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APOPTOSIS IN GLIOMAS, AND ITS ROLE IN THEIR CURRENT AND FUTURE TREATMENT

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1. ABSTRACT

Apoptosis has recently entered the spotlight in the continuing search for new therapeutic approaches to cancer because it plays a twofold role in this disease. As stated by Lowe and Lin: "(M)ost cytotoxic anticancer agents induce apoptosis …(and so) the same mutations that suppress apoptosis during tumor development also reduce treatment sensitivity" (1). Therefore, any strategy aimed at increasing the propensity of glioma cells to undergo apoptosis could be therapeutic in its own right, but has the added potential of enhancing their sensitivity to other, established, treatments. As a corollary, understanding apoptotic mechanisms at the molecular level will not only help to explain why gliomas arise, but also identify points of intervention. This review will focus on these points, with emphasis on two families of apoptotic molecules, death ligands and their receptors, and BCL-2 family proteins. Near-term strategies of how apoptosis can be exploited therapeutically are discussed.

2. INTRODUCTION

2.1. Concepts of Cell Death

Mammalian ontogenesis involves the loss of specific cell populations within most organ systems that have been studied systematically. This physiological type of cell death, which appears to be crucial in shaping the central nervous system, amongst other organs, is executed by the dying cell, and is often termed programmed cell death. It is assumed that most physiological or programmed cell deaths exhibit the morphological features of apoptosis, including condensation and fragmentation of chromatin and nuclear remnants, cytoplasmic compartmentalization, resulting in membrane blebbing, and the formation of apoptotic bodies (2). The latter are efficiently cleared from the tissue by "nonprofessional" phagocytes, such as astrocytes in the brain, which recognize signals displayed on the surface of apoptotic cells and cell fragments. Such signals are likely to account for the lack of inflammatory responses associated with physiological cell death during development. Of note, apoptosis is a purely morphological term, which does not imply a specific underlying biochemical pathway of cell death. One fundamental conceptual distinction between programmed and "non-programmed" (incidental) cell deaths is that the latter are triggered by the accidental as well as global disruption of the cellular processes whereas the former are believed to result from the activation of specific death pathways. Non-programmed cell death can therefore result from many insults including mechanical trauma, inflammation or ischemia, and is characterized by disturbances of energy metabolism, cell swelling, osmotic

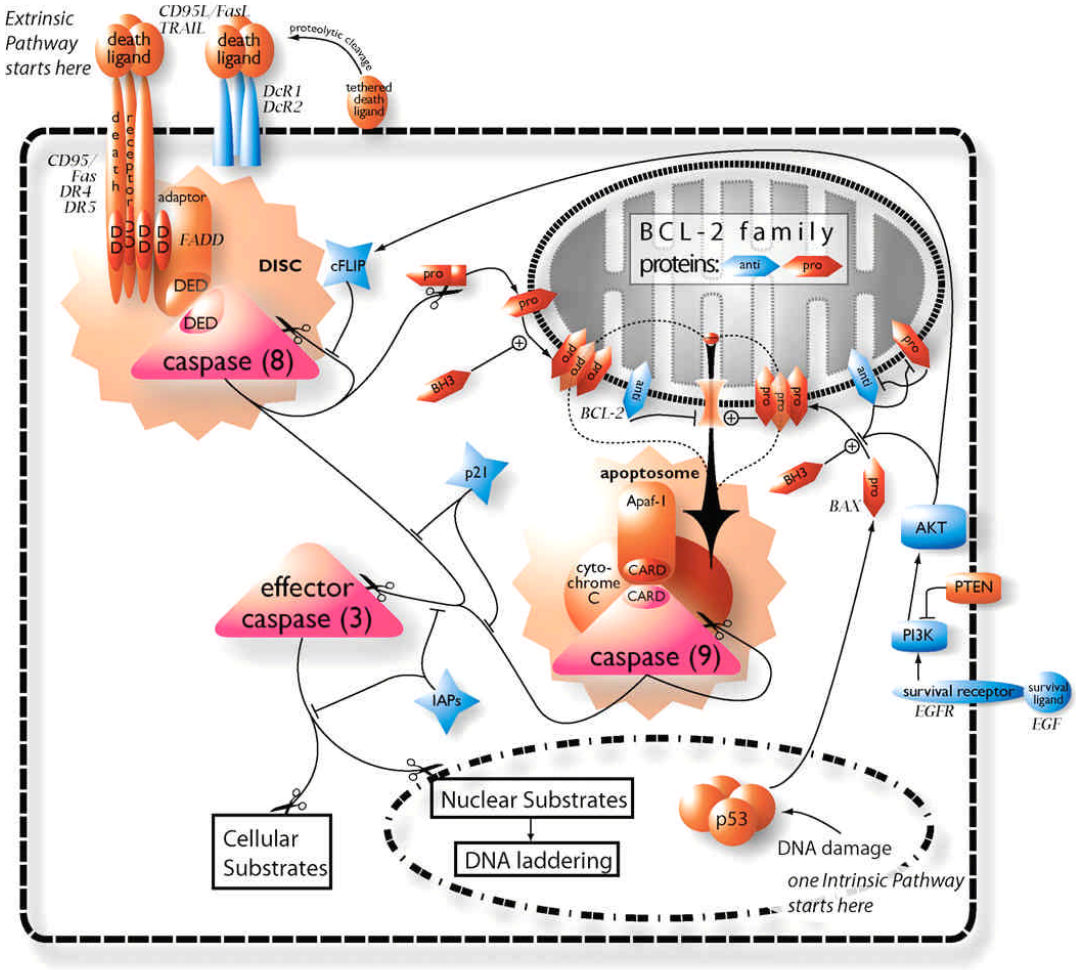

Figure 1. Schematic of Apoptosis Pathways.The extrinsic (top left) and intrinsic (bottom right) pathways of apoptosis are shown, converging on effector caspases and leading to dismantling of the cell by cutting cellular and nuclear substrates. Pro-apoptotic molecules are shown in red, and anti-apoptotic molecules are shown in blue. In some instances, examples of specific molecules are shown in italic serif font. In the extrinsic pathway the trimerization of death receptors by death ligand results in the formation of the death-inducing signaling complex (DISC) together with adaptor molecules, leading to the proteolytic activation of regulatory caspases, typically caspase 8, and thereby caspase 3. In one instance of the intrinsic pathway DNA damage, P53 activation, expression of a pro-apoptotic member of the bcl-2 family, for example BAX, or other unknown events causes mitochondrial pore formation and the release of cytochrome c. This in turn allows the formation of the apoptosome together with APAF-1 and the activation of a regulatory caspase, usually caspase 9, and thereby caspase 3. In addition survival signals emanating from survival ligands and receptors can inhibit the pro-apoptotic members of the bcl-2 family, or caspases by via caspase inhibitors such as IAP. Cross talk between the pathways is indicated by the proteolytic activation of a pro-apoptotic member of the bcl-2 family by caspase 8. For additional details and references see text.

lysis, degeneration of organelles, activation of catabolic enzymes such as proteases and endonucleases, and the release of pro-inflammatory mediators. The most commonly described morphological form of non-programmed cell death is necrosis. Importantly, however, all the above-mentioned stimuli may also promote apoptotic cell death, provided stimuli intensity is not too strong and allows the cell to maintain energy supplies and to respond actively to the noxious insult.

