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Review

Role of ion channels in ionizing radiation-induced cell death☆

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ABSTRACT

Neoadjuvant, adjuvant or definitive fractionated radiation therapy are implemented in first line anti-cancer treatment regimens of many tumor entities. Ionizing radiation kills the tumor cells mainly by causing double strand breaks of their DNA through formation of intermediate radicals. Survival of the tumor cells depends on both, their capacity of oxidative defense and their efficacy of DNA repair. By damaging the targeted cells, ionizing radiation triggers a plethora of stress responses. Among those is the modulation of ion channels such as Ca^{2+}-activated K^+ channels or Ca^{2+}-permeable nonselective cation channels belonging to the super-family of transient receptor potential channels. Radiogenic activation of these channels may contribute to radiogenic cell death as well as to DNA repair, glucose fueling, radiogenic hypermigration or lowering of the oxidative stress burden. The present review article introduces these channels and summarizes our current knowledge on the mechanisms underlying radiogenic ion channel modulation. This article is part of a Special Issue entitled: Membrane channels and transporters in cancers.

Contents

1. Introduction

Ionizing radiation kills or inactivates cells mostly by damaging the nuclear DNA and cell survival critically depends on successful repair of the DNA damage [1]. Ionizing radiation may lead to necrotic as well as apoptotic cell death depending on cell type, dose and fractionation protocols [2]. The major death pathway in this scenario in normal tissue cells is apoptosis. However, cancer cells which often have developed strategies to evade apoptosis [3] may either undergo (regulated) necrosis or reenter the cell cycle with accumulated DNA damages. During the subsequent cell divisions those cells will not be able to segregate the chromosomes and end up as multinucleated giant cells in mitotic catastrophe. Mitotic catastrophe again leads either to apoptotic or necrotic cell death. Another possible mechanism of radiation-induced death in cells with disturbed apoptosis machinery is excess autophagy. While autophagy is a survival strategy [4] excess autophagy overdigests the cytoplasm and cell organelles forcing the cell into apoptosis or necrosis [5].

Meanwhile, the evidence is overwhelming that ion channels fulfill pivotal functions in cell death mechanisms such as apoptosis (for review see the article by Annarosa Arcangeli in this special issue on "Membrane channels and transporters in cancers") as well as in stress response and survival strategies. Notably, tumor cells have been demonstrated to express a set of ion channels which is different to that of the parental normal cells. These channels may fulfill specific oncogenic functions in neoplastic transformation, malignant progression or tissue

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invasion and metastasis (for review see [1]). In addition, they may contribute to the cellular stress response for instance during fractionated radiation therapy and may confer radioresistance.

The present review intends to sum up data on ion channel function in the stress response to ionizing radiation. In particular, ion channels that may induce cell death in tumor cells and facilitate radiogenic cell killing are introduced. In addition, data on ion channels which, in contrast to the before mentioned, confer radioresistance are reviewed. Finally, ion channels of tumor cells that might contribute to acquired radioresistance, e.g. by promoting radiogenic hypermigration or transition into relatively radioresistant ***c***ancer ***s***tem (cell)-like ***c***ells (CSCs) are described. Prior to that, a brief introduction into radiotherapy and its radiobiological principles is given in the next paragraphs.

2. Radiotherapy

Radiation therapy together with surgery and systemic chemotherapy is the main pillar of anti-cancer treatment. About half of all cancer patients receive radiation therapy, half of all cures from cancer include radiotherapy [6]. Despite modern radiation techniques and advanced multimodal treatments, local failures and distant metastases often limit the prognosis of the patients, especially due to limited salvage treatments [7].

Ionizing radiation impairs the clonogenic survival of tumor cells mainly by causing double strand breaks in the DNA backbone. The number of double strand breaks increases linearly with the absorbed radiation dose. The intrinsic capacity to detoxify radicals formed during transfer of radiation energy to cellular molecules such as H_2O (giving rise to hydroxyl radicals, $^{\cdot}OH$) and the ability to efficiently repair DNA double strand breaks by non-homologous end joining or homologous recombination determines the radiosensitivity of a given tumor cell. Irradiated tumor cells which leave residual DNA double strand breaks un-repaired lose their clonogenicity meaning that these cells can not restore tumor mass (for review see [8]).

In addition to these intrinsic resistance factors, the microenvironment may lower the radiosensitivity of tumor cells. Hypoxic areas are frequent in solid tumors reaching a certain mass. Tumor hypoxia, however, decreases the efficacy of radiation therapy [9]. Ionizing radiation directly or indirectly generates radicals in the deoxyribose moiety of the DNA backbone. In a hypoxic atmosphere, cellular thiols can react with those DNA radicals resulting in chemical DNA repair. At higher oxygen partial pressure, in sharp contrast, radicals of the deoxyribose moiety are chemically transformed to strand break precursors [10]. By this mechanism, hypoxia increases radioresistance by a factor of two to three (oxygen enhancement ratio) [11].

Fractionated treatment regimens which improve recovery of the normal tissue after irradiation but not of the tumor have been established in radiotherapy [12]. In addition to limit normal tissue toxicity, killing of tumor mass by initial radiation fractions has been demonstrated to reoxygenate and thereby radiosensitize solid tumors during further fractionated radiotherapy. Beyond that, fractionated radiation regimens aim to redistribute tumor cells in a more vulnerable phase of the cell cycle in the time intervals between two fractions [13]. Accelerated repopulation of the tumor after irradiation is a frequently reported phenomenon. Possible mechanisms of accelerated repopulation include induction of CSCs: It has been proposed that radiation therapy induces CSCs to switch from an asymmetrical into a symmetrical mode of cell division; i.e., a CSC which is thought to normally divide into a daughter CSC and a lineage-committed progenitor cells is induced by the radiotherapy to divide symmetrically into two proliferative CSC daughter cells. This is thought to accelerate repopulation of the tumor after end of radiotherapy. Importantly, CSCs are thought be relatively radioresistant possibly due to i) high oxidative defense and, therefore, low radiation-induced insults, ii) activated DNA checkpoints resulting in fast DNA repair, and iii) an attenuated radiation-induced cell cycle redistribution [14].

Finally, fractionated radiation therapy, which applies fractions of sublethal radiation doses (usually 2 Gy per fraction), has been demonstrated in a variety of tumor entities *in vitro* and in animal models to stimulate hypermigration and hypermetastasis of tumor cells as well as infiltration of the tumor by CD11b-positive myeloid cells and subsequent vasculogenesis. It is tempting to speculate that radiogenic hypermigration boosts cellular interaction of tumor cells with non-tumor cells, e.g. endothelial cells. It has been proposed that CSCs lodge within perivascular niches where a complex regulatory network supports CSC survival [15]. As a matter of fact, CSCs but not non-CSCs gain radioresistance when transplanted orthotopically in mice [16] supporting the idea of a tumor microenvironment-dependent acquired radioresistance. Ion channels contribute to both, intrinsic and acquired radioresistance of tumor cells as discussed in the next paragraphs

3. Radiosensitizing ion channels

Member 2 of the melastatin family of transient receptor potential channel (TRPM2) is a Ca^{2+}-permeable nonselective cation channel. Heterologous expression of TRPM2 in human embryonic kidney cells [17] or A172 human glioblastoma cells [18] facilitates oxidative stress-induced cell death. Reactive oxygen species (ROS) have been demonstrated to trigger TRPM2 activation [19,20]. The principal activator, however, of TRPM2 is ADP-ribose (ADPR) that binds to a special domain located at the C-terminus of the channel [21,22]. Sources of ADPR are the mitochondria [23] or ADPR polymers. The latter are formed, e.g., during DNA repair by poly (ADP-ribose) polymerases (PARPs). ADPR is released from the ADPR polymers by glycohydrolases [21,24].

Expression of TRPM2 has been demonstrated in several tumor entities such as insulinoma [25], hepatocellular carcinoma [25], prostate cancer [26], lymphoma [27], leukemia [28] and lung cancer cell lines [29]. TRPM2 activity increases the susceptibility to cell death [30] probably by overloading cells with Ca^{2+} (Fig. 1A).

Remarkably, cancer cells may evade TRPM2-mediated cell death. In lung cancer cells, de-methylation of a GpC island within the TRPM2 gene gives rise to new promoters that regulate transcription of a non-functional truncated TRPM2 channel [29] and to a TRPM2 specific antisense RNA. This antisense RNA inhibits TRPM2 translation. Moreover, the truncated channel is non-functional and acts dominant negative, thus switching off the tumor-suppressing function of the full-length TRPM2 protein [29] (Fig. 1B).

