Clin Exp Med (2008) 8:141–145
DOI 10.1007/s10238-008-0170-2

ORIGINAL ARTICLE

Serum levels of angiogenic cytokines decrease after radiotherapy in non-Hodgkin lymphomas

Roberto Ria · Teresa Cirulli · Teresa Giannini · Santa Bambace · Gabriella Serio · Maurizio Portaluri · Domenico Ribatti · Angelo Vacca · Franco Dammacco

Received: 7 November 2007 / Accepted: 30 January 2008

Abstract
Purpose Serum levels of angiogenic cytokines decrease after radiotherapy in patients with cancer, and this may be relevant for treatment response and progression-free survival. Herein, we set out to determine whether circulating fibroblast growth factor-2 (FGF-2), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and platelet-derived growth factor-β (PDGF-β) decrease after radiotherapy in patients with non-Hodgkin lymphomas (NHLs) and if so, whether their decrease correlates with age, tumour histotype and stage, and radiation dose.
Material and methods The serum levels of FGF-2, VEGF, HGF and PDGF-β were evaluated before and after radiotherapy by an enzyme-linked immunosorbent assay (ELISA). These levels were correlated both reciprocally and with age, histotype, stage and radiation dose.
Results After radiotherapy, FGF-2, VEGF and PDGF-β, but not HGF, significantly decreased in relation to the radiation dose and response. No correlation was established between cytokine levels, except for VEGF and PDGF-β, which decreased in parallel. Haemoglobin levels did not decrease after radiotherapy, while FGF-2, VEGF, HGF and PDGF-β levels did not correlate with age, NHL stage and histotype.
Conclusions Soluble FGF-2, VEGF and PDGF-β levels decline after radiotherapy in NHLs, and may have predictive significance for response to treatment and recurrence.

Keywords Angiogenesis · Cytokines · Non-Hodgkin lymphoma · Prognosis · Radiotherapy

R. Ria (✉) · T. Cirulli · T. Giannini · A. Vacca · F. Dammacco
Department of Biomedical Sciences and Human Oncology,
Section of Internal Medicine,
University of Bari Medical School,
Policlinico, Piazza Giulio Cesare 11, I-70124 Bari, Italy
e-mail: ria@dimo.uniba.it

S. Bambace · M Portaluri
Operative Unit of Radiotherapy,
Di Summa General Hospital,
Brindisi, Italy

G. Serio
Department of Biomedical Sciences and Human Oncology,
Section of Medical Statistics,
University of Bari Medical School,
Bari, Italy

D. Ribatti
Department of Human Anatomy,
University of Bari Medical School,
Bari, Italy

Introduction

Angiogenesis, i.e., the formation of new vessels from existing ones, is an essential event for progression (growth, invasion and metastasis) of solid and haematological tumours, including non-Hodgkin lymphomas (NHLs), and has a prognostic value [1].

It is a multistep process that begins with the activation of resting endothelial cells, continues with degradation of the extracellular matrix, proliferation and migration of endothelial cells toward the angiogenic stimulus, and ends with the constitution of new blood vessels enveloped by a basement membrane [2].

A variety of angiogenic cytokines, including fibroblast growth factor-2 (FGF-2), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), platelet-derived growth factor-β (PDGF-β) and tumour necrosis factor-α (TNF-α), promote angiogenesis [3]. They are released by tumour cells and inflammatory cells, such as

mast cells [4] and macrophages [5], and stimulate resting endothelial cells by means of cognate-specific tyrosine-kinase receptors [2]. Receptor/cytokine binding activates endothelial cells and increases their proliferation, modifies cell adhesion proteins and increases the secretion of proteolytic enzymes, cell migration and invasion [6, 7].

Because FGF-2, VEGF, HGF and PDGF-β are soluble, they can be detected in plasma or serum, and their levels are related to the tumour stage [1]. Interestingly, serum levels of angiogenic factors decline after chemotherapy, and become undetectable in patients in complete remission, whereas they vary little or not at all in patients who have partial or no response [8]. Hence, they may have a predictive significance in terms of response to treatment and recurrence.

In the present study, we set out to determine whether circulating FGF-2, VEGF, HGF and PDGF-β decrease after radiotherapy in patients with NHLs and if so, whether their decrease correlates with the radiation dose, tumour type and age.