There have been tremendous advances in the field of apoptosis in recent years, particularly in the identification and cataloging of involved molecules. In the case of many of these, such as the death ligands and receptors, bcl-2 family proteins and caspases, progress has also been made in understanding function and pathways. It is beyond the scope of this review to describe all this information in detail, and it has been well reviewed elsewhere (1). However, a brief description of apoptotic pathways is provided, along with Figure 1, as a framework for the discussion of how apoptosis can be exploited in neuro-oncology.

2.2. A Brief Introduction to the Pathways of Apoptosis

Somewhat artificially, the current literature tends to distinguish two categories of apoptotic pathways. The

'extrinsic' pathways start with ligand-mediated activation of transmembrane death receptors, which recruit and activate caspases through intermediary adapter proteins. Death receptors, such as CD95 (also known as Fas or APO-1), are activated by trimerization of their ligands (e.g., CD95 ligand or CD95L) and have intracellular death domains (DD) that bind to homologous domains on adapter molecules, such as Fas-associated protein with death domain (FADD) (3). These in turn recruit regulatory pro-caspases, such as caspase-8, via another homophilic interaction, involving death effector domains (DED) (3). Cross-linking of death receptors by ligand leads to the formation of the death-inducing signaling complex (DISC), which is formed by the intracellular domains of the receptors, the adapters and the caspases. As a result of this aggregation, pro-caspases are then converted into active enzymes by proteolytic cleavage of the pro-domain and go on to proteolytically activate effector caspases, paradigmatically caspase-3. These in turn then dismantle the cell by proteolytic cleavage of structural proteins (4). Importantly it has remained unclear in any paradigm of caspase-dependent apoptotic cell death which specific substrates of caspases need to be cleaved to pass the point of commitment to cell death. Initial caspase activation may occur simply because the local concentration of weakly active pro-caspases is increased by the assembly of the DISC, pointing to the importance of adapter molecules and multiprotein aggregates in regulating apoptosis.

The 'intrinsic' pathways are triggered by intracellular events. In one paradigm, DNA damage activates the tumor suppressor protein P53, which is a key regulator of apoptosis, as well as many other processes in normal and cancer cells. Discussion of the role of P53 in gliomas is beyond the scope of this review, which will instead focus on molecules whose function appears at present to be more restricted to death pathways, such as bax, a pro-apoptotic member of the bcl-2 family which is expressed following P53 activation (5). Importantly the intrinsic pathway may be triggered in p53 mutant cells with comparable efficacy as in p53 wild-type cells, indicating that p53 is but one of the upstream regulators of the intrinsic apoptotic pathway.

The bcl-2 family of proteins, which has over 20 members, share protein-protein interaction motifs, called bcl-2 homology (BH) domains. BH domains mediate the complex homo- and heterodimerization (5), which are believed to form the basis of part or all of their activity. Some members are anti-apoptotic, such as the oncoprotein Bcl-2, while others are pro-apoptotic such as the p53-induced gene bax. It has been claimed that the relative expression levels of anti-apoptotic and pro-apoptotic members of this protein family determine the sensitivity of a given cell to undergo apoptosis. Recent evidence suggests that bcl-2 family proteins act by controlling the release of cytochrome c from mitochondria, possibly by regulating pore complexes formed by other proteins, or directly participating in pore formation (6). The release of cytochrome c from mitochondria in turn leads to the assembly of the apoptosome complex together with the apoptosis-activating factor (APAF)-1 protein, and the recruitment and activation of the regulatory caspase-9, via a caspase activation and recruitment domain (CARD)-mediated interaction (4). Activation of effector caspases and dismantling of the cell ensues.

Recently it has been suggested that members of the bcl-2 family that have only one bcl-2 homology domain, BH3, are important sensors of cellular damage and initiators of apoptosis by an intrinsic pathway that is independent of the nucleus (7). These BH3-only proteins include natural born killer (NBK), also known as BCL-2 interacting killer (BIK), BCL-XL/BCL-2-associated death promoter (BAD), BH3-interacting domain death agonist (BID) and Harakiri (HRK). In summary, the central role of the mitochondria and mitochondrial factors such as cytochrome c, apoptosis inducing factor (AIF) and second mitochondria-derived activator of caspase/direct IAP binding protein with low pI (SMAC/DIABLO) has led to the synonymous use of the terms intrinsic and mitochondrial pathway.

The extrinsic and intrinsic pathways also communicate with each other and may in fact not be separable. The death receptor-mediated pathways have been further sub-classified into type I and type II categories where type I cells are strong DISC generators which produce enough caspase activity at the DISC to undergo apoptosis. In contrast, type II cells require a mitochondrial amplification loop which generates mitochondria-mediated caspase activity, in turn amplifying caspase 8 cleavage and thus DISC-associated death-promoting activity (8). It now appears that most cells are type II cells, and that human glioma cells, where the mitochondrial pathway is involved in death receptor-mediated apoptosis, and BCL-2 or BCL-XL are protective, belong in this group (9). Furthermore, both pathways are subject to cross-talk from other signaling pathways, including the survival signals emanating from what were traditionally considered mitotic pathways. The most prominent example here is Akt or protein kinase B, which has been shown to promote survival following stimulation of tyrosine kinase growth factor receptors, including the epidermal growth factor receptor, and activation of PI3 kinase in many cell types, including glioma cells (10;11).

3. APOPTOSIS IN GLIOMAGENESIS

3.1. Life or death decisions

One widely held view of how the apoptotic process is triggered is built around the central role that p53 plays in apoptosis and cancer. It states that apoptotic cell death occurs when a proliferating cell sustains genomic damage that exceeds its capacity for repair (12;13). P53 plays a critical role in arresting the cell cycle under these conditions, and either promotes repair or triggers apoptosis. The single cell's suicide is beneficial to the organism in that the propagation of genomic alterations to ensuing generations of cells might promote cancer. According to this model apoptosis results from the internal conflict to proliferate and to arrest the cell cycle due to DNA damage. As outlined by Evan and Littlewood, this view does not fit with the observation that oncogenes can induce

proliferation while sensitizing cells to apoptosis (12). They propose an alternative hypothesis: stimulating cell division primes the cell for apoptosis, which must be countermanded by a direct survival signal. A physiological means to countermand such an apoptotic signal would be through transient activation of the PI3 Kinase and Akt pathway, and constitutive, high level activation of this pathway is often seen in cancer. In addition, pivotal oncogenes such as bcl-2 do not act to drive proliferation, but block pathways of apoptotic cell death, and in combination with enhanced expression or activity of promitotic genes, such as c-myc or cdk-4, would be a constellation likely to promote neoplastic transformation. An extension of this view, which is gaining acceptance, is that the decision to undergo apoptosis is the result of the integration by the cell of a range of inputs, including growth signals, death signals, cell-cell and cell-matrix contact, genome state, membrane and organelle states and direct survival signals. Accordingly, a cancer cell might experience pro-apoptotic signals that can come from a variety of sources. These include ligand-mediated activation of death receptors, such as the tumor necrosis factor (TNF) receptor family, loss of cell-matrix contacts, survival factor depletion, hypoxia, DNA damage, telomere shortening or oncogene activation (1;3;12;14;15). Survival signals also come from the outside, and often involve signaling pathways that also stimulate cell division (1;12). If the sum of these signals leads to the activation of the cascade of regulatory and effector caspases, then apoptosis results (4). However, even this view is a simplification in that there are probably also caspase-independent apoptotic or apoptosis-like cell death pathways, including the glioma cell death mediated by the synthetic p53 homolog, chimeric tumor suppressor (CTS)-1 (16) or depletion of HSP70 (Nylandsted *et al.* submitted for publication).

