

Current Clinical Pathology  
*Series Editor: Antonio Giordano*

Luigi Pirtoli  
Giovanni Luca Gravina  
Antonio Giordano *Editors*

# Radiobiology of Glioblastoma

Recent Advances and Related Pathobiology

 Humana Press

# CURRENT CLINICAL PATHOLOGY

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*SERIES EDITOR*

This series includes monographs dealing with important topics in surgical pathology, cytopathology hematology, and diagnostic laboratory medicine. It is aimed at practicing hospital based pathologists and their residents providing them with concise up-to- date reviews and state of the art summaries of current problems that these physicians may encounter in their daily practice of clinical pathology.

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Editors

# Radiobiology of Glioblastoma

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and Related Pathobiology

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*This book is dedicated to our patients, in particular to those affected by tumors with a very poor prognosis, as glioblastoma currently is, as research has unfortunately not provided favorable survival outcomes as of yet; to our mentors, who have oriented our lives toward professional and scientific commitments; and to our families, who have sustained our efforts in implementing this volume.*



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## Foreword

*Le vent se lève! ... il faut tenter de vivre!*

(Paul Valéry, Cimetière Marin, 1920)

Toward the end of 2013, Antonio Giordano suggested I should prepare for publication a book on radiobiology of glioblastoma, my main field of scientific interest. Initially, I had some qualms about the difficulty in implementing such an issue and on its possible usefulness, given the tremendous accumulation of knowledge on this subject in recent years and the present, modest impact of most of these disclosures on therapy outcome. Nevertheless, the idea appealed to me because many of the striking radiobiology experimental results presently achieved are not satisfactorily addressed by suitable clinical trials. A comprehensive coverage of the modern, mechanistic, pathobiology-grounded radiobiology topics of possible clinical relevance through selected contributions seemed to be appropriate, considering the possible translational perspectives. Therefore, I decided to take up the challenge after ensuring the cooperation for this task with Antonio Giordano himself, as a prominent pathobiology scientist in oncology, and with Giovanni Luca Gravina, a radiation oncologist and biologist of established reputation, both of whom have honored me with their friendship.

Glioblastoma patients may achieve, at most, median survivals of 12–15 months and 25 % and 5 % 2- and 5-year overall survival rates, respectively, after current therapy. From a radiobiological point of view, the most relevant problem is the radiation resistance of glioblastoma, which in the vast majority of cases recurs in the primary site after radiotherapy. The empirically developed principles of radiation dose fractionation and modulation over time, for improved tumor control/normal tissue damage ratios, do not enable improved tumor control with radiation doses higher than 60 Gy, differently from most other cancers. Technology alone does not seem able to overcome this situation. The impressive progress in medical imaging and in radiotherapy facilities, in fact, now safely provide a very precise tumor localization and radiation high-dose delivery in a few sessions, activating also cell-death pathways different from those involved in killing cancer cells with conventional schedules, but the results of specially designed clinical trials have been disappointing. However, hallmarks in the framework of molecular classification, genome-wide characterization, membrane receptors, epigenetic features, signal pathways, and immune domain are shown to be inherently related to the prognosis of glioblastoma and, in some cases, are predictive of



a response to current postoperative therapy methods, including radiotherapy. Targeting or modulating these determinants by “biological” agents is a promising approach for improving radiation effectiveness, as documented in numerous preclinical disclosures. However, glioblastoma is a complex and elusive disease, and these attempts have been unsuccessful in the clinical setting so far, probably due to molecular signaling redundancy, clonal selection (or emergence) of resistant phenotypes under treatment, or difficulty in penetrating blood–brain barrier by the drugs. In conclusion, further research is warranted, for a more fitting, “individually featured” radiotherapy of glioblastoma with radiation optimization and enhancement grounded on pathobiology knowledge, an approach which is more and more frequently implemented in other tumors. Translational aspects are relevant to this regard. Identification of suitable markers should continue through the use of large database collections including pathological, biological, and clinical data to establish reliable correlations with advanced statistical methods, so that pre-clinical research may select the most plausible working hypotheses for clinical trial design.

I hope this book will contribute to this purpose or at least in facilitating communication among the involved professionals and researchers.

Siena, Italy  
23 October 2015

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## Preface

There has been a clear-cut change in the mentality of oncologists in the last decades. Up to the end of the past century, in fact, any therapeutic approach to cancer could be assigned to one of the following two categories: treatment for definitive cure or otherwise palliation. In the former setting, the real possibility of achieving definitive healing of the disease sometimes justified treatment-related side effects or damages of not negligible severity, whereas in the latter orientation—whose intent was only a reasonable improvement in life expectancy and/or symptoms relief—a milder therapeutic conduct was more advisable. This scenario has been radically modified by the rise of more effective and safer surgical, radiation, and drug therapies, with the consequence that also in patients whose cancer could not be eradicated, it could be changed into a chronic disease in many cases with an approach more active than in the past, allowing survival times often exceeding the fateful 5-year threshold and a satisfactory quality of life.