Methods and materials

Patients

Twenty patients with NHLs (12 F, 8 M; mean age 61, range 39–74 years) were studied (Table 1). They were scheduled to receive radiotherapy for different NHL histotypes in the Radiotherapy Unit of the Di Summa General Hospital (Brindisi, Italy). Radiotherapy was delivered to lymph node sites in accordance with each schedule (Table 1). The median dose was 26 Gy (range 6–52). Patients were at different disease stages (7 stage I, 4 stage II, 4 stage III and 5 stage IV) and 13 patients had already undergone previous cycles of chemotherapy, which was ended about one month before starting radiotherapy. The study was approved by the local Ethics Committee, and all patients gave their informed consent.

Sample collection and storage

Blood samples were collected one week before and one week after radiotherapy. For FGF-2, HGF and PDGF-β, peripheral blood was processed immediately after venipuncture by centrifugation at 1500 rpm for 10 min and stored at –80°C. Samples for VEGF were collected in platelet-poor citrated plasma to avoid release of VEGF by platelets during coagulation [9], and stored as above.

Enzyme-linked immunosorbent assay (ELISA)

Aliquots of sample proteins (100 mg/100 ml) were measured by the Bradford method and tested in triplicate for FGF-2, VEGF, HGF and PDGF-β levels by applying a sandwich ELISA (Human Angiogenesis Array, TEMA Ricerca S.r.l., Pierce Biotechnology Inc., Rockford IL), according to the manufacturer's instructions. The colorimetric reaction was blocked and sent for reading to the TEMA Ricerca labs, where plates were read with a Search Light CCD Image and Analysis System.

Table 1 Details of patients evaluated for serum levels of FGF-2, VEGF and HGF before and after radiotherapy

Pat.	Sex	Age	Days from chemot.	Radiation site	LNH type	Stage	n.fr × Gy[a]	Radiation dose (Gy)	Status at 12 months
1	F	46	33	Neck	Follicular	II	15×2	30	ned
2	F	69	35	Neck	Follicular	IV	17×2	34	ned
3	M	71		Neck	Large cell	I	17×1.8	30	ned
4	M	56	30	Abdomen	Follicular	III	19×2	38	awd
5	M	72		Abdomen	Mantle cell	I	16×2	32	ned
6	F	64	35	Mediastinum	Large cell	III	13×2	26	ned
7	F	44	32	Mediastinum	Large cell	IV	17×2	34	awd
8	F	54		Neck	Follicular	I	16×2	32	ned
9	F	63	38	Abdomen	Large cell	IV	13×1.8	24	ned
10	F	73		Neck	Large cell	I	13×2	26	ned
11	M	71	36	Supraclaval	Mantle cell	II	16×2	32	ned
12	F	53		Neck	Large cell	I	14×1.8	25	ned
13	M	73	32	Inguinal	Large cell	II	11×2	22	dod
14	F	73	36	Supraclaval	Follicular	III	17×1.8	30	ned
15	F	56		Inguinal	Follicular	I	17×1.8	30	ned
16	F	74	30	Axilla	Large cell	II	15×3	45	ned
17	M	70	38	Supraclaval	Follicular	IV	14×1.8	26	ned
18	F	57	30	Axilla	Large cell	IV	15×3	45	ned
19	M	39		Supraclaval	Follicular	I	7×3	21	ned
20	F	40	32	Abdomen	Follicular	III	8×3	24	ned

[a] Number of fraction × radiation dosage
ned no evidence of disease, *awd* alive with disease, *dod* dead of disease