The initially described member of the vanilloid family of TRP channels, the nociceptive and heat receptor TRPV1, is reportedly expressed in several tumor entities such as uveal melanoma [31], pancreatic [32] and prostatic neuroendocrine tumors [33], glioblastoma [34] and urothelial cancer of human bladder [35]. At least in the latter two tumor entities, TRPV1 exerts anti-oncogenic effects [35,36]. TRPV1 expression inversely correlates with glioma grading [34]. Remarkably, neural precursor cells have been demonstrated to induce ER stress-mediated cell death of glioblastoma cells by activating glioblastoma TRPV1 channels through secretion of endogenous vanilloids [37]. Along those lines is the observation that a TRPV1 antagonist promotes tumorigenesis in mouse skin [38].

Notably, targeting of TRPM2 and TRPV1 by RNA interference has been demonstrated to decrease gamma irradiation-induced formation of nuclear γH2AX foci and further DNA damage response in A549 lung adenocarcinoma cells [39]. Since γH2AX foci are used as a surrogate for DNA double strand breaks, one might speculate that TRPM2 or TRPV1 may amplify ionizing radiation-induced insults (Fig. 1). Another interpretation which has been favored by the author of the study [39] would be that activity of TRPM2 and TRPV1 is required for the formation of DNA repair complexes. In combination, the data hint to the possibility of radiosensitizing cancer cells by pharmacologically activating TRPM2 or TRPV1 channels. Whether this might become a promising new strategy of tumor radiosensitization has to await animal studies.

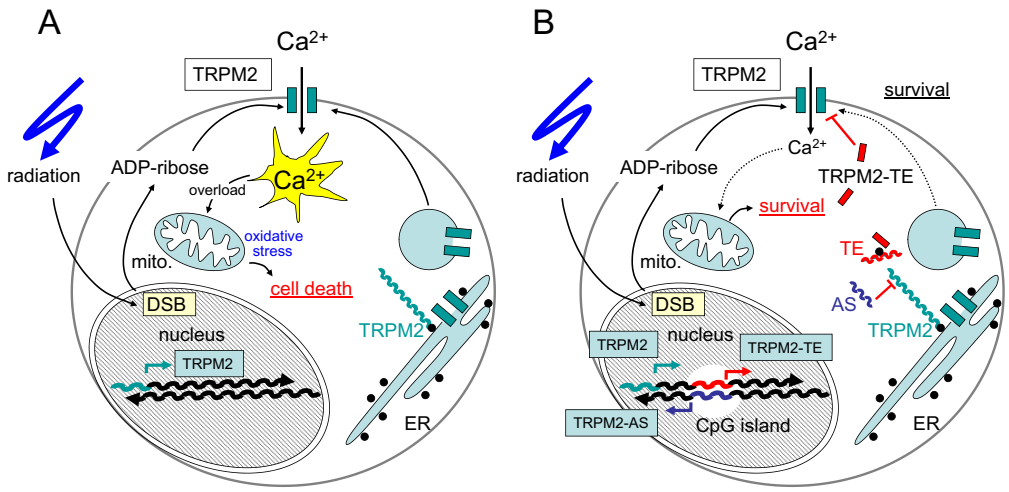

Fig. 1. Speculative mechanism of a putative TRPM2-mediated radiosensitization (A) and reported strategy [29] of lung cancer cells to avoid TRPM2-mediated susceptibility to cell death (B), for details see text. TRPM2-TE (TE): truncated TRPM2, TRPM2-AS (AS): TRPM2 antisense RNA, mito.: mitochondrion).

4. Ion channels conferring intrinsic radioresistance

DNA repair involves cell cycle arrest, chromatin relaxation and formation of repair complexes at the site of DNA damage. Moreover, radiation-induced formation of radicals requires activated radical detoxification pathways and increased oxidative defense to constrain the radiation-induced insults. All these processes of stress response lead to elevated ATP consumption which requires intensified energy supply. Recent *in vitro* observations suggest that these processes depend at least partially on radiation-induced ion channel activation.

Studies of our laboratory indicate that survival of irradiated human leukemia cells critically depends on Ca^{2+} signaling involving radiogenic activation of TRPV5/6-like nonselective cation and $K_v3.4$ voltage-gated K^+ channels [40,41]. The nonselective cation channels in concert with $K_v3.4$ generate radiogenic Ca^{2+} signals that contribute to G_2/M cell cycle arrest by CaMKII-mediated inhibition of the phosphatase cdc25B. Activity of the latter is required in these cells for release from radiation-induced G_2/M arrest via dephosphorylation and thereby activation of cdc2, a component of the mitosis promoting factor. Experimental interference with the radiogenic Ca^{2+} signals, e.g. by pharmacological inhibition or knock-down of $K_v3.4$ overrides cell cycle arrest resulting in increased apoptosis and decreased clonogenic survival of irradiated leukemia cells [40,41]. This radiosensitization by $K_v3.4$ targeting demonstrates the pivotal role of radiogenic $K_v3.4$ channel activation for cell cycle arrest and DNA repair.

Similar to leukemia cells, A549 lung adenocarcinoma cells reportedly respond to ionizing radiation with activation of K_v K^+ channels [42] and transient hyperpolarization of the plasma membrane. Later on, the membrane potential of the irradiated A549 cells strongly depolarizes. This depolarization is dependent on external glucose and inhibited by phlorizin, a sodium glucose cotransporter (SGLT) blocker. In parallel, irradiation induces phlorizin-sensitive ^{3}H-glucose uptake within few minutes after irradiation [43]. Combined, these data suggest that radiogenic activation of SGLT transporters and K_v K^+ channels cooperate in glucose fuelling of the irradiated A549 cells, the former by generating the glucose entry routes, the latter by increasing and maintaining the driving force for Na^+-coupled glucose entry. Glucose uptake by SGLTs is mainly driven by the inwardly directed electrochemical driving force for Na^+ which in turn is highly dependent on the K^+ channel-regulated membrane potential. SGLTs allow efficient glucose uptake even from a glucose-depleted microenvironment which is typical for malperfused solid tumors [44]. It is therefore not surprising that several tumor entities such as colorectal, pancreatic, lung, head and neck, prostate, kidney, cervical, breast, bladder and prostate cancer as well as chondrosarcomas and leukemia upregulate SGLTs [45–53].

SGLT has been shown to be in complex with the EGFR [50,53] and radiogenic SGLT activation depends on EGFR tyrosine kinase activity [43]. Importantly, radiogenic increase in glucose fuelling seems to be required for cell survival since the SGLT inhibitor phlorizin radiosensitizes A549 lung adenocarcinoma and FaDu head and neck squamous carcinoma cells [43].

Intracellular ATP concentration has been reported to drop in irradiated A549 cells indicative of an irradiation-caused energy crisis. Notably, recovery from radiation-induced ATP decline is EGFR/SGLT-dependent and associated with improved DNA-repair leading to increased clonogenic cell survival. This is evident from the fact that EGFR or SGLT blockade delays recovery of intracellular ATP concentration and histone modifications necessary for chromatin remodeling during DNA repair. *Vice versa*, inhibition of the histone H3 modification prevents chromatin remodeling as well as energy crisis [8]. Together, these data suggest that irradiation-associated interactions between SGLT1 and EGFR result in increased glucose uptake, which counteracts the energy crisis in tumor cells caused by chromatin remodeling required for DNA repair (Fig. 2) [8,43].

Besides plasma membrane ion channels, mitochondrial transport pathways have been shown to contribute to cellular stress response. Stress-induced upregulation of uncoupling proteins (UCPs) conveys hyperpolarization of the membrane potential across the inner mitochondrial membrane ($\Delta\Psi_m$,) and thereby formation of reactive oxygen species [54]. UCPs are reportedly upregulated in a number of aggressive human tumors (leukemia, breast, colorectal, ovarian, bladder, esophagus, testicular, kidney, pancreatic, lung, and prostate cancer) in which they are proposed to contribute to malignant progression (for review see [54]).