Unfortunately, this is not the case of glioblastoma: with current therapies, one half of the affected patients die within 1 year from the diagnosis and long-term survivors are extremely rare, amounting to no more than 5 % at 5 years. But resigning to a palliation outcome is generally seen as inappropriate by the medical community and by the patients and their relatives. However, due to the frailty of the brain, surgical ablation is necessarily incomplete in most cases, and radiation therapy can be delivered only within precise dose and volume constraints. Even the most advanced radiotherapy technology scarcely impacts on tumor control, and associated chemotherapy may improve outcome only to a limited extent. Furthermore, relevant treatment-related damages may occur. So, much effort is presently made to enhance the effectiveness and safety of the available therapeutic tools.

One major determinant of therapy failure in glioblastoma, in fact, is its inherent resistance to radiation that, in light of the most recent radiobiology disclosures, can be appropriately considered as an “adaptive strategy” of the tumor against the radiation threat, which is more effective than in other cancers. The enormously improved knowledge of the natural history of glioblastoma in the fields of gene and mechanistic molecular biology, achieved in the last decades, seems to point the way to effectively cope with radiation resistance, by specifically targeting its underlying molecular determinants. However, much research is still needed, as the first clinical trials on molecular targeting agents have produced modest results. This may be due sometimes to working hypotheses not thoroughly verified in the preclinical setting

but, in general, to the great complexity of the disease and the redundancy of its biological machinery. Another cause may be the insufficient communication between preclinical and clinical researchers. There is also the need of new translational tools, besides clinical trials, such as large database collection and advanced statistical methodology.

We collected in this book authoritative information by some authors of the highest repute, giving a context to, and focusing on, clinical, laboratory, and translational radiation biology research on glioblastoma and its related pathology field. Also subjects such as particle therapy, radiation tolerance of normal brain, immunology, and nanomaterial technology are dealt with, with special reference to their respective correlated radiobiology topics. Our intent was mainly to promote the reciprocal understanding and insight among researchers and professionals in radiation and medical oncology, pathology, biology, and medical physics, in order to improve cooperation among them. Glioblastoma, although relatively rare, is in the spotlight due to the extreme complexity of its biological machinery, which represents a challenge for research, due to the necessity to disclose multiple new targets suitable for innovative therapies and unconventional approaches. These investigations might elucidate aspects of relevant interest also in other neoplasms. We hope that the efforts and the time devoted to the accomplishment of this book have obtained a useful and stimulating state-of-the-art assessment for the readers.

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L.P., G.L.G., and A.G.



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Glioblastoma (GB) accounts for 54 % of primary brain tumors, with an incidence of about five new cases for every 100,000 per year, and after aggressive multimodal treatments, prognosis remains poor, with a 5-year Overall Survival (OS) rate barely reaching 5 %, as extensively documented in the section of this book dedicated to prognostic parameters of GB. Maximum achievable safe surgical resection, and limited-volume radiotherapy (RT) with concurrent and sequential chemotherapy (CHT) based on the alkylating agent Temozolomide (TMZ) [1], achieve 40, 15, and 7–8 % OS rates, respectively at 1-, 2-, and 3-years. These present standards of treatment mostly stem from studies dating back to the seventies of the

last century [2, 3], and progressively evolving through subsequent clinical trials.

A great deal of medical literature is dedicated to GB, with increasing frequency over time. Most recent articles on GB, in fact, begin with the statement that prognosis has not improved, despite the numerous research findings on its underlying genomic and molecular mechanisms. This is due at least in part to the difficulty in improving patient outcomes, given the elusive nature of this disease with respect to therapeutic innovations, including those in the RT domain. Radiation is one of the most used and useful tool against cancer, including GB, and knowledge of its mechanisms of action on biological substrates is of the utmost importance in oncology. Radioresistance of GB is one challenge for Radiation Biology (RB) that has emerged from the clinical setting, and important questions raised by clinical experiences are addressed by basic RB laboratory research. However, RB is a scarcely known discipline outside of the inner circle of the radiological science scholars, and we are convinced that a comprehensive and updated coverage of this subject is warranted, that is, the aim of this book. The researchers and the practitioners studying GB in the domains of radiation and medical oncology, pathology, biology, and physics may profit from reciprocal scientific contributions collected in a lineup fitting the present state-of-the-art.

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We dedicated the first section of the book to RB topics emerging from clinical studies on GB. These include research regarding RT dose, volume and fractionation, CHT associated with RT, RT modalities alternative to the current photon irradiation, mathematical modeling of treatment parameters, prognostic parameters and markers, and radiation tolerance of normal brain. The second part addresses preclinical research domains of particular relevance for GB. These include related basic experimental RB; immune system and GB microenvironment; genetic and epigenetic determinants in tumor initiation and progression; GB microenvironment in its relationship with hypoxia and glioma stem cell-related radiation resistance; cell-death pathways and radiation; miRNA manipulation in modifying radiation resistance of GB; and nanoparticle research. The third and last section of the book deals with translational issues, specifically preclinical models for GB RB, present attempts to correlate molecular RB with clinical RB, and the perspectives of large databases and ontologic models for the correlation of results derived from preclinical and clinical data. Many of these contributions are unavoidably overlapping, reflecting contiguous fields of research and the scientific interests of the authors, who are often watchful for collateral disclosures influencing their work. In our opinion, this is an added value and not redundancy.