Table 2 FGF-2, VEGF, HGF and PDGF-β serum levels before and after radiotherapy

Pat.	FGF-2		VEGF		HGF		PDGF-β	
	Pre-RT	Post-RT	Pre-RT	Post-RT	Pre-RT	Post-RT	Pre-RT	Post-RT
1	0.2 (0–0.3)	0.1 (0–0.3)	0.1 (0–0.2)	3.2 (2.2–4)	99.3 (82–105.2)	62.5 (59.1–71.2)	66.2 (58.7–73.1)	52.0 (50.1–58.2)
2	0.1 (0–0.1)	0.1 (0–0.3)	0.3 (0.1–0.3)	0.2 (0–0.3)	46.6 (41.3–48.8)	10.3 (9.7–11.2)	20.7 (19.8–21.4)	8.8 (8.1–9.2)
3	9.4 (9.1–9.7)	12.1 (11.6–12.3)	0.5 (0.5–0.6)	3.7 (3.5–3.8)	11.9 (11.7–12.2)	1.4 (1.1–1.5)	32.5 (31.7–32.8)	96.0 (95.1–97.2)
4	434.3 (421.1–441.2)	382.3 (375.6–390.2)	27.8 (26.6–28.3)	5.2 (4.6–6.1)	47.7 (46.9–47.9)	20.7 (19.6–21.3)	200.0 (196.6–211.3)	147.2(139.6–151.3)
5	7.2 (6.9–7.4)	7.7 (7.5–7.8)	2.7 (2.6–2.8)	3.0 (2.6–3.3)	22.4 (21.3–23.3)	35.1 (34.8–35.4)	58.2 (57.6–59.3)	24.9 (24.6–25.1)
6	8.9 (8.6–9.1)	7.0 (6.8–7.3)	5.0 (4.9–5.0)	3.9 (3.6–4.1)	10.9 (10.6–11.2)	48.7 (48.5–48.8)	5.1 (4.9–5.2)	10.9 (10.6–11.2)
7	500.0 (489.1–512.3)	400.0 (387.6–321.6)	18.9 (18.6–19.3)	9.4 (9.2–9.5)	72.2 (69.6–75.6)	28.9 (28.8–28.9)	65.4 (62.1–67.3)	115.5 (109.5–120.3)
8	86.0 (84.6–89.3)	50.3 (49.2–50.9)	0.2 (0–0.3)	0.2 (0.2–0.3)	7.2 (7.1–7.2)	43.3 (42.6–45.1)	17.3 (16.6–17.4)	80.5 (79.6–81.3)
9	551.9 (539.6–571.0)	412.9 (409.2–4151.3)	2.6 (2.6–2.7)	4.9 (4.5–5.0)	8.9 (8.6–9.1)	21.8 (19.6–23.3)	16.1 (15.9–16.3)	17.6 (17.1–17.8)
10	18.4 (17.6–19.4)	13.6 (13.3–13.8)	0.2 (0–0.3)	1.8 (1.6–2.3)	8.3 (8.1–8.3)	9.6 (9.2–10.3)	18.2 (18.0–18.5)	2.0 (1.9–2.0)
11	25.8 (24.9–26.5)	7.3 (7.2–7.3)	25.1 (25.0–25.3)	16.1 (15.2–16.8)	11.7 (11.5–12.0)	9.7 (9.6–9.9)	165.7 (155.2–171.2)	62.1 (59.6–64.3)
12	189.2 (179.6–201.2)	96.8 (92.4–97.9)	12.0 (11.6–12.2)	0.0 (0–0)	19.7 (19.1–20.8)	7.1 (7.0–7.1)	47.6 (45.6–49.5)	26.3 (24.9–28.4)
13	0.1 (0–0.3)	0.4 (0.4–0.4)	14.2 (13.6–14.5)	4.5 (4.4–4.8)	20.3 (19.6–21.3)	17.6 (17.2–17.8)	55.4 (51.9–57.6)	13.9 (13.8–14.3)
14	177.8 (169.4–179.9)	69.1 (67.8–71.0)	0.2 (0–0.2)	0.3 (0–0.3)	14.3 (13.6–14.8)	17.5 (16.9–17.8)	4.9 (4.9–5.0)	0.0 (0–0)
15	197.1 (196.0–198.3)	100.0 (95.6–102.3)	17.8 (17.7–18.1)	4.7 (4.6–4.7)	72.9 (72.0–73.4)	59.2 (58.2–6.3)	141.9 (139.6–143.6)	69.8 (65.6–72.3)
16	26.4 (24.6–27.3)	34.1 (31.8–36.2)	39.3 (39.1–41.3)	21.3 (19.6–21.9)	100.5 (95.4–104.2)	54.4 (53.6–57.1)	272.3 (252.8–281.3)	110.3 (109.6–113.3)
17	493.1 (482.1–499.3)	395.7 (389.6–401.2)	2.8 (2.6–2.9)	0.2 (0.2–0.3)	4.3 (4.3–4.3)	2.7 (2.6–2.7)	7.6 (7.6–7.6)	6.4 (6.1–6.5)
18	66.0 (59.6–68.9)	75.7 (72.5–77.8)	4.3 (4.2–4.3)	3.2 (3.2–3.2)	10.7 (10.6–10.7)	6.2 (6.2–6.3)	23.6 (22.6–24.1)	16.6 (16.4–16.9)
19	0.3 (0.2–0.3)	0.4 (0.4–0.5)	0.2 (0–0.3)	3.2 (3.2–3.3)	99.3 (89.6–103.8)	62.5 (60.6–64.0)	66.2 (64.8–66.9)	52.0 (50.4–53.7)
20	0.3 (0.2–0.3)	0.3 (0.1–0.3)	0.1 (0–0.3)	0.1 (0–0.1)	46.6 (41.8–49.7)	10.3 (10.1–10.3)	20.7 (19.6–21.3)	8.8 (8.6–8.9)