In addition to malignant progression, UCPs may alter the therapy sensitivity of tumor cells. UCP-2 expression has been associated with paclitaxel resistance of p53 wildtype lung cancer, CPT-11 resistance of colon cancer and gemcitabine resistance of pancreatic adenocarcinoma, lung adenocarcinoma, or bladder carcinoma. Accordingly, experimental targeting of UCPs has been demonstrated to sensitize tumor cells to chemotherapy *in vitro* (for review see [54]).

Notably, ionizing radiation induces up-regulation of UCP-2 expression in colon carcinoma cells [55] and in a radiosensitive subclone of B cell lymphoma [56], as well as UCP-3 expression in rat retina [57]. Radioprotection might result from lowering the radiation-induced burden of reactive oxygen species. As a matter of fact, multi-resistant subclones of leukemia cells reportedly show higher UCP-2 protein expression, lower $\Delta\Psi_m$, lower radiation induced formation of reactive oxygen species, and decreased DNA damage as compared to their parental sensitive cells [58].

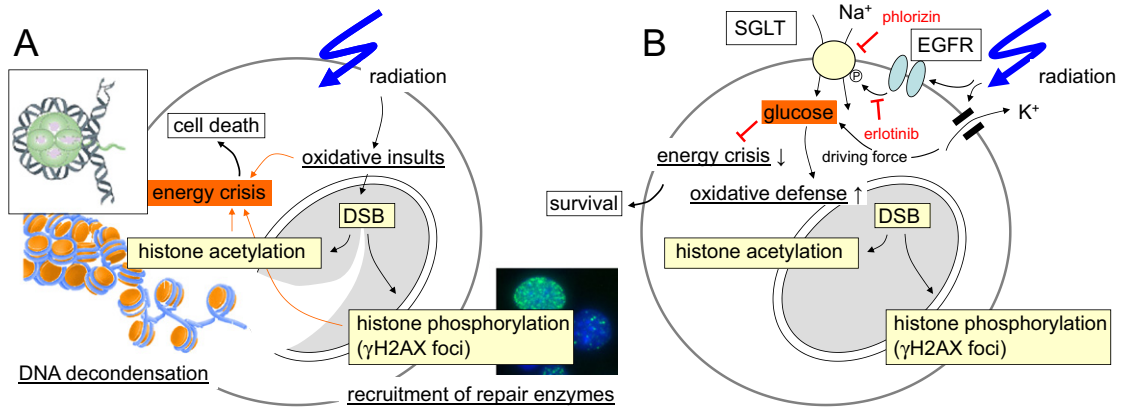

Fig. 2. Radiation-caused energy crisis (A) and functional significance of SGLT1-mediated glucose fueling for DNA repair and cell survival (B) of irradiated A549 lung adenocarcinoma cells (DSB: double strand breaks).

In summary, these data indicate that ion transports through channels may regulate processes that mediate intrinsic radioresistance. Only few laboratories worldwide including ours are working on the radiophysiology of tumor cells. The investigation of ion transports in irradiated cells therefore is at its very beginning and the few data available are mostly phenomenological in nature. The molecular mechanisms that underlie e.g. radiogenic channel activation are still ill-defined. Nevertheless, the data prove functional significance of ion transports and electrosignaling for the survival of irradiated tumor cells and might have translational implications for radiotherapy in the future.

5. Ion channels in acquired radioresistance

Microenvironmental stress such as hypoxia, interstitial nutrient depletion or low pH has been proposed to switch tumor cells from a "Grow" into a "Go" phenotype. By migration and tissue invasion "Go" tumor cells may evade the locally confined stress burden and resettle in distant and less hostile regions. Once resettled, tumor cells may readapt the "Grow" phenotype by reentering cell cycling and may establish tumor satellites in more or less close vicinity of the primary focus (for review see [54]).

In accordance with this hypothesis, sublethal ionizing irradiation as applied in single fractions of fractionated radiotherapy has been demonstrated *in vitro* and/or in rodent tumor models to induce migration, invasion and metastasis or spreading of cervix carcinoma [59], head and neck squamous cell carcinoma [60], lung adenocarinoma [61,62], colorectal carcinoma [62], breast cancer [62–64], meningioma [65], medulloblastoma [66] and glioblastoma. In particular, in glioblastoma the experimental evidence for such radiogenic hypermigration is meanwhile overwhelming [67–80]. Glioblastoma cells show a highly migrative phenotype that may "travel" large distances through the brain [81]. At least in theory, radiogenic hypermigration might, therefore, contribute to locoregional treatment failure by promoting emigration of tumor cells from the target volume during fractionated radiation therapy.

Migration and radiogenic hypermigration are well documented in glioma cells. They invade the surrounding brain parenchyma primarily by moving along axon bundles and the vasculature. During brain invasion along those tracks cells have to squeeze between very narrow interstitial spaces which requires effective local cell volume decrease and reincrease. Glioblastoma cells are capable of losing all unbound cell water [82]. The electrochemical driving force for this tremendous cell volume decrease is provided by an unusually high cytosolic Cl^- concentration (100 mM) [83,84] which is utilized as an osmolyte. During local regulatory volume decrease, extrusion of Cl^- and K^+ along their electrochemical gradients involves ClC-3 Cl^-- channels [85,86], Ca^{2+}-activated high conductance BK- [74,87,88] and intermediate

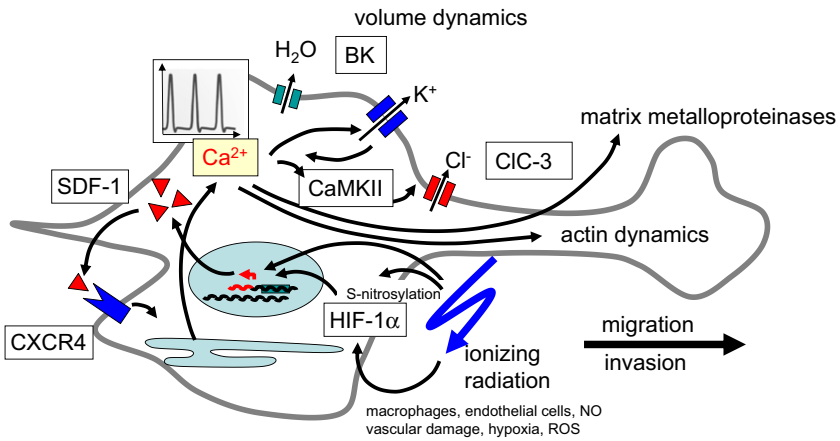

Fig. 3. Hypothetical signaling underlying radiogenic hypermigration of glioblastoma cells. SDF-1 is a HIF-1α target gene and hypoxia is a strong inductor of SDF-1 expression. IR-caused damage of the tumor vasculature and resultant tumor hypoxia has been proposed to induce SDF-1 expression [111]. Beyond that, ionizing radiation reportedly stimulates the generation of NO in tumor-associated macrophages leading to HIF-1α stabilization by S-nitrosylation [100]. Finally, radiation may directly stabilize HIF-1α as deduced from *in vitro* experiments (own unpublished results). SDF-1 induces Ca^{2+} signals through CXCR4 chemokine receptor that in turn contribute to the programming and mechanics of migration (for details see text) and possibly invasion, e.g., via calpain-dependent [112] activation of matrix metalloproteinases [113,114].