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## Prognostic Markers and Treatment Strategies

The largely incomplete information on tumor initiation and progression of GB and its almost universally fatal course have driven research for many years towards an analytic approach of both patient- and treatment-related prognostic factors conditioning life expectancy. Respectively, these include age, performance, and neurological status, as well as extent of surgical resection, RT and CHT [4], which have been analyzed in the past in an attempt to identify parameters for the best benefit/risk ratio of therapy. The traditional approach to biological and clinical radiation

oncology investigation in this field for a long time consisted mainly of mathematical modeling of *in vitro* and *in vivo* experimental results, or of data from clinical series. The vast majority of available reports show that RT acts as a prognostic factor just as a dichotomic parameter: the related survival advantage exists, as compared to surgery alone, but this is not dose-dependent according to a continuous dose-effectiveness function above 60 Gy, as normally happens in solid tumors. A recent mathematical analysis of GB patients undergoing RT-CHT seems to theoretically indicate that increments of outcome might occur up to a tumor control probability of 85 % with a RT total dose of 74 Gy in 30 daily fractions of 2.2 Gy each over 6 weeks [5]. This hypothesis needs to be confirmed in a clinical setting, but it is unlikely to deliver such an RT treatment without increasing the probability of normal tissue complication beyond acceptable levels, even using the most advanced irradiation techniques.

Only recently, pathobiology research has unveiled information that is conceivably suitable for identifying prognostic parameters. We are aware, in fact, that GB is a biologically complex disease, and that patient- and treatment-related prognostic parameters may reflect inherent tumor initiation and progression features, and different response to treatment. GB regrowth in the primary site, that is, in the full-dose RT region, is the most common failure of RT, even if it improves survival over surgery alone, as previously mentioned.

The recent assessment of the “genomic landscape” of GB [6], and the improved knowledge of signaling pathways, have led to great expectations from biologically targeted therapies, specifically monoclonal antibodies (mAb) and tyrosine-kinase inhibitors (TKI), as well as active and passive immune therapy [7, 8]. However, the numerous clinical trials undertaken on these grounds have generally yielded unsatisfactory results. Possible hypotheses for explaining these failures include molecular signaling redundancy and cross-talk; clonal selection (or emergence) of resistant phenotypes under treatment; preclinical studies mainly addressing tumor-initiating or

early growth factors and not late tumor progression mechanisms; difficulty of the drugs in penetrating the blood–brain barrier (BBB), etc. [9]. In addition, integrating the above-mentioned agents with radiation, as well as modern refinements of imaging and radiation-dose delivery techniques, have not produced substantially improved outcomes. However, molecular radiobiology, in general, has rapidly evolved over the last two decades, paralleling the improved knowledge of DNA damage and repair mechanisms, intra- and intercellular signaling pathways and microenvironmental factors, as well as tumor profiling biomarkers and molecular targeting [10]. GB, in particular, is presently the subject of much scientific discussion regarding ionizing radiation under this new perspective. The recent molecular classification of GB TCGA (The Genome Cancer Atlas) addressed recurrent genomic abnormalities in GB, which resulted in a gene-expression/molecular classification of GB into proneural, neural, classical, and mesenchymal subtypes [11]. An aggressive postsurgical therapy (that is, RT with >3 cycles of chemotherapy, vs. a less intensive management), yielded a significantly reduced mortality in the classical and mesenchymal subtype, a borderline impact on survival in the neural subtype, and no effect on the proneural subtype.

### **Inherent GB Radiation Resistance and Failure in Radiosensitizing GB by Targeting Key Signal Molecules**

Radioreistance of GB is attributable to both intracellular and microenvironmental factors [12]. Radiation-induced cell death in solid tumors is mostly due to DNA double-strand break (DSB), and enhanced DNA DSB repair may occur and improve radiation resistance: the PI3K-Akt pathway, downstream of several membrane receptors (particularly the erbB family members) may be activated and potentiate DSB repair after radiation, besides constitutively stimulating tumor growth and invasion [13]. *EGFR* amplification (present in about 40 % of GBs) promotes resistance to RT in preclinical studies through the activation of DNA PKcs (DNA-

dependent protein kinase catalytic subunit) leading mainly to nonhomologous end joining (NHEJ) DNA DSB repair. Furthermore, experimental evidence indicates that the link between EGFR signaling and DSB repair occurs by the PI3K-Akt or MAPK (mitogen-activated PK) pathways [14]. A frequent (30–50 %) mutant form of EGFR is expressed in GB, specifically the EGFR variant III (EGFRvIII or  $\Delta$ EGFR). Its deletion of the extracellular domain (exons 2–7) constitutively activates a high stimulation of the PI3K/Akt/mTOR pathway, and confers radiation resistance [15]. Some authors [16–18] have considered the relationship between increased cell-survival signaling by EGFR and *TP53* mutations and apoptosis. For a long time, in fact, apoptosis has been supposed to be the main type of programmed cell death (PCD) after anticancer treatments, including RT [19–22]. However, evidence exists that other types of PCD are induced by CHT and RT, such as autophagy-related or type-II PCD, and regulated necrosis (including necroptosis, type III PCD) [21–23]. These pathways are not necessarily mutually exclusive, as previously believed. Even if autophagy is important in many cancers as a protective mechanism against radiation [10], it can act both as a pro-survival mechanism and as a pro-death mechanism, the latter observed in GB [24]. Autophagy-related cell death is one of the metabolic pathways inhibited by EGFR, which can act via mTOR or by direct inhibition of Beclin1, a cytoplasmic protein that induces autophagy by binding to the Vps34-Vps15 core [25, 26]. We could experimentally demonstrate, for instance, that combined EGFR and autophagy modulation impact on radiation and TMZ sensitivity (that is, clonal inhibition) in human GB cell lines [27]. Similarly, in patients undergoing RT and TMZ, low-EGFR- and high Beclin1-expressing GBs have a significantly better median survival, as compared to other ones showing high-EGFR and both high- and low-Beclin1 expression, after standard RT-TMZ [28].