Results are expressed as mean and (range)

Statistical analysis

Non-parametric tests were applied because variables were not normally distributed. The analysis was performed with the statistical software MedCalc. The Wilcoxon signed-rank test was employed to compare pre- and post-radiotherapy cytokine serum levels. The Spearman rank correlation test was used to evaluate the correlation between cytokine variation. The linear regression was used to evaluate the relation between each cytokine variation with age and the dose of radiation therapy. The Wilcoxon rank unpaired test was employed to compare cytokine variations between NHL stage (stage I and II *vs*. stage III and IV) and histotype (follicular *vs*. large and mantle cell).

Results

The serum levels of FGF-2, VEGF, HGF and PDGF-β decreased significantly after radiotherapy for FGF-2

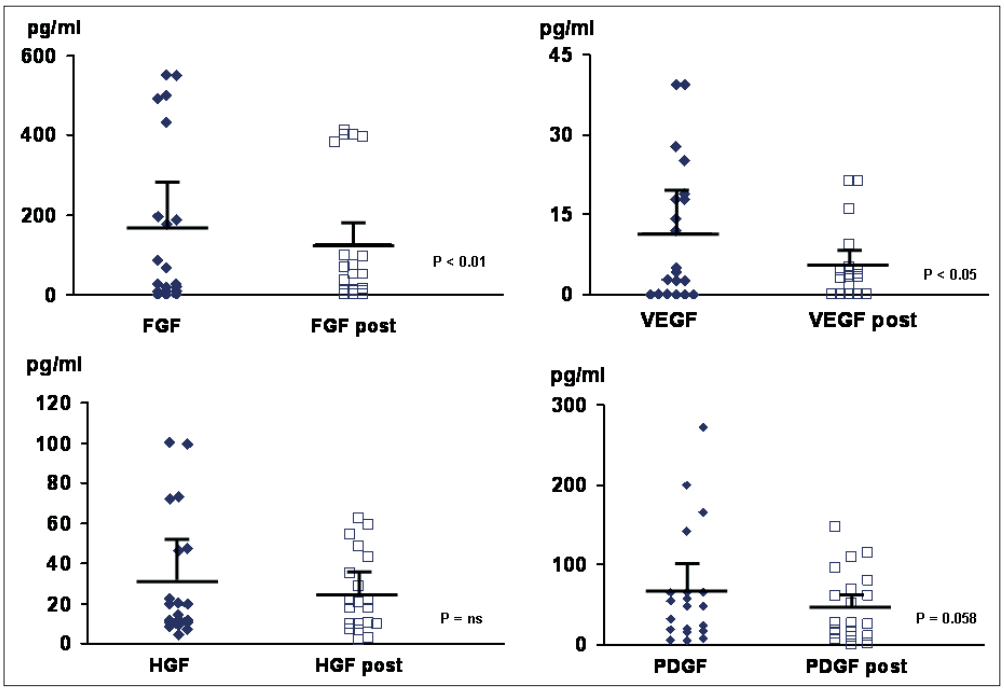

Fig. 1 Pre- and post-radiotherapy distribution of patients for FGF-2, VEGF, HGF and PDGF-β

(median 40.38 ± 11.9 pg/ml; $p = 0.009$, by Wilcoxon signed-rank test) and VEGF (median 4.8 ± 1.8 pg/ml; $p = 0.0129$), weakly for PDGF-β (median 24.12 ± 8.9 pg/ml; $p = 0.034$) and not significantly for HGF (median 7.39 ± 5.7 pg/ml; $p = 0.058$) (Table 2).