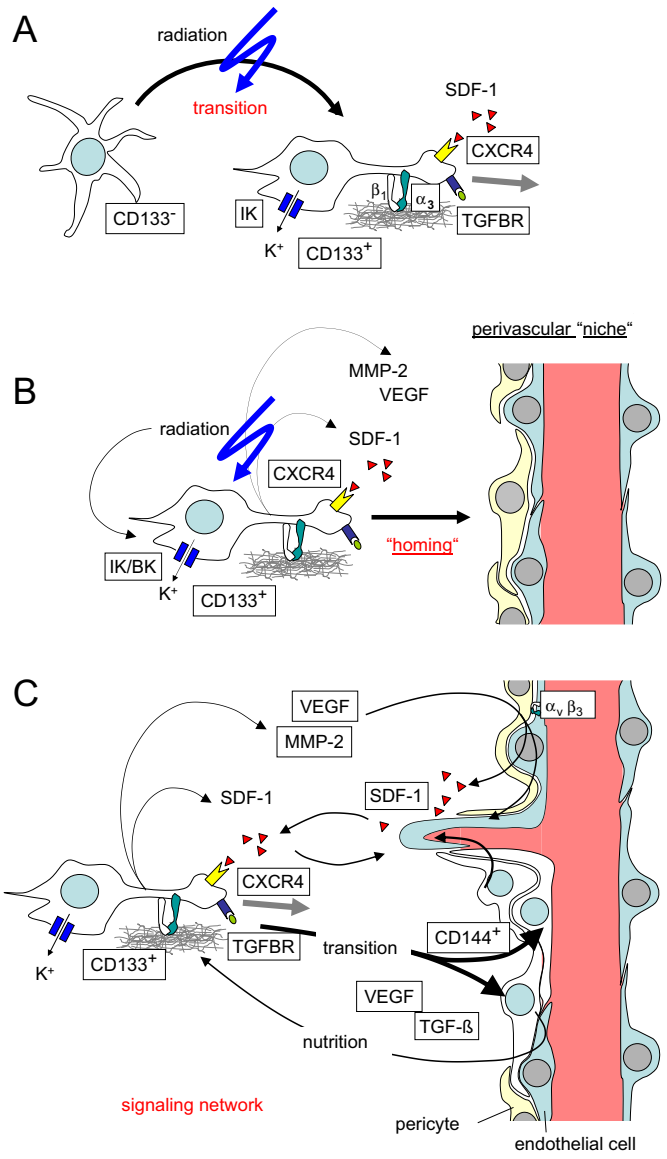

Fig. 4. Synopsis of the signaling network in glioblastoma conferring radioresistance and speculative role of ionizing radiation herein. A. Irradiation induces secretion of SDF-1 [80,95–97] and transition of $CD133^-$ "differentiated" glioblastoma cells to $CD133^+$ GSCs with up-regulated CXCR4 [106], β1/α3 integrins [108], TGFB2 receptor [115], TGF-β responsiveness [115], and IK Ca^{2+}-activated K^+ channel-dependent highly migratory and invasive phenotype [109] B. Irradiation promotes "homing" of GSCs to perivascular niches by stimulating cell migration. C. The reciprocal interaction between glioblastoma and endothelial cells strongly depends on matrix metalloproteinase-2 (MMP-2) expression by glioblastoma [114] and SDF-1 signaling of endothelial cells [110]. Importantly, irradiation induces upregulation of MMP-2 in glioblastoma cells (B) which is required for tissue invasion [67,71,72,79,114] and VEGF secretion (B) [71,75] which reportedly may promote angiogenesis [116]. In addition, transition of glioblastoma cells into endothelial cells [117] and pericytes [15] reconstruct the glioblastoma vasculature which supports both, vessel function and tumor growth.

conductance IK K^+ channels [86,89]. Inhibition of either of these channels attenuates glioblastoma cell migration or invasion [83,90–94] confirming their pivotal function in these processes.

Ionizing radiation has been demonstrated in our laboratory to activate BK K^+ channels in glioblastoma cells *in vitro* [74]. Radiogenic BK channel activity, in turn, is required for Ca^{2+}/calmodulin kinase II (CaMKII)- [74] and consecutive CaMKII-dependent ClC-3 channel activation (own unpublished observation and [85]). Inhibition of BK or CaMKII abolishes radiogenic hypermigration [74] indicating BK channel activation as key event of radiogenic hypermigration of glioblastoma cells. Radiogenic hypermigration is paralleled by radiogenic expression of the chemokine SDF-1 (stromal cell-derived factor-1, CXCL12) in different tumor entities including glioblastoma [80,95–97]. Glioblastoma cells reportedly express CXCR4 chemokine receptors and SDF-1 stimulates glioblastoma cell migration via CXCR4-mediated Ca^{2+} signaling [93]. CXCR4 receptors reportedly signal through phospholipase C and BK channels have been shown to be functionally coupled with IP_3 receptors in the ER [98] suggesting (and confirmed by own unpublished observations) that radiogenic SDF1/CXCR4 signaling is upstream of BK channel activation. SDF-1, in turn, is a target gene of the transcription factor HIF-1α which reportedly becomes stabilized, e.g. by S-nitrosylation, upon irradiation [95,99–101] (Fig. 3). Together, this gives a good example of radiogenic signaling which integrates biochemical signaling, electrosignaling (i.e., BK-dependent regulation of membrane potential) and Ca^{2+} signaling modules (more details are given in the legend to Fig. 3).

Ionizing radiation has been demonstrated to select stem (cell)-like glioblastoma cells (GSCs) or even induce transition of "differentiated" cancer cells to GSCs/CSCs in glioblastoma [102–104] and other tumor entities [14]. Notably, "stemness" is associated with SDF-1 secretion [105] and markedly increased CXCR4 chemokine expression [106]. Importantly, CXCR4 upregulation is required to maintain "stemness" of non-small cell lung cancer [107] and glioblastoma cells [105]. In accordance to CXCR4 upregulation, GSCs show a highly migratory/invasive phenotype [108,109]. Most importantly, this phenotype is highly dependent on the Ca^{2+}-activated IK K^+ channel [89,109]. Furthermore, IK channels have been demonstrated to be overexpressed in about one third of the glioma patients with IK protein expression correlating with poor patient survival [89].

Unexpectedly, a previous report demonstrated that *xeno*grafted $CD133^+$ stem-like subpopulations of glioblastoma exhibit a higher radioresistance than *xeno*grafted $CD133^-$ cells while radiosensitivity of both subpopulations does not differ *in vitro* [16]. This clearly indicates a function of the brain microenvironment for radioresistance. In particular, endothelial cells have been postulated to promote glioblastoma therapy resistance [110]. Part of the reported reciprocal interaction between glioblastoma cells and endothelial cells as well as of the complex signaling network in perivascular "niches" is schematically summarized in Fig. 4.

Albeit merely speculative, the idea that radiogenic hypermigration might promote "homing" of (CXCR4-highly-expressing stem-like) glioblastoma cells to perivascular niches is highly attractive. The subsequent reciprocal modifications of glioblastoma and endothelial cells might eventually induce radioresistance of glioblastoma cells. Together, these data suggest that radiogenic hypermigration might contribute to the apparently high radioresistance of glioblastoma cells either by promoting evasion from the radiation target volume or by stimulating the chemotaxis of glioblastoma cells to "radioprotective" perivascular niches.

6. Concluding remarks

The radiation physiology of cancer cells is yet a neglected research field. While the number of reports on ion channel function in neoplastic transformation, malignant progression or metastasis of cancer cells increases constantly only little is known about the role of ion channels in radiotherapy. The few data available strongly suggest that ionizing radiation-induced ion channel modifications are a common phenomenon. Importantly, these modifications impact on the stress response and survival of irradiated tumor cells. By modulating intracellular Ca^{2+} signals radiosensitive ion channels may directly crosstalk with the biochemical signaling of the DNA damage response. By driving local cell volume changes radiogenic ion channel modifications may promote cell migration and stress evasion of irradiated tumor cells. By stabilizing the membrane potential ionizing radiation-induced K^+ channel activity might facilitate Na^+-coupled glucose uptake providing the energy for DNA-repair. Finally, mitochondrial channels upregulated

by ionizing radiation might lower the oxidative insults associated with ionizing radiation. Given the aberrant and partly specific ion channel expression of tumor cells, a more profound understanding of the mechanisms underlying radiogenic ion channel modifications might be harnessed in the future to develop new strategies for the radiosensitization of tumors.

