preoperative radiation therapy for rectal cancer. *Oncol Rep* 2001 Nov;8(6):1247-9.
- (63) Bevacizumab. Anti-VEGF monoclonal antibody, avastin, rhumab-VEGF. *Drugs R D* 2002;3(1):28-30.