Some failures of mAb or of TKI against EGFR in achieving favorable results in clinical trials might be due, at least in part, to the lack of a concurrent inhibition of the downstream cell-death pathway's activity. PI3K-mTOR and EGFR inhibitors, as well as PDGFR, VEGF, and p53



inhibitors, are the subject of very recent, extensive, and thorough reviews (e.g., [9]). Some authors considered, in particular, the relationships of these pathways with the related genomic significant mutations [8], as identified in TGCA of GB [6].

However, these studies do not primarily address RT enhancement, but in general the effectiveness of biological targeting drugs, both as single- or dual-agents, or in combination with current RT-CHT schedules. From a radiobiological point of view, further study is necessary: in fact, many trials associate “targeting” drugs with RT without previous preclinical *in vitro* and *in vivo* investigations exhaustively grounding their effectiveness as radiation enhancers on sound proofs-of-principle [12].

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## Glioma Stem Cells

Another main factor causing GB resistance to radiation therapy is its intrinsic composition including heterogeneous cell populations—that is, a cellular hierarchy deriving from glioma stem cells (GSCs) through multiple genetic and epigenetic events [29–31]: inducing quiescence, altered cell-cycle control, activation of the DNA-repair pathways and complex interactions with the tumor microenvironment. Irradiated GBs contain more GSCs than unexposed ones, thus suggesting that GSCs have a role in radiation resistance [32]. GSCs may also have a fundamental role in promoting tumor neo-angiogenesis [33, 34], as suggested by high VEGF expression, and by their possibility to differentiate into endothelial tumor cells [35, 36] or pericytes [37]. Neo-angiogenesis may also depend on hypoxia-inducible factor (HIF)-mediated recruitment of bone marrow-derived cells restoring GB vascularity damaged by radiation [38].

Hypoxia, due to its general and well-known property of reducing the effect of radiation-induced reactive oxygen species (ROS) damage on DNA by restraining their combination with oxygen [39], has a relevant role in radiation resistance of GB, which is a highly hypoxic tumor. Furthermore, specialized hypoxic sites (the so-

called “niches”) composed of GB-associated stromal cells, immune cells, and non-cellular components provide signals promoting the GSC phenotype [40–43]. GSCs located in these niches usually express the CD133/prominin-1 marker, used for their identification, enrichment, and as a prognostic marker. However, it is questioned whether glioma and normal brain stem cells can be univocally discriminated by CD133 positivity. Genome-wide-based analyses have demonstrated that GSCs express a multiple-gene signature, existing in GSCs but not in normal brain SCs, correlating with survival [44]. This report also shows that characteristic Hedgehog- and Wnt-pathway alterations, such as active  $\beta$ -catenin, were present only in GB GSCs. Interestingly  $\beta$ -catenin, as well as Gli-1 enhanced immunohistochemistry expression level, negatively conditioned GB patients’ survival after standard RT-TMZ in our experience [45].

The clinical implications of the radiobiological research on cancer SC are currently the subject of ongoing studies, both for predictive bioassays and for combination of novel systemic treatments with RT [46].

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## Epigenetic Events

Radiation and CHT resistance, as well as other features of the aggressiveness of GB, may result from epigenetic events, such as alterations in the gene methylation status, conditioning the radiation or CHT effect in DNA gene sequence disruption and repair. Different DNA methylation alterations exist between radiation-sensitive and -resistant cells [47]. Furthermore, radiation may induce modifications, such as phosphorylation or changes in the methylation status of histones [48]. About half of GBs harbor somatic mutations determining DNA methylation, histone modification, and nucleosome positioning [49].

Methylation of the O6-methylguanine-DNA methyl-transferase (MGMT) gene showed a significant median survival benefit for GB patients undergoing RT-TMZ, as compared to those with the same feature undergoing RT only (21.7 vs. 15.3 months, respectively;  $p=0.007$ ). On the con-

trary, the difference between the same treatment groups, out of nonmethylated-MGMT GB patients, did not attain statistical significance, in 206 patients included in an EORTC-NCIC trial [50]. A meta-analysis study on 2018 high-grade glioma patients included in 20 reports showed that MGMT gene silencing was significantly associated with improved survival in patients undergoing RT-TMZ; this advantage was less significant in those receiving only RT, and null in those receiving neither TMZ nor RT [51]. However, causal interpretation of these results requires caution: a sample classification only according to the methylated and nonmethylated status for a gene may be dependent on the relationship between the overall CpG island methylation, the CpG methylation at individual sites, and the effectiveness of gene silencing, that is dependent in turn on the location within the gene [52]. DNA methylation may also involve other epigenetic modifications of chromatin, and the methyl-CpG-binding domain (MBD) proteins connected with histone deacetylases (HDACs) and histone methyl-transferase (HMTs), functionally affecting the regulation of transcription. Furthermore, these events may regulate HIF effects at the DNA and histone levels, as extensively reported by Cimini et al. in a dedicated section of this book.