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References

[1] S.M. Huber, Oncochannels, Cell Calcium 53 (2013) 241–255.
[2] M. Verheij, Clinical biomarkers and imaging for radiotherapy-induced cell death, Cancer Metastasis Rev. 27 (2008) 471–480.
[3] D. Hanahan, R.A. Weinberg, The hallmarks of cancer, Cell 100 (2000) 57–70.
[4] A. Apel, I. Herr, H. Schwarz, H.P. Rodemann, A. Mayer, Blocked autophagy sensitizes resistant carcinoma cells to radiation therapy, Cancer Res. 68 (2008) 1485–1494.
[5] S. Palumbo, S. Comincini, Autophagy and ionizing radiation in tumors: the "survive or not survive" dilemma, J. Cell. Physiol. 228 (2013) 1–8.
[6] German-Cancer-Aid, Information Booklet, http://www.krebshilfe.de/fileadmin/Inhalte/Downloads/PDFs/Blaue_Ratgeber/053_strahlen.pdf 2013.
[7] A.C. Muller, F. Eckert, V. Heinrich, M. Bamberg, S. Brucker, T. Hehr, Re-surgery and chest wall re-irradiation for recurrent breast cancer: a second curative approach, BMC Cancer 11 (2011) 197.
[8] K. Dittmann, C. Mayer, H.P. Rodemann, S.M. Huber, EGFR cooperates with glucose transporter SGLT1 to enable chromatin remodeling in response to ionizing radiation, Radiother. Oncol. (2013), http://dx.doi.org/10.1016/j.radonc.2013.03.016 (pii: S0167-8140(13)00145-X).
[9] H. Harada, How can we overcome tumor hypoxia in radiation therapy? J. Radiat. Res. 52 (2011) 545–556.
[10] P.M. Cullis, G.D.D. Jones, J. Lea, M.C.R. Symons, M. Sweeney, The effects of ionizing radiation on deoxyribonucleic acid. Part 5. The role of thiols in chemical repair, J. Chem. Soc. Perkin Trans. 2 (1987) 1907–1914.
[11] M. Langenbacher, R.J. Abdel-Jalil, W. Voelter, M. Weinmann, S.M. Huber, *In vitro* hypoxic cytotoxicity and hypoxic radiosensitization. Efficacy of the novel 2-nitroimidazole N, N, N-tris[2-(2-nitro-1H-imidazol-1-yl)ethyl]amine, Strahlenther. Onkol. 189 (2013) 246–254.
[12] B. Jones, R.G. Dale, A.M. Gaya, Linear quadratic modeling of increased late normal-tissue effects in special clinical situations, Int. J. Radiat. Oncol. Biol. Phys. 64 (2006) 948–953.
[13] T.M. Pawlik, K. Keyomarsi, Role of cell cycle in mediating sensitivity to radiotherapy, Int. J. Radiat. Oncol. Biol. Phys. 59 (2004) 928–942.
[14] F. Pajonk, E. Vlashi, W.H. McBride, Radiation resistance of cancer stem cells: the 4 R's of radiobiology revisited, Stem Cells 28 (2010) 639–648.
[15] L. Cheng, Z. Huang, W. Zhou, Q. Wu, S. Donnola, J.K. Liu, X. Fang, A.E. Sloan, Y. Mao, J.D. Lathia, W. Min, R.E. McLendon, J.N. Rich, S. Bao, Glioblastoma stem cells generate vascular pericytes to support vessel function and tumor growth, Cell 153 (2013) 139–152.
[16] M. Jamal, B.H. Rath, P.S. Tsang, K. Camphausen, P.J. Tofilon, The brain microenvironment preferentially enhances the radioresistance of $CD133^{+}$ glioblastoma stem-like cells, Neoplasia 14 (2012) 150–158.
[17] Y. Hara, M. Wakamori, M. Ishii, E. Maeno, M. Nishida, T. Yoshida, H. Yamada, S. Shimizu, E. Mori, J. Kudoh, N. Shimizu, H. Kurose, Y. Okada, K. Imoto, Y. Mori, LTRPC2 Ca^{2+}-permeable channel activated by changes in redox status confers susceptibility to cell death, Mol. Cell 9 (2002) 163–173.
[18] M. Ishii, A. Oyama, T. Hagiwara, A. Miyazaki, Y. Mori, Y. Kiuchi, S. Shimizu, Facilitation of H2O2-induced A172 human glioblastoma cell death by insertion of oxidative stress-sensitive TRPM2 channels, Anticancer Res. 27 (2007) 3987–3992.
[19] M. Naziroglu, A. Luckhoff, A calcium influx pathway regulated separately by oxidative stress and ADP-Ribose in TRPM2 channels: single channel events, Neurochem. Res. 33 (2008) 1256–1262.
[20] M. Naziroglu, A. Luckhoff, Effects of antioxidants on calcium influx through TRPM2 channels in transfected cells activated by hydrogen peroxide, J. Neurol. Sci. 270 (2008) 152–158.
[21] E. Fonfria, I.C. Marshall, C.D. Benham, I. Boyfield, J.D. Brown, K. Hill, J.P. Hughes, S.D. Skaper, S. McNulty, TRPM2 channel opening in response to oxidative stress is dependent on activation of poly(ADP-ribose) polymerase, Br. J. Pharmacol. 143 (2004) 186–192.
[22] F.J. Kuhn, I. Heiner, A. Luckhoff, TRPM2: a calcium influx pathway regulated by oxidative stress and the novel second messenger ADP-ribose, Pflugers Arch. 451 (2005) 212–219.
[23] A.L. Perraud, C.L. Takanishi, B. Shen, S. Kang, M.K. Smith, C. Schmitz, H.M. Knowles, D. Ferraris, W. Li, J. Zhang, B.L. Stoddard, A.M. Scharenberg, Accumulation of free ADP-ribose from mitochondria mediates oxidative stress-induced gating of TRPM2 cation channels, J. Biol. Chem. 280 (2005) 6138–6148.
[24] J. Eisfeld, A. Luckhoff, Trpm2, Handb. Exp. Pharmacol. (2007) 237–252.
[25] K. Inamura, Y. Sano, S. Mochizuki, H. Yokoi, A. Miyake, K. Nozawa, C. Kitada, H. Matsushime, K. Furuichi, Response to ADP-ribose by activation of TRPM2 in the CRI-G1 insulinoma cell line, J. Membr. Biol. 191 (2003) 201–207.
[26] X. Zeng, S.C. Sikka, L. Huang, C. Sun, C. Xu, D. Jia, A.B. Abdel-Mageed, J.E. Pottle, J.T. Taylor, M. Li, Novel role for the transient receptor potential channel TRPM2 in prostate cancer cell proliferation, Prostate Cancer Prostatic Dis. 13 (2010) 195–201.
[27] W. Zhang, I. Hirschler-Laszkiewicz, Q. Tong, K. Conrad, S.C. Sun, L. Penn, D.L. Barber, R. Stahl, D.J. Carey, J.Y. Cheung, B.A. Miller, TRPM2 is an ion channel that modulates hematopoietic cell death through activation of caspases and PARP cleavage, Am. J. Physiol. Cell Physiol. 290 (2006) C1146–C1159.
[28] W. Zhang, X. Chu, Q. Tong, J.Y. Cheung, K. Conrad, K. Masker, B.A. Miller, A novel TRPM2 isoform inhibits calcium influx and susceptibility to cell death, J. Biol. Chem. 278 (2003) 16222–16229.
[29] U. Orfanelli, A.K. Wenke, C. Doglioni, V. Russo, A.K. Bosserhoff, G. Lavorgna, Identification of novel sense and antisense transcription at the TRPM2 locus in cancer, Cell Res. 18 (2008) 1128–1140.
[30] S. McNulty, E. Fonfria, The role of TRPM channels in cell death, Pflugers Arch. 451 (2005) 235–242.
[31] S. Mergler, R. Derckx, P.S. Reinach, F. Garreis, A. Bohm, L. Schmelzer, S. Skosyrski, N. Ramesh, S. Abdelmessih, O.K. Polat, N. Khajavi, A.I. Riechardt, Calcium regulation by temperature-sensitive transient receptor potential channels in human uveal melanoma cells, Cell. Signal. 26 (2014) 56–69.
[32] S. Mergler, M. Skrzypski, M. Sassek, P. Pietrzak, C. Pucci, B. Wiedenmann, M.Z. Strowski, Thermo-sensitive transient receptor potential vanilloid channel-1 regulates intracellular calcium and triggers chromogranin A secretion in pancreatic neuroendocrine BON-1 tumor cells, Cell. Signal. 24 (2012) 233–246.
[33] S. Malagarie-Cazenave, N. Olea-Herrero, D. Vara, C. Morell, I. Diaz-Laviada, The vanilloid capsaicin induces IL-6 secretion in prostate PC-3 cancer cells, Cytokine 54 (2011) 330–337.
[34] C. Amantini, M. Mosca, M. Nabissi, R. Lucciarini, S. Caprodossi, A. Arcella, F. Giangaspero, G. Santoni, Capsaicin-induced apoptosis of glioma cells is mediated by TRPV1 vanilloid receptor and requires p38 MAPK activation, J. Neurochem. 102 (2007) 977–990.
[35] G. Santoni, S. Caprodossi, V. Farfariello, S. Liberati, A. Gismondi, C. Amantini, Antioncogenic effects of transient receptor potential vanilloid 1 in the progression of transitional urothelial cancer of human bladder, ISRN Urol. 2012 (2012) 458238.
[36] C. Amantini, P. Ballarini, S. Caprodossi, M. Nabissi, M.B. Morelli, R. Lucciarini, M.A. Cardarelli, G. Mammana, G. Santoni, Triggering of transient receptor potential vanilloid type 1 (TRPV1) by capsaicin induces Fas/CD95-mediated apoptosis of urothelial cancer cells in an ATM-dependent manner, Carcinogenesis 30 (2009) 1320–1329.
[37] K. Stock, J. Kumar, M. Synowitz, S. Petrosino, R. Imperatore, E.S. Smith, P. Wend, B. Purfurst, U.A. Nuber, U. Gurok, V. Matyash, J.H. Walzlein, S.R. Chirasani, G. Dittmar, B.F. Cravatt, S. Momma, G.R. Lewin, A. Ligresti, L. De Petrocellis, L. Cristino, V. Di Marzo, H. Kettenmann, R. Glass, Neural precursor cells induce cell death of high-grade astrocytomas through stimulation of TRPV1, Nat. Med. 18 (2012) 1232–1238.
[38] S. Li, A.M. Bode, F. Zhu, K. Liu, J. Zhang, M.O. Kim, K. Reddy, T. Zykova, W.Y. Ma, A.L. Carper, A.K. Langfald, Z. Dong, TRPV1-antagonist AMG9810 promotes mouse skin tumorigenesis through EGFR/Akt signaling, Carcinogenesis 32 (2011) 779–785.
[39] K. Masumoto, M. Tsukimoto, S. Kojima, Role of TRPM2 and TRPV1 cation channels in cellular responses to radiation-induced DNA damage, Biochim. Biophys. Acta 1830 (2013) 3382–3390.
[40] N. Heise, D. Palme, M. Misovic, S. Koka, J. Rudner, F. Lang, H.R. Salih, S.M. Huber, G. Henke, Non-selective cation channel-mediated Ca^{2+}-entry and activation of Ca^{2+}/calmodulin-dependent kinase II contribute to G2/M cell cycle arrest and survival of irradiated leukemia cells, Cell. Physiol. Biochem. 26 (2010) 597–608.
[41] D. Palme, M. Misovic, E. Schmid, D. Klumpp, H.R. Salih, J. Rudner, S. Huber, Kv3.4 potassium channel-mediated electrosignaling controls cell cycle and survival of irradiated leukemia cells, Pflugers Arch. (2013), http://dx.doi.org/10.1007/s00424-013-1249-5.
[42] S.S. Kuo, A.H. Saad, A.C. Koong, G.M. Hahn, A.J. Giaccia, Potassium-channel activation in response to low doses of gamma-irradiation involves reactive oxygen intermediates in nonexcitatory cells, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 908–912.
[43] S.M. Huber, M. Misovic, C. Mayer, H.P. Rodemann, K. Dittmann, EGFR-mediated stimulation of sodium/glucose cotransport promotes survival of irradiated human A549 lung adenocarcinoma cells, Radiother. Oncol. 103 (2012) 373–379.
[44] V. Ganapathy, M. Thangaraju, P.D. Prasad, Nutrient transporters in cancer: relevance to Warburg hypothesis and beyond, Pharmacol. Ther. 121 (2009) 29–40.
[45] J.A. Nelson, R.E. Falk, The efficacy of phloridzin and phloretin on tumor cell growth, Anticancer Res. 13 (1993) 2287–2292.
[46] N. Ishikawa, T. Oguri, T. Isobe, K. Fujitaka, N. Kohno, SGLT gene expression in primary lung cancers and their metastatic lesions, Jpn. J. Cancer Res. 92 (2001) 874–879.
[47] B.M. Helmke, C. Reisser, M. Idzko, G. Dyckhoff, C. Herold-Mende, Expression of SGLT-1 in preneoplastic and neoplastic lesions of the head and neck, Oral Oncol. 40 (2004) 28–35.
[48] L.C. Yu, C.Y. Huang, W.T. Kuo, H. Sayer, J.R. Turner, A.G. Buret, SGLT-1-mediated glucose uptake protects human intestinal epithelial cells against Giardia duodenalis-induced apoptosis, Int. J. Parasitol. 38 (2008) 923–934.
[49] V.F. Casneuf, P. Fonteyne, N. Van Damme, P. Demetter, P. Pauwels, B. de Hemptinne, M. De Vos, C. Van de Wiele, M. Peeters, Expression of SGLT1, Bcl-2 and p53 in primary pancreatic cancer related to survival, Cancer Invest. 26 (2008) 852–859.