3.2. Does apoptosis correlate with malignancy in brain tumors?

The hypothesis that the loss of normal apoptotic processes is one element in cancer progression predicts that rates of apoptosis should vary with cancer grade, if not type. More importantly, the implied relationship between treatment sensitivity and apoptosis might be apparent in studying the pre-treatment rates of spontaneous apoptosis. While the spontaneous cell death in malignant gliomas can be extensive, necrosis is the major modality of cell death, notably in glioblastoma (GB) where necrosis is a defining histological feature (17). There may be large areas of necrosis in the tumor center and small foci of necrosis often surrounded by pseudopalisading tumor cells (18). Necrosis is thus clearly the major mode of spontaneous cell death in human gliomas *in vivo*. The current view suggests that rapid tumor growth produces a demand for blood vessel supply that cannot be met despite strong angiogenic properties, resulting in hypoxic/ischemic damage to central tumor areas. These observations fit well with the rule that necrosis affects contiguous tissue areas whereas apoptosis affects single cells in the context of viable tissue. However, numerous studies have also determined levels of apoptotic cell death in malignant gliomas, defined by light microscopic and ultrastructural criteria, including nuclear condensation and pyknosis, formation of apoptotic bodies, and *in situ* detection of DNA fragmentation using *in situ* terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) (19). TUNEL is not entirely specific for apoptosis since TUNEL-sensitive degradation can occur in dividing cells and in the process of necrosis. Nevertheless, TUNEL is a suitable marker to differentially assess the extent of apoptosis in tumor specimens.

All studies reported to date for gliomas show that rates of apoptosis vary a great deal between tumors of the same histological type and sometimes across regions of a single tumor. These variations are likely to reflect micro-environmental differences experienced by tumor cells as well as probabilistic aspects of the entry into the cell death program. The influence of the microenvironment can clearly be seen in the case of TUNEL-positive tumor cells preferentially localized among perinecrotic pseudopalisading cells (19;20). These cells are expressing vascular endothelial growth factor (VEGF), consistent with hypoxia which is not severe enough to induce necrotic cell death (21). Cells in adjacent areas that are surrounded by the pseudopalisading cells typically die by necrosis, probably because the steeper decline of O2 partial pressure and nutrient depletion prevents them from entering apoptosis, which is an ATP-dependent process (22). Local delivery of the anti-angiogenic agent endostatin induces apoptosis and necrosis (23), further corroborating the idea that different levels of the same insult, here hypoxia, produce different modes of cell death.

Despite the regional variation in apoptosis within individual tumors and among types of tumors, some interesting relationships between apoptosis and other tumor behaviors have been found. Among astrocytomas, the rate of apoptosis has been found to correlate with grade. For example, in a study of 59 astrocytomas of grades II, III and IV using TUNEL, the pre-treatment apoptotic indices (AI) were 0.21±0.05%, 0.27±0.13% and 0.70±0.13% respectively (24). In another study, the rates for these tumors were 0.3%, 0.8% and 1.7% respectively, showing differences in absolute terms, but a similar trend (25). Similar data were obtained using H&E staining (19) or an antibody to single stranded DNA (26). In one study, cell division rates were also analyzed using the MIB-1 antibody to determine a Ki-67 labeling index which revealed a positive correlation with AI and tumor grade (25). Therefore, in higher grade tumors increased proliferation is accompanied by increased rates of spontaneous apoptosis, as predicted by the hypothesis that the induction of cell division primes cells for apoptosis (12). The fact that the more malignant gliomas achieve a clinically observable higher net growth rate implies, however, that increases in AI do not compensate for the increases in cell division rate.

When considering differences in AI in a group of otherwise similar tumors, a correlation between rates of apoptosis and patient survival can be observed. Korshunov and colleagues used TUNEL to study grade IV astrocytomas with a group of 168 patients, prior to radiotherapy and chemotherapy. They found a mean AI of 0.77% (27), in good agreement with the figure of 0.7% reported by Carroll *et al* (24). In their study all patients subsequently received the same treatment of 60 Gy

radiation and nitrosourea chemotherapy, and under these circumstances a clear correlation between AI and outcome was seen. Median survival of patients whose tumor showed an AI of less than 0.5% was 10 months compared with 15 months for those with an AI of greater than 0.5%, implying that the rate of spontaneous apoptosis in a naïve tumor is an indicator of rate of progression, or treatment sensitivity, or both. Interestingly, glioblastomas (grade IV astrocytomas) with an AI of less than 0.5% tended to show apoptotic nuclei that were concentrated around necrotic areas as opposed to being distributed throughout the tumor (27), suggesting that in these tumors the propensity of cells to undergo apoptosis was overall lower, but that apoptosis could be triggered under appropriate circumstances. The combined assessment of proliferation and an AI to calculate a tumor growth index (PI/AI) revealed that, among 39 astrocytic tumors of different grades, cases with lower PI than AI had a better survival than expected, while cases with more proliferation than apoptosis had a poor outcome (28). However it must be noted that AI did not have a prognostic value in several other studies examining malignant gliomas (19;25;26).

The clinical implications of AI and PI in other glioma types have remained controversial as well. A correlation between AI and cell division has been observed in oligodendrogliomas. In one study of 98 tumors, mitotic index and AI correlated, such that more malignant tumors had higher values in both categories (29). Additionally a very weak positive correlation of AI with survival was also observed across tumor grades. However, another study of 32 tumors uncovered no variation of AI with grade (30). Likewise for medulloblastoma, two studies have reported conflicting evidence of the relationship between AI and outcome. In the first report, 43 tumors classified as high or low risk, depending on the degree of resection and whether any tumor spread was observed at the time of operation, were treated with radiotherapy and chemotherapy or radiotherapy alone, respectively (31). When the quartile with the highest pre-treatment AI (above 85.6%) was compared to the remainder, it was found that patients with these tumors had longer progression-free survival, regardless of initial risk and treatment received, suggesting that strongly elevated levels of AI may be an indicator of treatment sensitivity in this tumor group (31). In contrast, another study showed that higher AI was associated with poor outcome (32). Here an AI of greater than 1.5%, which was close to the median value in a range of 0.1 to 8.2%, was found to be associated with higher rates of metastasis and shorter survival. Although it is difficult to explain how these studies could report such different AI ranges (the median in Haslam *et al.* of 7.55% is close to the upper range in Korshunov *et al.*), it is possible that the correlations with patient outcome are based on different phenomena. Accordingly, the cases in the upper quartile in the first study would represent cases with extremely elevated rates of spontaneous apoptosis, which would be predicted to be more sensitive to treatment than the average (31). In contrast, in the second study no such cases were found, and so here AI correlates simply with more aggressive tumors, that naturally have a poorer outcome, as had been shown for other glioma types (32).