Antiepileptic drugs may affect therapeutic outcome of GB patients undergoing current RT-TMZ schedules, by MGMT-independent mechanisms, and due to HDAC inhibition and the consequent histone acetylation that loosens up the chromatin structure, making DNA more accessible to anticancer drugs and enhancing the cytotoxic effect of radiation. This is the case of Valproic acid (VPA) [53], which also induces apoptosis independently of the p53 status [54], induces autophagy as a cell-death pathway in GB, and may increase the bioavailability of TMZ by reducing the clearance of the metabolite that methylates DNA. GB patients submitted to RT-TMZ and treated with VPA, in fact, have enjoyed a better survival benefit, as compared to those not undergoing VPA medication or receiving other antiepileptic drugs in an EORTC/NCIC trial [55]. Further studies are warranted, in order to assess whether the activity of VPA in enhanc-

ing RT-TMZ in GB is mainly due to HDAC inhibition, or to an increased TMZ bioavailability or to other bioeffects, as indicated above. Other antiepileptic drugs are presently under evaluation in this area of research, but the main interest at the moment is focused on TMZ- and not on radiation-enhancement, which, however, deserves consideration.

MicroRNAs (or miRs, small noncoding RNA sequences of an average of 23 nucleotides) may exert an epigenetic downregulation of target genes. Overexpression of miR-181a sensitizes U87-MG (malignant glioma) cells to radiation and downregulates mRNA and protein expression of BCL-2, a protein that regulates apoptotic cell death. MiRNA expression profiles after IR exposure in the U87-MG cells showed downregulation of miR-181a. Transient overexpression of miR-181a sensitized these cells to IR and led to downregulation of mRNA and protein level of BCL-2. BCL-2 is associated with radioresistance but also it plays a protective role against apoptotic cell death and is frequently overexpressed in human tumor cells [56, 57]. Growth arrest and apoptosis, due to radiation, can be enhanced by inhibition of miR-21 in U251GBM cells through overriding G2-M arrest [58]. GB cell line radiation resistance can be mediated through regulation of cell-cycle genes, such as PDCD4 and hMSH2 by miR-21 [59]. There is sound preclinical evidence showing that also many other miRNAs may modulate the radiation resistance of GB, conditioning downstream both the PI3K/Akt and the ATM/Chk2/p53 pathways, as reported by Comincini et al. in this book. These authors speculate that, given the short time in which a large number of radiobiological studies on miRNAs in GB have been published (that is, over the past 10 years or so) it is reasonable to expect rapid and significant clinical developments.

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## Immunity and Radiation Response of Glioblastoma

Differently from a former concept, brain is not immune-privileged, particularly if a breakdown of the BBB takes place, like in the case of GB,

which develops abnormal vasculature and tumor-associated inflammation. Immunotherapy of GB has been developed, through passive (mAb, cytokine-mediated therapies and adoptive cell transfer) and active immunity agents (peptide- and cell-based approaches). Immunology subjects and immunotherapy are dealt with in most recent updates on emerging strategies against GB [60–63] and many prospective phase-I to -III clinical trials are presently ongoing on this subject. However, the topic of an immunity-based approach to overcome GB refractoriness to radiation is specifically addressed more rarely, both in laboratory and clinical experimental contexts. In this book, Cooper et al. deal specifically with radiation-induced immune response against GB, as well as with the interference of the brain/tumor microenvironment with effective antitumor immunity. Radiation may have several adverse effects on immunity, such as those systemically occurring during limited-volume, fractionated RT for GB, due to exposure to circulating lymphocytes. Over a complete RT course (60 Gy in 6 weeks, 5 fractions of 2 Gy per week) lymphocytes may drop by 50 % of the baseline count [64] due to radiation-induced apoptosis. Immunosuppressive effects may also be due to TMZ- and steroid-induced leukopenia. Furthermore, the GB microenvironment itself may exert an immunosuppressive influence, and immune checkpoints may inhibit immune cell proliferation and activity.

These effects make it difficult to detect a possible antitumor immunity in the clinical setting and the role of radiation in its modulation. Preclinical experimental radiobiology approaches are therefore necessary, such as those undertaken in mice submitted to a focally collimated, stereotactic single-fraction 10 Gy irradiation of an orthotopic tumor deriving from GL261 glioma cells, followed by activation of 4-1BB (or CD137, a member of TNF superfamily, a co-stimulatory molecule), and blockade of CTLA-4 (or CD 152, Cytotoxic T-lymphocyte Antigen 4, an immune checkpoint downregulating the immune system) [65]. This triple-therapy schedule achieved a median survival of 66.5 days, vs. 22.5 days ( $p < 0.05$ ) in mice undergoing only the 4-1BB/CTLA-4 manipulation, and 24 days

( $p < 0.01$ ) in those submitted to irradiation alone. The primary tumor site showed increased CD4+ and CD8+ infiltrating lymphocytes after triple-therapy; depletion by monoclonal Abs of CD4+ inhibited the antitumor efficacy of triple-therapy, whereas depletion of CD8+ did not interfere with triple-therapy efficacy and allowed a longer survival compared with controls. Long-term-surviving animals achieved also memory response and rejected a subsequent growth of GL261 glioma cells, implanted in the flank. Some clinical trials are presently ongoing, taking into account also similar co-signal balances in other animal-model experiments [66, 67], and adopting programmed cell death (PD-1) immune checkpoint-inhibiting monoclonal Abs, such as Pidilizumab and Nivolumab. However, RT exerts multiple favorable and sometimes unfavorable effects on GB, based on different domains of cell-mediated and humoral immunity, which need in-depth evaluation and are the subject of intensive research [7].