Figure 1 shows the scatter distribution of cytokines pre- and post-radiotherapy per patient. No significant differences in baseline values were present for patients who underwent chemotherapy compared to those who underwent radiotherapy alone. Moreover, no significant differences in the baseline cytokine levels were present with respect to histology and stage.

There was no interrelation between the cytokine reductions, except for VEGF and PDGF-β, which decreased in parallel ($rs = 0.53$, $p = 0.02$, by Spearman correlation test). The linear regression model shows that the variation of levels of FGF-2, VEGF, HGF and PDGF-β after radiotherapy were not related with previous chemotherapy, age and the radiation dose (data not shown).

No statistically significant difference (Wilcoxon sum rank test) was found between the cytokine levels and histotype (follicular *vs*. large and mantle cell). In contrast, the variation of FGF-2 levels was significantly different ($p = 0.004$) for the NHL stage (stage I and II *vs*. stage III and IV).

The outcome of patients did not show any positive trend for patients who received previous chemotherapy; indeed, two patients who showed residual disease after treatment were in the group that received chemotherapy. However, this may be related to the advanced stage of these patients.

Discussion

A variety of angiogenic cytokines, including FGF-2, VEGF, HGF, PDGF-β and TNF-α, promote angiogenesis [3]. There is evidence that soluble angiogenic cytokines (i.e., FGF-2, VEGF, HGF and PDGF-β) are related to the tumour stage [1], and can be measured in the patients' plasma or serum. Moreover, their serum levels decline after chemotherapy, becoming undetectable in patients in complete remission, but not in non-responder patients or in those who reach only a partial response [8]. This decrease may represent a predictive sign of response to treatment and recurrence.

It has been shown that the levels of angiogenic factors rise with tumour progression and correlate with a more aggressive phenotype, and fall during response to treatment and long-term disease control [10]. In our series, we found that FGF-2, VEGF, HGF and PDGF-β levels did not decrease in relation to the radiation dose. Others have found that in patients with head and neck tumours, FGF-2 and VEGF decrease after radiotherapy, which has an impact on response and progression-free survival [11, 12]. VEGF also declined in patients undergoing chemotherapy for advanced breast cancer, in whom the better the response, the greater the drop recorded [13]. Similar results were observed in patients receiving high-dose chemotherapy for acute myeloid leukaemia, in whom serum levels of angiogenin and FGF-2 decreased with response [14]. Finally, serum FGF-2, VEGF and HGF are reported to rise with progression of multiple myeloma and to decrease with response to chemotherapy, and thus mark response and can predict relapse [15].

In a previous work, we demonstrated that levels of FGF-2 and VEGF, but not HGF, decrease significantly in various tumour types and that the extent of their diminution is related to the radiation dose and response [16]. Herein, we show that in NHLs, serum levels of all angiogenic cytokines decrease after radiotherapy. These reductions did not correlate between the different cytokines, except for VEGF and PDGF, whose decrease was closely interrelated. This suggests that these two cytokines may have a linked action on endothelial cells and coordinated release by tumour cells.

The antiangiogenic effects of radiotherapy, exerted through its effects on endothelial cell survival, proliferation, apoptosis and cell surface molecule expression, which reduce the tumour oxygen supply and nutrients, have been previously described [17–20]. X-rays give an antiangiogenic activity in the chick embryo chorioallantoic membrane assay through direct injury to the subendothelial basement membrane [21]. Hence radiotherapy may be responsible for the reduction of angiogenic cytokines through inhibition of their sources, such as the inflammatory infiltrate and tumour cells. Low oxygenation increases cell resistance to radiotherapy and leads to an incomplete response [22]. It may be postulated that if serum levels of angiogenic cytokines remain high or start to rise within a short period of time after radiotherapy, patients will probably respond poorly to therapy and will undergo relapse or disease progression relatively quickly.

Further studies are needed to elucidate the role of angiogenesis in NHL progression and response to radiotherapy, and to determine whether antiangiogenic drugs administered shortly after radiotherapy can prolong disease-free survival.