Although more work needs to be done to complete the picture of the role of apoptosis in brain tumors, the studies performed to date provide some intriguing clues that this process is deregulated. While at one level increased malignancy, with its concomitant increase in cell division, appears to be accompanied by increased rates of apoptosis, at another level AI may allow the identification of tumors that will respond to treatment, from within a closely defined group. It must also be considered that the apoptosis discussed so far is spontaneous. It is not difficult to imagine that there may be a great deal of variation in the level of environmental influences promoting cell death in tumors as heterogeneous as the gliomas themselves. Therefore, tumors that show a similar spontaneous AI may have quite different sensitivities to stimuli that promote apoptosis, begging the question "what is the relationship between apoptosis and cancer treatment?"

3.3. Do existing treatments for glioma rely on the induction of apoptosis?

Radiotherapy is the cornerstone of postoperative therapy for malignant glioma and prolongs the median survival for glioblastoma by 6-8 months. The role of chemotherapy is less well defined, but metaanalyses indicate a significant survival advantage for patients treated with nitrosourea-based chemotherapy compared with radiotherapy alone (33;34) In addition to nitrosoureas, temozolomide has recently been approved for the treatment of recurrent malignant glioma (35;36), and its role in the primary management of malignant glioma is currently been evaluated in EORTC trial 26981. To what degree current modalities of cancer treatment act via the induction of apoptosis remains an area of intense investigation, and great interest, that has recently been comprehensively reviewed (37). For malignant gliomas, specifically, there is little information on the extent and mode of cell death induced by radiotherapy and chemotherapy because tissue removal typically precedes these treatments. Radiotherapy is the most important component of current glioma treatment, and its ability to induce DNA damage and apoptosis has been documented in several cell types. However, examination of the short-term effects of radiation on cultured glioma cells has failed to uncover the rapid induction of apoptosis found in some other cells. In one study treating 14 glioma-derived cell lines with up to 25 Gy failed to show any of the hallmarks of apoptosis within the first 30 hours (38). The observation that cell lines showing this acute radioresistance can be induced to rapidly enter caspase-mediated cell death when treated with CD95L or ceramide (39;40) suggests that this is not due to an overall inability to undergo apoptosis. Indeed, if apoptosis is measured 3 to 8 days after irradiation some cells do show a significant increase in the rates of apoptosis (41). This delayed apoptosis is not observed in all cell lines, however, including U87MG cells previously found to be refractory to the rapid induction of apoptosis (38). One defect that may contribute to the radioresistance of glioma cells is an inability to induce ceramide following irradiation, which in many cell types triggers CD95 signalling (40).

Radiation does consistently reduce the clonogenic potential of glioma cells (38;41;42), which may mean that in those cells in which apoptosis is not triggered

either early or late, a permanent cell cycle arrest or other form of cell death ensues. While a simple interpretation of these data would be that glioma cells have incurred a defect in the intrinsic pathway responsible for triggering apoptosis in response to radiation-induced genotoxicity, this does not explain all findings. Particularly intriguing is the observation that irradiation can delay apoptosis induced by cis-diammino-dichloroplatinum (II) (CDDP), another genotoxic agent, or even activation of CD95 which triggers the extrinsic apoptotic pathway (43). That this delay is itself transitory and that irradiation must be administered before apoptosis is stimulated (43), suggests that it is the result of a short-lived alteration of the cellular state, possibly due to the destruction of a radiolabile mediator of apoptosis. This finding has important implications for treatments that combine irradiation and chemotherapy, and also for the combined modality strategies that are currently being devised, and will be discussed towards the end of this article.

Several *in vitro* studies have demonstrated that high concentrations of various chemotherapeutic agents can, in principle, induce apoptosis in human malignant glioma cells (44-46) as has been established for many forms of chemotherapy in other systems (37). These include agents that damage DNA, perturb replication, transcription or protein synthesis, arrest the cell cycle, inhibit protein kinases, as well as pleiotropic drugs that alter gene expression. (For a review of current chemotherapeutic agents being developed for glioma see (47).) That the induction of apoptosis is also important to the action of chemotherapy in patients is largely inferred from these cell culture experiments, but some studies have provided direct evidence for this hypothesis. In one example, 10 patients were given estramustine, which arrests cells in G2/M by preventing microtubule function, prior to surgery (48). Analysis of glioma tissue removed at surgery showed an increase in AI from 1-3% in control cases to 5 to 50% in treated cases, and that apoptosis was induced in correlation with the accumulation of drug in the tumor.

Most chemotherapeutic agents appear to act via the intrinsic apoptosis pathways. Although it has been demonstrated in some cases that such drugs can alter the expression of death receptors and ligands in glioma cells, this appears not to be central to their activity. For example, teniposide (VM26), camptothecin, vincristine, cytarabine, CDDP and BCNU, as well as the RNA and protein synthesis inhibitors actinomycin D and cycloheximide, do not act via death receptors, nor is their activity inhibited by the caspase 8 inhibitor crm-A, which blocks the extrinsic pathway which relies on caspase 8 activity generated at the DISC (46). Inhibitors of topoisomerases induce apoptosis in glioma cells *in vitro*, presumably by inducing genome damage. In one study, the topoisomerase I inhibitors camptothecin and β-lapachone both induced the expression of the pro-apoptotic protein Bax, which did not require wild-type p53 activity and could not be inhibited by overexpression of bcl-2 (49). In contrast, etoposide, which is an inhibitor of topoisomerase II and thus also a DNA-damaging agent, was reported to induce apoptosis in cultured glioma cells via a p53 dependent mechanism (50), but dominant-negative p53 did not block etoposide-induced cell death in another study (51). Analysis of death pathways involved in chemotherapy-induced apoptosis has also demonstrated the involvement of cytochrome c release from mitochondria (45), another component of the intrinsic pathway, although not exclusive to it. Protein kinase inhibitors have also been shown to induce apoptosis of glioma cells *in vitro*. For example, a range of protein kinase C inhibitors acting by different mechanisms on this family of proteins, have this effect, which may rely at least in part on the inhibition of other kinases (52;53).

Several other drugs, which are either actively used in the treatment of cancer or are being evaluated for this, have been shown to induce apoptosis in glioma cells. Doxorubicin, a DNA intercalating drug with pleiotropic effects on the cell, causes cultured glioma cells to enter apoptosis (54). Fenretinide, a synthetic retinoid with potential as a chemopreventive agent and relative of 13-cis-retinoic acid which has shown some effect *in vitro* and in clinical trials, can induce apoptosis in glioma cells in culture (55). Farnesyltransferase inhibitors, which target post-translational modification of proteins, including ras which is highly active in glioma, induce apoptosis in astrocytoma cells in culture, perhaps in part by causing the upregulation of pro-apoptotic members of the bcl-2 family, such as bax and bak (56).