Vaccination with DCs loaded with an *EGFRvIII* (a mutant form of *EGFR* present in about 30–40 % of GBs) specific peptide, induced immune response and a relevant improvement in prognosis out of a small series of patients [68]. This led to the development of a prospective trial in the adjuvant setting after chemoradiation [69], showing good results in a comparison with matched controls. The preliminary results of the ACT-III trial, addressing Rindopepimut (a vaccine consisting of the unique *EGFRvIII* peptide sequence conjugated with keyhole limpet hemocyanin), delivered in conjunction with TMZ and after chemoradiation in GB, were published very recently [70]. This study raises remarkable interest, due to a median overall survival of 21.8 months and 3-year survival of 26 %, out of 65 *EGFRvIII*+ GB patients, to a fourfold anti-*EGFR* antibody increase in 85 % of patients, and to the *EGFRvIII*+/*EGFRvIII*-conversion in 4/6 recurring patients. These outcomes are under evaluation in a random phase-III trial (ACT-IV). However, the above results derive primarily from investigating the subject of vaccine therapy against *EGFRvIII* in GB, with no particular radiobiological meaning. The subject of immunotherapy against *EGFRvIII* in conjunction with

radiation is stimulating, and not yet sufficiently addressed in preclinical experiments that are suitable for specific therapeutic developments.

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## **Evolving Radiation Techniques, Particle Therapy, and Immunity**

The recent evolution of image tools (CT, MRI, radionuclide methods) and radiation therapy planning and dose delivering has generally provided high conformality in RT of GB. Ionizing particle beams, as compared to photons, have the peculiarity of more selective dose deposition at a definite depth (Bragg's peak). Proton beam irradiation of GB has a better conformity index (CI, which is the ratio between the planned target volume of a tumor and the healthy tissue volume that receives a significant dose as regards radiation tolerance) compared to the most sophisticated photon RT techniques presently available [71]. This may spare critical structures of the healthy brain from severe damage, thus allowing very high-dose irradiation of GB and possibly improving local tumor control. Heavy ion beams (e.g., carbon ions) add to this selective dose deposition, also producing the advantage of a high ionization density (expressed as Linear Energy Transfer, or LET). This achieves a high relative biological effectiveness (RBE), due to inactivating events very close to each other along particles' paths, spaced out ranges comparable to the size of biological molecules like DNA. Therefore, more effects of charged particle irradiation are direct and irreparable, with a lesser dependence on parameters like dose fractionation, oxygenation, stem cell resistance, etc., than X- or  $\gamma$ -ray photon irradiation. However, it is difficult to demonstrate the clinical benefits of ion-beam methods, mainly due to the very limited availability of dedicated facilities. Nevertheless, the present trend towards hypofractionated photon RT, which derives from the selective, high-gradient linear-accelerator-based RT techniques and image-guided irradiation, might further develop in the near future with charged particles.

From a radiobiological standpoint, the focal RT high-dose deposition with stereotactic RT or particle therapy is attractive for many reasons. At very high doses, vascular radiation damage may become dominant, impairing tumor nutrient supply and oxygenation. Endothelial cell apoptosis steeply increases above fractions of 10 Gy [72], and a devascularizing effect becomes evident at image studies after doses of 18–24 Gy [73]. Further, radiation may induce cell necrosis in tumors [74] besides apoptosis and autophagy-related cell death, especially after high-dose delivery, and inflammation response is always present in this case. Immunity is a main pathophysiological domain involved in this context, as inflammatory status may promote the antigen-specific immunity through DC maturation, internalization of apoptosis- and necrosis-derived tumor cell molecules, and presentation of antigens to T cells, thus countering the poor immunogenicity of clinically developed tumors [75]. The presence in the microenvironment, after irradiation, of the so-called DAMPs (damage-associated molecular patterns) [76], like ATP and the high-mobility group protein 1 (HMGB1), activates TLR4 (toll-like receptor 4) in CD8+ T-lymphocytes (shown to be correlated with radiation success). Calreticulin translocation to the cell surface (CRT) may in turn induce the capture of tumor antigens by dendritic cells (DC), which also mature due to HMGB1, thus initiating an immune response against the tumor [77]. In tumors characterized by systemic metastases, these processes are involved in the so-called “abscopal effect”, which is a regression effect beyond direct cytotoxicity of radiation on tumor cells, occurring on primary or metastatic sites after focal irradiation of a single tumor site (revised in [78]). However, many mechanisms are involved in radiation-induced immunity against cancer, which are the subject of intensive preclinical research for its enhancement also in the clinical setting, e.g., through vaccination, immunomodulation, and adoptive cell transfer for a synergic approach with RT. These studies are ongoing also for GB [77] and are the subject of a dedicated section of this book.