[50] Z. Weihua, R. Tsan, W.C. Huang, Q. Wu, C.H. Chiu, I.J. Fidler, M.C. Hung, Survival of cancer cells is maintained by EGFR independent of its kinase activity, Cancer Cell 13 (2008) 385–393.

[51] N. Leiprecht, C. Munoz, I. Alesutan, G. Siraskar, M. Sopjani, M. Foller, F. Stubenrauch, T. Iftner, F. Lang, Regulation of Na^+-coupled glucose carrier SGLT1 by human papillomavirus 18 E6 protein, Biochem. Biophys. Res. Commun. 404 (2011) 695–700.

[52] E.M. Wright, D.D. Loo, B.A. Hirayama, Biology of human sodium glucose transporters, Physiol. Rev. 91 (2011) 733–794.

[53] J. Ren, L.R. Bollu, F. Su, G. Gao, L. Xu, W.C. Huang, M.C. Hung, Z. Weihua, EGFR-SGLT1 interaction does not respond to EGFR modulators, but inhibition of SGLT1 sensitizes prostate cancer cells to EGFR tyrosine kinase inhibitors, Prostate 73 (2013) 1453–1461.

[54] S.M. Huber, L. Butz, B. Stegen, D. Klumpp, N. Braun, P. Ruth, F. Eckert, Ionizing radiation, ion transports, and radioresistance of cancer cells, Frontiers in Physiology | Membrane Physiology and Membrane Biophysics 4 (2013) 212.

[55] A. Sreekumar, M.K. Nyati, S. Varambally, T.R. Barrette, D. Ghosh, T.S. Lawrence, A.M. Chinnaiyan, Profiling of cancer cells using protein microarrays: discovery of novel radiation-regulated proteins, Cancer Res. 61 (2001) 7585–7593.

[56] D.W. Voehringer, D.L. Hirschberg, J. Xiao, Q. Lu, M. Roederer, C.B. Lock, L.A. Herzenberg, L. Steinman, L.A. Herzenberg, Gene microarray identification of redox and mitochondrial elements that control resistance or sensitivity to apoptosis, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 2680–2685.

[57] X.W. Mao, J.D. Crapo, D.S. Gridley, Mitochondrial oxidative stress-induced apoptosis and radioprotection in proton-irradiated rat retina, Radiat. Res. 178 (2012) 118–125.

[58] M.E. Harper, A. Antoniou, E. Villalobos-Menuey, A. Russo, R. Trauger, M. Vendemelio, A. George, R. Bartholomew, D. Carlo, A. Shaikh, J. Kupperman, E.W. Newell, I.A. Bespalov, S.S. Wallace, Y. Liu, J.R. Rogers, G.L. Gibbs, J.L. Leahy, R.E. Camley, R. Melamede, M.K. Newell, Characterization of a novel metabolic strategy used by drug-resistant tumor cells, FASEB J. 16 (2002) 1550–1557.

[59] W.H. Su, P.C. Chuang, E.Y. Huang, K.D. Yang, Radiation-induced increase in cell migration and metastatic potential of cervical cancer cells operates via the K-Ras pathway, Am. J. Pathol. 180 (2012) 862–871.

[60] A.C. Pickhard, J. Margraf, A. Knopf, T. Stark, G. Piontek, C. Beck, A.L. Boulesteix, E.Q. Scherer, S. Pigorsch, J. Schlegel, W. Arnold, R. Reiter, Inhibition of radiation induced migration of human head and neck squamous cell carcinoma cells by blocking of EGF receptor pathways, BMC Cancer 11 (2011) 388.

[61] J.W. Jung, S.Y. Hwang, J.S. Hwang, E.S. Oh, S. Park, I.O. Han, Ionising radiation induces changes associated with epithelial–mesenchymal transdifferentiation and increased cell motility of A549 lung epithelial cells, Eur. J. Cancer 43 (2007) 1214–1224.