If apoptosis plays a role in the efficacy of currently used treatments, then therapy-related alterations of apoptotic pathways might be expected to lead to acquired resistance. In that regard, a single comparative study of primary and recurrent lesions from the same patients demonstrated that recurrent lesions exhibited patterns of bcl-2 family protein expression favoring resistance to apoptosis (57). Most importantly, this trend was independent from concurrent therapeutic interventions, including radiotherapy and chemotherapy, as it was also seen in a small group of patients who did not have specific treatment for their primary lesion. This suggests that the natural history of glioblastoma already effectively selects for cellular resistance to apoptosis. Whether this is also true in less aggressive and so less rapidly progressing gliomas awaits further study.

4. APOPTOSIS PATHWAYS – NEW MOLECULES, NEW TARGETS

In the next sections, molecular pathways identified over the last decade or so that are primarily involved in the regulation of apoptosis will be discussed. For each of these groups we will examine three issues: whether these proteins are present in brain tumors; how they contribute to the apoptotic mechanisms in glioma cells; and how they are being or may be exploited for therapeutic benefit. Because much of the work to date has been performed in glioma cell lines, we are providing a summary of some of what is known about them pertaining to apoptosis (Table 1).

Table 1. A summary of the expression of major regulators of apoptosis in commonly used glioma cell lines

Cell line	CD95	CD95L	CD95L sensitivity	DR4	DR5	DcR1	DcR2	TRAIL	TRAIL sensitivity	bcl-2	bax	origin	P53[l]
U87MG	+[a]	-[a]	sens[g]	+[i]/-[j]	+[i, j]	-[i, j]	-[i]/+[j]		sens[i]	-[d,k]	+[d, k]	*de novo* GB	wt/wt
U138MG	+[a]	+[a]	res[i]	-[i, j]	+[i, j]	-[i]/+[j]	-[i]/+[j]	+[h]	res[i]	-[k]	+[k]	*de novo* GB or AA	wt/wt
U251MG	+[i]			+[i]/-[j]	+[i, j]	+[i]/-[j]	-[i]/+[j]	+[h]	sens[i]	-[k]	+[k]	*de novo* GB	273/273
U343MG	+[a]	+[a]										*de novo* AA	wt/wt
U373MG	+[i]		res[i]	-[i, j]	+[i, j]	-[i]/+[j]	-[i]/+[j]	+[h]	res[i]	-[k]	-[k]	*de novo* GB	273/273
LN-18	+[c]	+[b]	sens[c]; w/o prot./ mRNA synth. inh.	+[i, j]	+[i, j]	-[i]/+[j]	-[i]/+[j]	+[h]	sens[i]; w/o inhibitors of protein synthesis	-[c, k]	-[k]	*de novo* GB	238/wt
LN-215	+[c]		sens[c]							+[c]		*de novo* GB	mutant
LN-229	+[c]	+[b]	sens[c]	+[i]/-[j]	+[i, j]	+[i]/-[j]	-[i]/+[j]		sens[i]	+[c, e, k]	+[e, k]		wt/wt
LN-308	-[c], +[i]	+[b]	res[c]	-[i, j]	+[i, j]	-[i, j]	-[i]/+[j]	+[h]	sens[i]	+[c, k]	+[k]	*de novo* GB	null
LN-319	-[c], +[i]		res[c]	+[i, j]	+[i, j]	+[i]/-[j]	-[i]/+[j]	+[h]		-[c, k]	+[k]	*de novo* AA	175/175
LN-405	-[c]		res[c]							-[c]		*de novo* GB	282/282
LN-428	+[i]			+[i, j]	-[i]/+[j]	-[i]/+[j]	-[i]/+[j]	+[h]	sens[i]	+[k]	+[k]	*de novo* GB	173/282
LN-443		-[b]										*de novo* GB	wt/wt
A172	+[i]			+[i]/-[j]	+[i, j]	-[i, j]	+[i, j]		sens[i]	-[k]	+[k]	GB	wt/wt
D247MG	+[i]			+[i, j]	+[i, j]	-[i]/+[j]	-[i]/+[j]		sens[i]	-[k]	+[k]	gliosarcoma	wt/wt
9L gl.src.	+[a]	-[a]										N/A	-
T98G	+[a,c]	+[a]	sens[c]	-[i, j]	+[i, j]	+[i]/-[j]	+[i, j]	+[h]	sens[i]	+[c, d, k]	+[d, k]	N/A	-
HYQC	+[a]	+[a]										N/A	-
MGH238	+[a]	+[a]										N/A	-
MT539MG mouse		+[b]										Spontaneous	-
C6 rat		+[b]										ENU induced	-

For each cell line the expression status for CD95, CD95L, DR4, DR5, DcR1, DcR2, TRAIL, bcl-2 and bax is shown, when known. A "+" indicates expression and a "-" no expression. See references for details of how expression was measured. In addition the sensitivity ("sens") or resistance ("res") to CD95L or TRAIL is indicated. LN-18 cells do not require treatment with inhibitors of protein or mRNA synthesis for these death ligands to be effective. 9L cells are derived from a gliosarcoma. In addition the histology of the tumor of origin, as well as the p53 status is shown. For details see references below and the text. a: total protein studied (66); b: total protein studied (83); c: cell surface protein studied (59;80); d: (50); e: (49); f: (75); g: (40); h: protein (91); i: mRNA for DR and DcR, and protein by flow cytometry for CD95 (90); j: protein by flow cytometry for DR1 through 4, with overall very low levels of DR1, 3 and 4 detected, except for U87MG which had higher levels of DR 4 (95); k: protein levels by western (106); l: (124).

4.1. Death Ligands, Receptors and Downstream Effectors

In this category, most attention to date has focused on CD95, also known as Fas or APO-1, and its ligand, CD95L. More recently attention has shifted to Apo2 ligand (Apo2L)/TNF-related apoptosis-inducing ligand (TRAIL) and its death signaling receptors, death receptors (DR) 4 and 5, also known as TRAIL-R1 and TRAIL-R2, because of the greater likelihood that this molecule will make a clinical contribution (58).

Analysis of CD95 mRNA and protein in astrocytic gliomas (59) revealed that the proportion of tumors expressing the gene increased with grade, reaching nearly universal expression in glioblastoma (60;61). Histologically, CD95 expression in glioblastoma was associated predominantly with areas of necrosis (62) where higher rates of apoptosis are also consistently seen (27). Furthermore, the association with necrosis means that CD95 is more commonly seen in primary glioblastoma than in the secondary form of the disease progressing from WHO grade II or III lesions (18). While the correlation of CD95 and necrosis is not evidence of causation, it suggests that CD95-mediated cell death may be involved in generating these histological hallmarks of high-grade glioma. The observation that CD95, unlike VEGF, was not upregulated by hypoxia in a glioma cell line, suggests that its peri-necrotic presence may not simply be due to a drop in local oxygen levels (18). Yet, hypoxia has been proposed to selectively eliminate p53 wild-type cancer cells (which form the majority of cells in primary glioblastoma (63)), and p53 does enhance the expression of CD95 in glioma cells (16;64). These observations indicate that the complex interrelations between hypoxia, p53, CD95 and cell death in glioblastoma have not been fully elucidated. Moreover, malignant glioma cells appear to be resistant to fratricidal killing even though they co-express CD95 and CD95L at the cell surface (see below), further questioning the role of CD95 in spontaneous apoptosis or necrosis in glioma cells *in vivo* (58). Interestingly, in that regard, malignant glioma cells release a soluble decoy receptor, DcR3 for CD95L which blocks CD95L-induced apoptosis (65).