## The State-of-the-Art in GB Radiobiology, Related Pathobiology, and Their Clinical Relevance

It is becoming a truism to state that the progress made in clinical and molecular oncology and radiobiology has made it possible to switch from a population-based approach to a personalized treatment. The main advantage of combining information derived from both preclinical and clinical settings lies in the real opportunity of selecting specific molecular-oriented subjects, who will most likely benefit from a particular treatment in accordance with their “molecular profile”, or to select patients at risk of adverse events. For instance, the close integration between molecular biology and imaging may favor a reliable functional clinical evaluation of a number of biological events, previously identified only by pathology or laboratory assays, allowing a proper patient selection for the most effective therapeutic approach.

We now have a better understanding of the mechanisms sustaining the processes responsible, at the biological and clinical levels, for the aggressive radioresistant phenotypes of GB. At the same time, the important advances being made in our knowledge of biological processes might ground strategies for enhanced radiation response, as well as reduced toxicity of organs at risk. With particular regard to GB, progress in characterization, quantification, and timing of biological processes might improve the growing body of current evidence in the diagnostic and therapeutic fields, such as imaging and RT. This hopefully will allow for both the identification of subjects with specific molecular profiles and for this reason more responsive to ionizing radiation and strategies suitable for enhancing GB radiation sensitivity in radioresistant phenotypes.

However, advances in molecular-based approaches presently have the most striking consequences in an overwhelming amount of new drugs, able to modify cellular systems at the genetic, epigenetic, and signaling pathway levels in the preclinical setting, and in the introduction of a multitude of diagnostic tools able to monitor

individual molecular and biological processes with improving sensitivity and specificity. These achievements have dramatically augmented our understanding of the molecular bases of GB, and putatively should improve clinical outcomes, but this is not yet the rule. The presently available, enormous body of biological knowledge likely requires reliable processes for translation into the clinical setting. This might be a major challenge for the near future. In this regards, as previously stated, the aim of this book is to provide some selected contributions that might facilitate reciprocal understanding and communication among the main players in radiation research and clinical management of GB.

## References

1. Stupp R, Tonn JC, Brada M, et al. On behalf of the ESMO Guidelines Working Group: high-grade malignant glioma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2010;21:190–3.
2. Salazar OM, Rubin P, Donald JF, Feldstein ML. High-dose radiation therapy in the treatment of glioblastoma multiforme. *Int J Radiat Oncol Biol Phys*. 1976;1:717–27.
3. Walker MD, Strike TA, Sheline GE. Analysis of dose-effect relationship in the radiotherapy of malignant gliomas. *Int J Radiat Oncol Biol Phys*. 1979;5:1715–31.
4. Curran Jr WJ, Scott CB, Horton J, et al. Recursive partitioning analysis of prognostic factors in three Radiation Therapy Oncology Group malignant glioma trials. *J Natl Cancer Inst*. 1993;85:704–10.
5. Pedicini P, Fiorentino A, Simeon V, et al. Clinical radiobiology of glioblastoma multiforme: estimation of tumor control probability from various radiotherapy fractionation schemes. *Strahlenther Onkol*. 2014;190:925–32.
6. Brennan C, Verhaak RGW, McKenna A, et al. The somatic genomic landscape of glioblastoma. *Cell*. 2013;155:462–77.
7. Patel MA, Kim JE, Ruzevick J, et al. The future of glioblastoma therapy: synergism of standard of care and immunotherapy. *Cancers*. 2014;6:1953–85.
8. Prados MD, Byron SA, Tran NL, et al. Towards precision medicine in glioblastoma: the promise and the challenges. *Neuro Oncol*. 2015;17:1051–63. doi:10.1093/neuronc/nov031:1-10.
9. Bastien JL, McNeill KA, Fine HA. Molecular characterization of glioblastoma, target therapy, and clinical results to date. *Cancer*. 2014;121:502–10.
10. Baumann M, Bodis S, Dikomey E, et al. Molecular radiation biology/oncology at its best: cutting edge research presented at the 13th international Wofsborg