[62] Y.C. Zhou, J.Y. Liu, J. Li, J. Zhang, Y.Q. Xu, H.W. Zhang, L.B. Qiu, G.R. Ding, X.M. Su, S. Mei, G.Z. Guo, Ionizing radiation promotes migration and invasion of cancer cells through transforming growth factor-beta-mediated epithelial–mesenchymal transition, Int. J. Radiat. Oncol. Biol. Phys. 81 (2011) 1530–1537.

[63] S. Biswas, M. Guix, C. Rinehart, T.C. Dugger, A. Chytil, H.L. Moses, M.L. Freeman, C.L. Arteaga, Inhibition of TGF-beta with neutralizing antibodies prevents radiation-induced acceleration of metastatic cancer progression, J. Clin. Invest. 117 (2007) 1305–1313.

[64] D.M. Kambach, V.L. Sodi, P.I. Lelkes, J. Azizkhan-Clifford, M.J. Reginato, ErbB2, FoxM1 and 14-3-3zeta prime breast cancer cells for invasion in response to ionizing radiation, Oncogene 33 (2014) 589–598.

[65] O. Kargiotis, C. Chetty, V. Gogineni, C.S. Gondi, S.M. Pulukuri, A.P. Kyritsis, M. Gujrati, J.D. Klopfenstein, D.H. Dinh, J.S. Rao, uPA/uPAR downregulation inhibits radiation-induced migration, invasion and angiogenesis in IOMM-Lee meningioma cells and decreases tumor growth *in vivo*, Int. J. Oncol. 33 (2008) 937–947.

[66] S. Asuthkar, A.K. Nalla, C.S. Gondi, D.H. Dinh, M. Gujrati, S. Mohanam, J.S. Rao, Gadd45a sensitizes medulloblastoma cells to irradiation and suppresses MMP-9-mediated EMT, Neuro Oncol. 13 (2011) 1059–1073.

[67] C. Wild-Bode, M. Weller, A. Rimner, J. Dichgans, W. Wick, Sublethal irradiation promotes migration and invasiveness of glioma cells: implications for radiotherapy of human glioblastoma, Cancer Res. 61 (2001) 2744–2750.

[68] W. Wick, A. Wick, J.B. Schulz, J. Dichgans, H.P. Rodemann, M. Weller, Prevention of irradiation-induced glioma cell invasion by temozolomide involves caspase 3 activity and cleavage of focal adhesion kinase, Cancer Res. 62 (2002) 1915–1919.

[69] B. Hegedus, J. Zach, A. Czirok, J. Lovey, T. Vicsek, Irradiation and Taxol treatment result in non-monotonous, dose-dependent changes in the motility of glioblastoma cells, J. Neuro Oncol. 67 (2004) 147–157.

[70] S. Rieken, D. Habermehl, A. Mohr, L. Wuerth, K. Lindel, K. Weber, J. Debus, S.E. Combs, Targeting alphanubeta3 and alphanubeta5 inhibits photon-induced hypermigration of malignant glioma cells, Radiat. Oncol. 6 (2011) 132.

[71] A.V. Badiga, C. Chetty, D. Kesanakurti, D. Are, M. Gujrati, J.D. Klopfenstein, D.H. Dinh, J.S. Rao, MMP-2 siRNA inhibits radiation-enhanced invasiveness in glioma cells, PLoS One 6 (2011) e20614.

[72] S.Y. Kwak, J.S. Yang, B.Y. Kim, I.H. Bae, Y.H. Han, Ionizing radiation-inducible miR-494 promotes glioma cell invasion through EGFR stabilization by targeting p190B rhoGAP, Biochim. Biophys. Acta 1843 (2014) 508–516.

[73] A. Canazza, C. Calatozzolo, L. Fumagalli, A. Bergantin, F. Ghielmetti, L. Fariselli, D. Croci, A. Salmaggi, E. Ciusani, Increased migration of a human glioma cell line after *in vitro* CyberKnife irradiation, Cancer Biol. Ther. 12 (2011) 629–633.

[74] M. Steinle, D. Palme, M. Misovic, J. Rudner, K. Dittmann, R. Lukowski, P. Ruth, S.M. Huber, Ionizing radiation induces migration of glioblastoma cells by activating BK K^+ channels, Radiother. Oncol. 101 (2011) 122–126.

[75] W.J. Kil, P.J. Tofilon, K. Camphausen, Post-radiation increase in VEGF enhances glioma cell motility *in vitro*, Radiat. Oncol. 7 (2012) 25.

[76] I. Vanan, Z. Dong, E. Tosti, G. Warshaw, M. Symons, R. Ruggieri, Role of a DNA damage checkpoint pathway in ionizing radiation-induced glioblastoma cell migration and invasion, Cell. Mol. Neurobiol. 32 (2012) 1199–1208.

[77] W.T. Arscott, A.T. Tandle, S. Zhao, J.E. Shabason, I.K. Gordon, C.D. Schlaff, G. Zhang, P.J. Tofilon, K.A. Camphausen, Ionizing radiation and glioblastoma exosomes: implications in tumor biology and cell migration, Transl. Oncol. 6 (2013) 638–648.

[78] W. Zhou, Y. Xu, G. Gao, Z. Jiang, X. Li, Irradiated normal brain promotes invasion of glioblastoma through vascular endothelial growth and stromal cell-derived factor 1alpha, Neuroreport 24 (2013) 730–734.

[79] A. Shankar, S. Kumar, A. Iskander, N.R. Varma, B. Janic, A. Decarvalho, T. Mikkelsen, J.A. Frank, M.M. Ali, R.A. Knight, S. Brown, A.S. Arbab, Subcurative radiation significantly increases proliferation, invasion, and migration of primary GBM *in vivo*, Chin. J. Cancer 33 (2013) 148–158.

[80] S.C. Wang, C.F. Yu, J.H. Hong, C.S. Tsai, C.S. Chiang, Radiation therapy-induced tumor invasiveness is associated with SDF-1-regulated macrophage mobilization and vasculogenesis, PLoS One 8 (2013) e69182.

[81] J. Johnson, M.O. Nowicki, C.H. Lee, E.A. Chiocca, M.S. Viapiano, S.E. Lawler, J.J. Lannutti, Quantitative analysis of complex glioma cell migration on electrospun polycaprolactone using time-lapse microscopy, Tissue Eng. C Methods 15 (2009) 531–540.

[82] S. Watkins, H. Sontheimer, Hydrodynamic cellular volume changes enable glioma cell invasion, J. Neurosci. 31 (2011) 17250–17259.

[83] B.R. Haas, H. Sontheimer, Inhibition of the sodium-potassium-chloride cotransporter isoform-1 reduces glioma invasion, Cancer Res. 70 (2010) 5597–5606.

[84] C.W. Habela, N.J. Ernest, A.F. Swindall, H. Sontheimer, Chloride accumulation drives volume dynamics underlying cell proliferation and migration, J. Neurophysiol. 101 (2009) 750–757.

[85] V.A. Cuddapah, H. Sontheimer, Molecular interaction and functional regulation of ClC-3 by Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) in human malignant glioma, J. Biol. Chem. 285 (2010) 11188–11196.

[86] V.A. Cuddapah, K.L. Turner, S. Seifert, H. Sontheimer, Bradykinin-induced chemotaxis of human gliomas requires the activation of KCa3.1 and ClC-3, J. Neurosci. 33 (2013) 1427–1440.

[87] C.B. Ransom, H. Sontheimer, BK channels in human glioma cells, J. Neurophysiol. 85 (2001) 790–803.

[88] H. Sontheimer, An unexpected role for ion channels in brain tumor metastasis, Exp. Biol. Med. (Maywood) 233 (2008) 779–791.

[89] K.L. Turner, A. Honasoge, S.M. Robert, M.M. McFerrin, H. Sontheimer, A proinvasive role for the Ca^{2+}-activated K^+ channel KCa3.1 in malignant glioma, Glia 62 (2014) 971–981.

[90] N.J. Ernest, A.K. Weaver, L.B. Van Duyn, H.W. Sontheimer, Relative contribution of chloride channels and transporters to regulatory volume decrease in human glioma cells, Am. J. Physiol. Cell Physiol. 288 (2005) C1451–C1460.

[91] M.B. McFerrin, H. Sontheimer, A role for ion channels in glioma cell invasion, Neuron Glia Biol. 2 (2006) 39–49.