While the discovery of CD95 on glioma cells suggested that these cells might be killed by therapeutic stimulation of this receptor, subsequent studies revealed that in many cases these tumors expressed not only CD95, but also the corresponding death ligand, CD95L. For example, in a study of 22 astrocytic gliomas, 19 expressed CD95, and of these 15 also expressed both receptor and ligand (66). Similar data was obtained for cell lines where 4 out of 7 expressed both (see Table 1). Co-expression of ligand and receptor is also the rule, rather than the exception, for ependymoma and oligodendroglioma (67). Recent careful analysis of implanted mouse tumors showed that most of the CD95L expressed was derived from host macrophages, rather than the tumor cells themselves (68). Such an analysis has yet to be performed for gliomas. Regardless of the source of the soluble ligand, CD95 and CD95L are both present in glioma, yet these cells evidently

are not subject to high levels of apoptosis, begging the question of whether there is a defect in CD95 signaling or whether there is an effective counter-signal. A survey of freshly isolated (69) or established (see Table 1) glioma cells shows that in many exogenous agonistic CD95 antibodies (59), soluble CD95L (44) or viral vectors encoding CD95L (70) induce apoptosis, and so that this pathway can function properly. Interestingly, human fetal astrocytes may be more resistant to CD95L than neoplastic astrocytes (71), suggesting a link between transformation and sensitivity to CD95-mediated apoptosis. In order for CD95 signaling to be effective on glioma cells, most require simultaneous treatment with an inhibitor of either protein or mRNA synthesis, suggesting that they express a labile protein that prevents the CD95 signal from being carried out (59). This putative protective factor may well be specific to the extrinsic, death receptor-mediated, pathways since inhibition of RNA or protein synthesis do not sensitize to irradiation-induced apoptosis (40). Two likely candidates, BCL-2 and the x-linked inhibitor of apoptosis protein (XIAP), were excluded since their half-life in the presence of RNA and protein synthesis is far too long to explain the sensitization to death receptor-mediated apoptosis (72;73). In contrast, we have obtained some compelling evidence that p21WAF/Cip1 is the target of cycloheximide-mediated protein synthesis inhibition in glioma cell lines (9). Moreover, attenuation of p21 WAF/Cip1 expression by antisense oligonucleotides or antisense adenovirus also sensitizes these cells to BCNU (9;74).

Examination of the mechanism of CD95-mediated cell killing in glioma cells has shown it to be overall similar to that described in other cells. For example, it is mediated by caspases 8 and 3 and leads to rapid cleavage of the caspase substrate poly(ADP-ribose)polymerase (75). As expected CD95-mediated apoptosis can be inhibited by the pox-virus derived serpin cytokine response modifier (crm)-A, at the level of caspase 8 activation, and bcl-2, at the level of mitochondrial cytochrome c release (75). In addition, reactive oxygen species, more typically associated with necrotic cell death, have been implicated in CD95-mediated killing of glioma cells (76). This study also demonstrated the ability of superoxide dismutase and catalase to protect glioma cells from CD95-mediated apoptosis. Support for a generalized role for redox state in glial cell survival comes, for example, from the observation that the anti-oxidant N-acetyl-L-cysteine can protect oligodendrocytes from apoptosis induced by growth factor withdrawal (77). Whether the importance of redox state is more pronounced in glial cell apoptosis than in that of other cell types remains to be seen. It should be noted that not all studies found a role for free radical formation in CD95-mediated glioma cell apoptosis (78).

The therapeutic potential of the CD95/CD95L system in glioma has been extensively studied (58). Briefly, while the co-expression of CD95 and CD95L by gliomas and their apparent resistance to suicidal or fratricidal CD95-mediated apoptosis remains incompletely understood, it is clear that the application of exogenous CD95L has the potential to kill fresh *ex vivo* brain tumor cells (69;79). Furthermore, the combination of CD95 signaling with other compounds has significant potential. In one study, the upregulation of CD95 by cytokines was shown to increase cellular sensitivity to CD95-mediated apoptosis (59). More recent approaches have included the use of gene transfer to modulate this signaling pathway. It has long been appreciated that glioma cell sensitivity to CD95 cross-linking could be significantly enhanced by increasing the expression levels of CD95 (80). It is also possible to elevate the levels of CD95L using adenoviral vectors. When this was done in experimental tumors derived from the CD95-sensitive rat glioma cell line F98, a significant increase in apoptosis and survival time was obtained (81). Adenovirus-mediated expression of CD95L has also been shown to induce cell death in primary human glioma-derived cell cultures which are resistant to the chemotherapeutic agent CCNU (82). A confounding aspect of the expression of CD95L in brain tumors is that it appears to be involved in suppressing the T-cell mediated immune response (83), as has been proposed as a physiological function in the eye . In addition, CD95L is considered too toxic for application in humans because the systemic activation of the CD95 system results in fulminant liver failure within few hours (84), making local therapy the only possible option.

More recently attention has shifted to the death ligand Apo2L/TRAIL which interacts with death receptors (DR) 4 and 5 to transduce a signal by a biochemical pathway similar to CD95L acting via CD95 (3). One reason for this shift is that TRAIL does not induce the severe toxicities caused by CD95L and so is thought to have much more potential in the clinic (85). There are reports that some normal cells in humans may be more susceptible to TRAIL than their animal counterparts, including hepatocytes (86) and neural cells (87), raising the possibility that TRAIL may also have limitations in the clinic. However, these studies may be questioned in that artificial TRAIL preparations were used which would not be introduced into the clinic, e.g., His-tagged TRAIL (86) or Flag-tagged TRAIL cross-linked by FLAG antibody (83). In contrast, our own data indicate that nonmodified TRAIL retains its anti-human glioma xenograft in nude mice, in the absence of any neurotoxicity (Fulda *et al.* submitted for publication). Further, it is possible that spatially restricted treatment strategies, such as convection enhanced delivery by local infusion (88;89), could be used to circumvent any toxicities that do manifest when (or if) TRAIL is tested in the clinic.