- meeting on molecular radiation biology/oncology. *Radiother Oncol.* 2013;108:357–61.
11. Verhaak RGW, Hoadley KA, Purdom E, et al. An integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR and NF1. *Cancer Cell.* 2010;17(1):98. doi:[10.1016/j.ccr.2009.12.020](https://doi.org/10.1016/j.ccr.2009.12.020).
  12. Cohen-Jonathan Moyal E. Du laboratoire vers la clinique: expérience du glioblastoma pur moduler la radiosensibilité tumorale. *Cancer Radiother.* 2012;16:25–8.
  13. Toulany M, Rodemann HP. Potential of Akt mediated repair in radioresistance of solid tumors overexpressing erbB-PI3K-Akt pathway. *Transl Cancer Res.* 2013;2:190–202.
  14. Hatampaa KJ, Burma S, Zhao D, Habib AA. Epidermal growth factor receptor in glioma: signal transduction, neuropathology, imaging, and radioresistance. *Neoplasia.* 2010;12:675–84.
  15. Mukherjee B, McEllin B, Camacho CV, et al. EGFRvIII and DNA double strand break repair: a molecular mechanism for radioresistance in glioblastoma. *Cancer Res.* 2009;69:4252–9.
  16. Mellinghoff IK, Wang MY, Vivanco I, et al. Molecular determinants of the response of glioblastoma to EGFR kinase inhibitors. *N Engl J Med.* 2005;353:2012–24.
  17. Krakstad C, Chekenya M. Survival signaling and apoptosis resistance in glioblastomas: opportunities for target therapies. *Mol Cancer.* 2010;9:135. <http://www.molecular-cancer.com/content/9/1/135>
  18. Taylor TE, Furnari FB, Cavanee WK. Targeting EGFR for treatment of glioblastoma: molecular basis to overcome resistance. *Curr Cancer Drug Targets.* 2012;12:197–209.
  19. Wong R. Apoptosis in cancer: from pathogenesis to treatment. *J Exp Clin Cancer Res.* 2011;30:87. doi:[10.1186/1756-9966-30-87](https://doi.org/10.1186/1756-9966-30-87).
  20. Balcer-Kubiczek EK. Apoptosis in radiation therapy: a double edged sword. *Exp Oncol.* 2012;34:277–85.
  21. Ma H, Rao L, Wang HL, Mao ZW, et al. Transcriptome analysis of glioma cells for the dynamic response to irradiation and dual regulation of apoptosis genes: a new insight into radiotherapy for glioblastoma. *Cell Death Dis.* 2013;4, e895. doi:[10.1038/cddis.2013.412](https://doi.org/10.1038/cddis.2013.412).
  22. Mirzayans R, Andrais B, Scott A, et al. Ionizing radiation-induced responses in human cells with differing TP53 status. *Int J Mol Sci.* 2013;14:22409–35.
  23. Galluzzi L, Vitale I, Abrams JM, et al. Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. *Cell Death Differ.* 2012;19:107–20.
  24. Miracco C, Palumbo S, Pirtoli L, Comincini S. Autophagy in human brain cancer: therapeutic implications. In: Hayat MA, editor. *Autophagy: cancer, other pathologies, inflammation, immunity, infection, and aging*, vol. 5. San Diego: Elsevier/Academic Press; 2015. p. 105–20.
  25. Kang R, Zeh HJ, Lotze MT, Tang D. The beclin1 network regulates autophagy and apoptosis. *Cell Death Differ.* 2011;18:571–80.
  26. Wei Y, Zou Z, Becker N, et al. XEGFR-mediated beclin1 phosphorylation in autophagy suppression, tumor progression, and tumor chemoresistance. *Cell.* 2013;154:1269–84.
  27. Palumbo S, Tini P, Toscano M, et al. Combined EGFR and autophagy modulation impairs cell migration and enhances radiosensitivity in human glioblastoma cells. *J Cell Physiol.* 2014;229:1863–73.
  28. Tini P, Belmonte G, Toscano M et al. Combined epidermal growth factor receptor and beclin1 autophagic protein expression analysis identifies different clinical presentations, responses to chemo- and radiotherapy, and prognosis in glioblastoma. *BioMed Res Int.* 2015; ID 208076. <http://dx.doi.org/10.1159/2015/208076>
  29. Schonberg DL, Lubelski D, Miller TE, Rich JN. Brain tumor stem cells: molecular characteristics and their impact on therapy. *Mol Aspects Med.* 2014;39:82–101.
  30. Carrasco-Garcia E, Sampron N, Aldaz P, et al. Therapeutic strategies targeting glioblastoma stem cells. *Recent Pat Anticancer Drug Discov.* 2013;8:216–27.
  31. Altaner C. Glioblastoma and stem cells. *Neoplasia.* 2008;55:369–74.
  32. Bao S, Wu Q, McLendon RE, Hao Y, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature.* 2006;444:756–60.
  33. Bao S, Wu Q, Sathornsumetee S, Hao Y, et al. Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor. *Cancer Res.* 2006;66:7843–8.
  34. Wang J, Ma Y, Cooper MK. Cancer stem cells in glioma: challenges and opportunities. *Transl Cancer Res.* 2013;2:429–41.
  35. Wang R, Chadalavada K, Wilshire J, et al. Glioblastoma stem-like cells give rise to tumour endothelium. *Nature.* 2010;468:829–33.
  36. Ricci-Vitiani L, Pallini R, Biffoni M, et al. Tumour vascularization via endothelial differentiation of glioblastoma stem-like cells. *Nature.* 2010;468:824–8.
  37. Cheng L, Huang Z, Zhou W, et al. Glioblastoma stem cells generate vascular pericytes to support vessel function and tumor growth. *Cell.* 2013;153:139–52.
  38. Kioi M, Vogel H, Schultz G, et al. Inhibition of vasculogenesis, but not angiogenesis, prevents the recurrence of glioblastoma after irradiation in mice. *J Clin Invest.* 2010;120:694–705.
  39. Horsman MR, Overgaard J. The oxygen effect and tumor microenvironment. In: Steel G, editor. *Basic clinical radiobiology*. London: Arnold; 2002. p. 158–68.
  40. Persano L, Rampazzo E, Della Puppa A, Pistollato F, et al. The three-layer concentric model of glioblastoma: cancer stem cells, microenvironmental regulation, and therapeutic implications. *Scientific World Journal.* 2011;11:1829–41.
  41. Shiao SL, Ganesan AP, Rugo HS, Coussens LM. Immune microenvironments in solid tumors: new targets for therapy. *Genes Dev.* 2011;25:2559–72.
  42. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. *Cancer Cell.* 2012;21:309–402.