[92] L. Catacuzzeno, F. Aiello, B. Fioretti, L. Sforna, E. Castigli, P. Ruggieri, A.M. Tata, A. Calogero, F. Franciolini, Serum-activated K and Cl currents underlay U87-MG glioblastoma cell migration, J. Cell. Physiol. 226 (2010) 1926–1933.

[93] M. Sciaccaluga, B. Fioretti, L. Catacuzzeno, F. Pagani, C. Bertollini, M. Rosito, M. Catalano, G. D'Alessandro, A. Santoro, G. Cantore, D. Ragozzino, E. Castigli, F. Franciolini, C. Limatola, CXCL12-induced glioblastoma cell migration requires intermediate conductance Ca^{2+}-activated K^+ channel activity, Am. J. Physiol. Cell Physiol. 299 (2010) C175–C184.

[94] V.C. Lui, S.S. Lung, J.K. Pu, K.N. Hung, G.K. Leung, Invasion of human glioma cells is regulated by multiple chloride channels including ClC-3, Anticancer Res. 30 (2010) 4515–4524.

[95] G. Tabatabai, B. Frank, R. Mohle, M. Weller, W. Wick, Irradiation and hypoxia promote homing of haematopoietic progenitor cells towards gliomas by TGF-beta-dependent HIF-1alpha-mediated induction of CXCL12, Brain 129 (2006) 2426–2435.

[96] M. Kioi, H. Vogel, G. Schultz, R.M. Hoffman, G.R. Harsh, J.M. Brown, Inhibition of vasculogenesis, but not angiogenesis, prevents the recurrence of glioblastoma after irradiation in mice, J. Clin. Invest. 120 (2010) 694–705.

[97] S.V. Kozin, W.S. Kamoun, Y. Huang, M.R. Dawson, R.K. Jain, D.G. Duda, Recruitment of myeloid but not endothelial precursor cells facilitates tumor regrowth after local irradiation, Cancer Res. 70 (2010) 5679–5685.

[98] A.K. Weaver, M.L. Olsen, M.B. McFerrin, H. Sontheimer, BK channels are linked to inositol 1,4,5-triphosphate receptors via lipid rafts: a novel mechanism for coupling $[Ca^{2+}]_i$ to ion channel activation, J. Biol. Chem. 282 (2007) 31558–31568.

[99] C. Garcia-Moruja, J.M. Alonso-Lobo, P. Rueda, C. Torres, N. Gonzalez, M. Bermejo, F. Luque, F. Arenzana-Seisdedos, J. Alcami, A. Caruz, Functional characterization of SDF-1 proximal promoter, J. Mol. Biol. 348 (2005) 43–62.

[100] F. Li, P. Sonveaux, Z.N. Rabbani, S. Liu, B. Yan, Q. Huang, Z. Vujaskovic, M.W. Dewhirst, C.Y. Li, Regulation of HIF-1alpha stability through S-nitrosylation, Mol. Cell 26 (2007) 63–74.

[101] B.J. Moeller, Y. Cao, C.Y. Li, M.W. Dewhirst, Radiation activates HIF-1 to regulate vascular radiosensitivity in tumors: role of reoxygenation, free radicals, and stress granules, Cancer Cell 5 (2004) 429–441.

[102] M.J. Kim, R.K. Kim, C.H. Yoon, S. An, S.G. Hwang, Y. Suh, M.J. Park, H.Y. Chung, I.G. Kim, S.J. Lee, Importance of PKCdelta signaling in fractionated-radiation-induced expansion of glioma-initiating cells and resistance to cancer treatment, J. Cell Sci. 124 (2011) 3084–3094.

[103] C. Lagadec, E. Vlashi, L. Della Donna, C. Dekmezian, F. Pajonk, Radiation-induced reprogramming of breast cancer cells, Stem Cells 30 (2012) 833–844.
[104] K. Tamura, M. Aoyagi, N. Ando, T. Ogishima, H. Wakimoto, M. Yamamoto, K. Ohno, Expansion of CD133-positive glioma cells in recurrent de novo glioblastomas after radiotherapy and chemotherapy, J. Neurosurg. 119 (2013) 1145–1155.
[105] M. Gatti, A. Pattarozzi, A. Bajetto, R. Wurth, A. Daga, P. Fiaschi, G. Zona, T. Florio, F. Barbieri, Inhibition of CXCL12/CXCR4 autocrine/paracrine loop reduces viability of human glioblastoma stem-like cells affecting self-renewal activity, Toxicology 314 (2013) 209–220.
[106] G. Liu, X. Yuan, Z. Zeng, P. Tunici, H. Ng, I.R. Abdulkadir, L. Lu, D. Irvin, K.L. Black, J.S. Yu, Analysis of gene expression and chemoresistance of CD133^{+} cancer stem cells in glioblastoma, Mol. Cancer 5 (2006) 67.
[107] M.J. Jung, J.K. Rho, Y.M. Kim, J.E. Jung, Y.B. Jin, Y.G. Ko, J.S. Lee, S.J. Lee, J.C. Lee, M.J. Park, Upregulation of CXCR4 is functionally crucial for maintenance of stemness in drug-resistant non-small cell lung cancer cells, Oncogene 32 (2013) 209–221.
[108] M. Nakada, E. Nambu, N. Furuyama, Y. Yoshida, T. Takino, Y. Hayashi, H. Sato, Y. Sai, T. Tsuji, K.I. Miyamoto, A. Hirao, J.I. Hamada, Integrin alpha3 is overexpressed in glioma stem-like cells and promotes invasion, Br. J. Cancer 108 (2013) 2516–2524.
[109] P. Ruggieri, G. Mangino, B. Fioretti, L. Catacuzzeno, R. Puca, D. Ponti, M. Miscusi, F. Franciolini, G. Ragona, A. Calogero, The inhibition of KCa3.1 channels activity reduces cell motility in glioblastoma derived cancer stem cells, PLoS One 7 (2012) e47825.
[110] S. Rao, R. Sengupta, E.J. Choe, B.M. Woerner, E. Jackson, T. Sun, J. Leonard, D. Piwnica-Worms, J.B. Rubin, CXCL12 mediates trophic interactions between endothelial and tumor cells in glioblastoma, PLoS One 7 (2012) e33005.
[111] J.P. Greenfield, W.S. Cobb, D. Lyden, Resisting arrest: a switch from angiogenesis to vasculogenesis in recurrent malignant gliomas, J. Clin. Invest. 120 (2010) 663–667.
[112] H.S. Jang, S. Lal, J.A. Greenwood, Calpain 2 is required for glioblastoma cell invasion: regulation of matrix metalloproteinase 2, Neurochem. Res. 35 (2010) 1796–1804.
[113] C.M. Park, M.J. Park, H.J. Kwak, H.C. Lee, M.S. Kim, S.H. Lee, I.C. Park, C.H. Rhee, S.I. Hong, Ionizing radiation enhances matrix metalloproteinase-2 secretion and invasion of glioma cells through Src/epidermal growth factor receptor-mediated p38/Akt and phosphatidylinositol 3-kinase/Akt signaling pathways, Cancer Res. 66 (2006) 8511–8519.
[114] D.R. Maddirela, D. Kesanakurti, M. Gujrati, J.S. Rao, MMP-2 suppression abrogates irradiation-induced microtubule formation in endothelial cells by inhibiting alphavbeta3-mediated SDF-1/CXCR4 signaling, Int. J. Oncol. 42 (2011) 1279–1288.
[115] X.Z. Ye, S.L. Xu, Y.H. Xin, S.C. Yu, Y.F. Ping, L. Chen, H.L. Xiao, B. Wang, L. Yi, Q.L. Wang, X.F. Jiang, L. Yang, P. Zhang, C. Qian, Y.H. Cui, X. Zhang, X.W. Bian, Tumor-associated microglia/macrophages enhance the invasion of glioma stem-like cells via TGF-beta1 signaling pathway, J. Immunol. 189 (2012) 444–453.
[116] S. Bao, Q. Wu, S. Sathornsumetee, Y. Hao, Z. Li, A.B. Hjelmeland, Q. Shi, R.E. McLendon, D.D. Bigner, J.N. Rich, Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor, Cancer Res. 66 (2006) 7843–7848.
[117] R. Wang, K. Chadalavada, J. Wilshire, U. Kowalik, K.E. Hovinga, A. Geber, B. Fligelman, M. Leversha, C. Brennan, V. Tabar, Glioblastoma stem-like cells give rise to tumour endothelium, Nature 468 (2010) 829–833.