Many normal tissues are resistant to TRAIL-induced apoptosis. It has been proposed, although never convincingly demonstrated, that this resistance is linked to the expression of high levels of the decoy receptors, DcR1 and DcR2, also referred to TRAIL-R3 and TRAIL-R4 (3). These proteins lack the intracellular death domains required to transmit the apoptotic signal and might act to compete for ligand with the agonistic receptors, DR4 and DR5. Tumor cells, including those from gliomas, preferentially express the agonistic receptors (see Table 1; (90)). However, gliomas and cell lines derived from them also

make TRAIL itself (91), suggesting that analogous to the situation with CD95L, the presence of ligand and receptor at the cell surface is not enough to result in significant levels of apoptosis in tumors. This again raises the issue of whether there is a defect in the way the TRAIL signal is transduced in glioma cells. TRAIL-induced cell death is similar to that induced by CD95L in that it is attenuated by bcl-2 and blocked by crm-A overexpression, inhibited by dexamethasone, and not related to p53 status (90). In addition its effect is also enhanced by co-exposure to cycloheximide in most cell lines (92), suggesting that the modulation of TRAIL sensitivity may share a mechanism with that of CD95L, possibly inhibition by p21 WAF/Cip1 (9). Differential susceptibility to Apo2L/TRAIL-induced apoptosis may also involve differences in the expression of the apoptosis inhibitor phosphoprotein enriched in diabetes/phosphoprotein enriched in astrocytes-15 kDa (PED/PEA-15) (93).

A study of the effect of TRAIL on cell lines derived from primitive neuro-ectodermal tumors (PNETs) showed that resistance to TRAIL correlated with the absence of caspase 8, suggesting a global defect in the extrinsic pathway (92). These authors showed that sensitivity to TRAIL was restored by demethylating agents, suggesting that the caspase 8 gene had been transcriptionally silenced. However, loss of caspase 8 expression is not observed in malignant glioma cell lines (45). An alternative hypothesis would be that there is not enough TRAIL in brain tumors to trigger apoptosis. Evidence for this comes from the fact that most glioma cells are sensitive to exposure to exogenous TRAIL (see Table 1), suggesting that in many cell lines there is no deficit in the signal transduction machinery. Importantly, the efficacy of TRAIL in the treatment of intracranial U87MG xenografts in nude mice suggests that the application of sufficient TRAIL can induce apoptosis very effectively in glioma cells (94). This study also suggested that there was little neurotoxicity from TRAIL exposure of the normal brain, giving hope that this approach may work in the clinic. . Further, Apo2L/TRAIL acts in synergy with the nitrosourea lomustine (CCNU) to induce apoptosis in glioma cell lines (95), and even systemic co-treatment of nude mice bearing intracranial glioma xenografts with Apo2L/TRAIL and CDDP significantly extended survival.

Additional efforts of therapeutically exploiting the extrinsic death pathway have focused on downstream effectors. Transferring FADD into glioma cells controls their growth *in vitro* and *in vivo* (96). Further, TNF receptor-1-associated death domain protein (TRADD) was proposed to mediate p53-independent radiation-induced apoptosis of glioma cells, and TRADD overexpression sensitized glioma cells to irradiation (97). As these pathways become more completely described, additional points of intervention will be identified.

4.2. Bcl-2 family

Leaving aside intrinsic pathway triggers such as P53 and other sensors of genomic damage or cell stress including, more recently, the BH3-only proteins (7), most attention on this side of apoptosis has focused on the bcl-2-related proteins. The bcl-2 family encompasses over a dozen members, and includes proteins that pro- and anti-apoptotic, as well as proteins that sense the cellular state. Most attention in the context of neuro-oncology has been paid to the founder, bcl-2, and those members discovered soon after it, such as the pro-apoptotic protein bax.

Immunohistochemical analyses of astrocytomas have shown that almost all astrocytic tumors have some staining for bcl-2. In contrast to the expectation that expression of antiapoptotic members of the bcl-2 family increases with malignancy, stronger staining in astrocytoma and anaplastic astrocytoma compared with glioblastoma has been observed in most studies (98), although in other studies no overall correlation with prognosis or grade could be made (24;27;73;99). In some studies a correlation of higher levels of bcl-2 and expression in a higher proportion of tumor cells with higher grades of astrocytoma has been reported (73), but much variation exists.

There was also no regional correlation between the pattern of bcl-2 staining and necrosis, previously shown for CD95 (100), nor between bcl-2 and neo-vascularization (99). Yet, when the proportion of cells stained for bcl-2 in astrocytoma grade III was measured, a positive correlation with shorter survival was observed, and bcl-2 positivity was found to be a negative prognostic marker (101). Interestingly, this did not hold for glioblastoma, suggesting that in these tumors additional factors contribute to defects in apoptosis. A small series showed an association of high expression of the Mcl-1 protein with shortened progression-free survival in malignant glioma patients receiving adjuvant radiochemotherapy (102). Increased expression of bcl-2, and the anti-apoptotic relatives bcl-X and mcl-1 was higher in recurrent glioblastoma (57), suggesting that these proteins can make a significant contribution to the resistant phenotype associated with recurrence.

In contrast to astrocytomas, overall BCL-2 staining in oligodendrogliomas was low (29). Furthermore, attempts to correlate expression with clinical characteristics have not resulted in a clear indication of whether BCL-2 functionally contributes to tumor behavior. For example, in grade III oligodendrogliomas low levels of bcl-2 expression correlated with shorter survival (103), while it would be expected that tumors with less BCL-2 might be more treatment-sensitive. Overall increased expression of bcl-2 family members in the course of progression was observed in these tumors (103), leaving open the question of whether the balance between the pro- and anti-apoptotic sides of the family shifts.

That BCL-2 can protect glioma cells, such as T98G cells, was demonstrated when its overexpression protected them against CDDP, BCNU, paclitaxel, vincristine, doxorubicin and herpes simplex virus thymidine kinase-mediated killing (73;101). In addition to resistance to drugs, overexpression of bcl-2 can protect glioma cells from CD95 mediated killing, suggesting that it may contribute to the observed variability in sensitivity to CD95L among CD95-positive glioma cells (73).

Therapeutic exploitation of the bcl-2 gene would have to reduce the activity of the gene or its product. No pharmacological modalities to influence BCL-2 protein function have yet shown clinical promise, making gene therapy-based approaches the currently most promising approach, setting aside the issues of how to effectively deliver anti-sense bcl-2 to the tumor. One study using antisense oligonucleotides showed that glioma cells *in vitro* could be induced to undergo apoptosis when levels of BCL-2 protein were reduced (104).

Not all members of the bcl-2 gene family are anti-apoptotic. Proteins that share sequence motifs with bcl-2 can be pro-apoptotic, as exemplified by the BAX protein. Observations suggesting that these proteins interact in complexes have given rise to the idea that the balance between the two sides of this complex family may be a key determinant of entry into apoptosis. However, attempts to correlate the expression levels of pro- and anti-apoptotic bcl-2 family members with sensitivity in cells has met with limited success. In one study of cells from a variety of pediatric tumors, including glioma, levels of the anti-apoptotic BCL-2, BCL-XL and MCL-1 as well as the pro-apoptotic BAX, BAK and BCL-xS were examined. Only levels of BAX correlated with sensitivity to exposure to doxorubicin and actinomycin D (105). Among a panel of 12 malignant glioma cells, the expression patterns of bcl-2 family proteins correlated with radiosensitivity (42) but sensitivity to a series of chemotherapeutic drugs appeared to be unrelated to bcl-2 family protein expression (106). *In vivo*, reduced expression of BAX protein was also associated with recurrence in glioblastoma (57), and with shorter times to progression in oligodendroglioma (103). That mutational inactivation of Bax may play an important role in cancer progression has been suggested by studies in colon carcinoma, where the loss of functional Bax promoted clonal outgrowth and progression (107;108), and similar analyses in brain tumors could be of interest. This observation underlines that the balance between anti- and pro-apoptotic members of the bcl-2 family is what determines a cell's sensitivity to induction of cell death, and suggests that alterations of this balance by manipulation of either side, could be of therapeutic advantage.