Acknowledgements This work was supported by Associazione Italiana per la Ricerca sul Cancro (AIRC, National and Regional Funds), Milan, Ministry of Education, Universities and Research (MUR, PRIN Projects 2005; and CARSO Project n. 72/2) and Ministry for Health – Regione Puglia (grant BS2 and "Convenzione n. 131/Ricerca Finalizzata IRCCS"), Rome, Italy. T.C. is the recipient of a fellowship from Fondazione Italiana per la Ricerca sul Cancro (FIRC), Milan, Italy.

Conflict of interest statement The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

1. Folkman J (2001) Angiogenesis-dependent diseases. Semin Oncol 28:536–542
2. Carmeliet P (2000) Mechanisms of angiogenesis and arteriogenesis. Nat Med 6:389–395

3. Carmeliet P, Moons L, Luttun A et al (2001) Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. Nat Med 7:575–583
4. Ribatti D, Vacca A, Nico B et al (2001) The role of mast cells in tumour angiogenesis. Br J Haematol 115:514–521
5. Yu JL, Rak JW (2003) Host microenvironment in breast cancer development: inflammatory and immune cells in tumour angiogenesis and arteriogenesis. Breast Cancer Res 5:83–88
6. Singh S, Sadanandam A, Singh RK (2007) Chemokines in tumor angiogenesis and metastasis. Cancer Metastasis Rev 26:453–467
7. Papetti H, Herman IM (2002) Mechanisms of normal and tumor-derived angiogenesis. Am J Physiol Cell Physiol 282:C947–970
8. Poon RT, Fan ST, Wong J (2001) Clinical implications of circulating angiogenic factors in cancer patients. J Clin Oncol 19:1207–1225
9. Molica S, Vacca A, Ribatti D et al (2002) Prognostic value of enhanced bone marrow angiogenesis in early B-cell chronic lymphocytic leukemia. Blood 100:3344–3351
10. Ferrara N, Alitalo K (1999) Clinical Applications of angiogenic growth factors and their inhibitors. Nat Med 5:1359–1364
11. Bachtiary B, Selzer E, Knocke TH et al (2002) Serum VEGF levels in patients undergoing primary radiotherapy for cervical cancer: impact on progression-free survival. Cancer Lett 179:197–203
12. Dietz A, Rudat V, Conradt C et al (2000) Prognostic relevance of serum levels of the angiogenic peptide bFGF in advanced carcinoma of the head and neck treated by primary radiochemotherapy. Head Neck 22:666–673
13. Chala E, Manes C, Iliades H et al (2006) Insulin resistance, growth factors and cytokine levels in overweight women with breast cancer before and after chemotherapy. Hormones 5:137–146
14. Glenjen N, Mosevoll KA, Oystein B (2002) Serum levels of angiogenin, basic fibroblast growth factor and endostatin in patients receiving intensive chemotherapy for acute myelogenous leukemia. Int J Cancer 101:86–94
15. Urba ska-Rys H, Wierzbowska A, Robak T (2003) Circulating angiogenic cytokines in multiple myeloma and related disorders. Eur Cytokine Netw 14:40–51
16. Ria R, Portaluri M, Russo F et al (2004) Serum levels of angiogenic cytokines decrease after antineoplastic radiotherapy. Cancer Lett 216:103–107
17. Barr MP, Bouchier-Hayes DJ, Harmey JJ (2008) Vascular endothelial growth factor is an autocrine survival factor for breast tumour cells under hypoxia. Int J Oncol 32:41–48
18. Shi W, Teschendorf C, Muzyczka N et al (2003) Gene therapy delivery of endostatin enhances the treatment efficacy of radiation. Radiother Oncol 66:1–9
19. Rich JN (2007) Cancer stem cells in radiation resistance. Cancer Res 67:8980–8984
20. Duda DG, Jain RK, Willett CG (2007) Antiangiogenics: the potential role of integrating this novel treatment modality with chemoradiation for solid cancers. J Clin Oncol 25:4033–4042
21. Giannopoulou E, Katsoris P, Hatziapostolou M et al (2001) X-rays modulate extracellular matrix in vivo. Int J Cancer 94:690–698
22. Harada H, Kizaka-Kondoh S, Li G et al (2001) Significance of HIF-1-active cells in angiogenesis and radioresistance. Oncogene 26:7508–7516