The expression of bax has been implicated as a key element in their response to treatment. In an investigation into the mechanism underlying the inherent radioresistance of glioblastoma cells and radiosensitivity of medulloblastoma cells, it was found that the ability of wild-type p53 to induce BAX expression in response to radiation was the key determinant (109). Bax naturally makes a more attractive tool for therapy than do the anti-apoptotic family members. However, these antiapoptotic proteins must be considered in this context, as the balance between the pro- and anti-apoptotic bcl-2 family proteins will determine whether a tumor cell undergoes apoptosis or not (110). While it might not be surprising that the introduction of BAX protein into glioma cells induces apoptosis or sensitizes them to treatments that induce apoptosis (110;111) our own study on bax gene therapy for glioma was negative in that BAX neither induced cell death nor enhanced sensitivity to chemotherapy (112), even though moderate radiosensitization was obtained (42). The future will probably focus increasingly on the transfer of BH3-only genes, for example NBK which is highly active against glioma cells (Naumann *et al.* in preparation), provided that transgene expression can be limited to cancer cells.

4.3. Treatment combinations

In addition to using modulators of apoptosis as stand-alone therapies, the possibility that novel treatments aimed at restoring apoptotic potential to glioma cells may sensitize them to existing modalities is also likely to yield new potential therapies. One good example is that altering the balance between pro- and anti-apoptotic bcl-2 family members can sensitize cells to apoptosis. For example, the introduction of bax using a herpes virus vector enhanced the response of a rat glioma model to BCNU (113). However, while these approaches may be powerful, it should not be expected that increasing one pro-apoptotic factor would necessarily shift sensitivity to all inducers of apoptosis.

Another approach to shifting the apoptotic balance in a cell is to attenuate the survival signal. Signaling through growth factor receptors provides such survival signals, and many of them are now being targeted by small molecule drug design. Of particular importance to glioma is the overexpression and amplification of the epidermal growth factor receptor (EGFR), which is a hallmark of primary glioblastoma (114). Enhanced EGFR expression results in resistance to apoptosis induced by cytotoxic drugs as well as enhanced survival of glioma cells *in vivo* (115-117). In addition there is the potential that new regulators of apoptosis, such as the glioma associated adaptor protein SETA (also known as ruk and CIN85, and renamed SH3-domain kinase binding protein 1) (118;119) which is also involved in other signal transduction mechanisms (120-122) may help identify pathways that can be modulated to therapeutic advantage.

That the converse, where other modalities can render glioma cells sensitive to apoptosis induced by apoptosis pathway modulators, can also be fruitful has been shown in several instances. For example, the treatment of glioma cells in culture with the topoisomerase I inhibitor camptothecin sensitizes them to apoptosis induced by CD95L (49). Similarly, antisense-oligonucleotide mediated repression of the apoptosis inhibitor p21WAF/Cip1 sensitizes glioma cells to CD95L (9). That this manipulation may engender a more generalized sensitivity to cell death, including intrinsic pathways, is suggested by the observation that reducing p21WAF/Cip1 levels by antisense oligonucleotides also enhances apoptosis induced by irradiation in radioresistant glioma cells (123). p21WAF/Cip1 inhibition may therefore evolve to be a major target for strategies aiming to sensitize malignant glioma to radiotherapy, chemotherapy and death ligand therapy. On the contrary, there are some indications of potential interference between conventional therapies and induction of apoptosis. In one study radiation delayed the apoptosis induced by CDDP, another genotoxic agent, or

even activation of CD95, which works via the extrinsic pathway (43).

5. PERSPECTIVE

Spontaneous apoptosis in human malignant glioma is the result rather than the cause of different grades of malignancy. More aggressive tumors often have higher rates of apoptosis, which are most likely a result of other tumor processes such as genomic aberrations and enforced cell division plus hypoxic/ischemic tumor cell damage because of insufficient angiogenesis. However, it seems likely that the stresses that induce the observed cell death also continuously select for clones of cells that have the lowest possible levels of apoptosis, and so engender a significant degree of treatment resistance. This may be part of the explanation for why current therapeutic strategies, including radiotherapy and chemotherapy, can induce apoptosis efficiently in culture or animal models of glioma but fail to do so to a large extent in human malignant gliomas *in vivo*. Nevertheless, enough apoptotic signaling pathways appear to be present and active in glioma cells to hold out the hope that their exploitation may shift responses to existing therapies significantly, ushering in a new spectrum of combined therapies with less toxicity and more efficacy. In addition, exciting novel strategies that induce apoptosis by application of soluble death ligands, such as through therapy with Apo2L/TRAIL, perhaps delivered locally, will hopefully soon be evaluated in the clinic, hopefully beginning a new era in the treatment of glioma.

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Abbreviations:
AI: apoptotic index
AIF: Apoptosis inducing factor
Apo2L: Apo2 ligand
BAD: BCL-XL/BCL-2-associated death promoter
BCNU: 1,3-bis(2-chloroethyl)-1-nitrosourea
BID: BH3-interacting domain death agonist
BIK: BCL-2 interacting killer
CARD: caspase activation and recruitment domain
CDDP: cis-diammino-dichloroplatinum (II)
CCNU: 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea
CRM-A: cytokine response modifier A
CTS: chimeric tumor suppressor
DcR1/2/3: decoy receptor 1, 2 or 3
DD: death domains
DED: death effector domains
DIABLO: direct IAP binding protein with low pI
DISC: death-inducing signaling complex
DR4/5: death receptor 4 or 5
EGF: epidermal growth factor
EGFR: EGF receptor
FADD: Fas-associated protein with death domain
FLIP: FADD-like IL-1? -converting enzyme (FLICE) like inhibitory protein
GB: glioblastoma
HRK: Harakiri
NBK: natural born killer
PED/PEA-15: phosphoprotein enriched in diabetes/phosphoprotein enriched in astrocytes-15 kDa
PI: proliferation index
PNET: primitive neuro-ectodermal tumor
SMAC: second mitochondria-derived activator of caspase
TUNEL: Terminal deoxynucleotidyl transferase UTP nick end labeling;
TNF: tumor necrosis factor
TRAIL: TNF-related apoptosis-inducing ligand
TRADD: TNF receptor-1-associated death domain protein
VEGF: vascular endothelial growth factor
XIAP: x-linked inhibitor of apoptosis protein

Key Words: Glioma, Hypoxia, Apoptosis, p21, Apo2L/TRAIL, CD95L, EGFR, Bcl-2, Bax, Review

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