

Review

## Apoptosis in Brain Tumors: Prognostic and Therapeutic Considerations

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**Abstract.** *The direct and indirect regulation, as well as the mechanisms of apoptosis in the nervous tissue and their implications in the treatment and prognosis of brain tumors are reviewed.*

A large body of evidence demonstrates that apoptosis and necrosis represent the extreme ends of a wide range of possible cell death mechanisms based on preservation or loss of energy production in the cell. The same stimulus can elicit both classical types of cellular demise and the intensity of the initial insult often decides the prevalence of either apoptosis or necrosis. In neurons, intracellular energy levels and mitochondrial function are rapidly compromised in necrosis but not in apoptosis, and it has recently been shown that depleting ATP from human T-cells switches the predominant type of demise to necrosis. Necrosis affects groups of cells, is associated with the inflammatory response and primarily involves cytoplasmic organelles, whereas apoptosis is a programmed cell death which affects single cells, presupposes integrity of cellular organelles and requires activation of specific genes (1). Apoptosis, as a programmed cell death, plays a key role in the removal of redundant neurons during CNS development, immune regulation, brain tumor development and, supposedly, in neurodegenerative diseases. It can be directly demonstrated in tissues by morphological-immunohistochemical procedures and biochemical analysis of DNA laddering, and indirectly through the demonstration of activation or deactivation of the relevant pathways (2).

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The tremendous volume of contributions to the study of apoptosis has produced such a quantity of data that it is difficult to insert them into a general theoretical scheme of the phenomenon and its regulation. Apoptosis can be seen, on the one side, as a phenomenon belonging to the biological foundations of life, mainly of interest to biology philosophers, and, on the other, as a biological event technically useful in the hands of pathologists/ neuropathologists for establishing tumor diagnosis and prognosis, understanding tumor development, the recognition of the effects of therapies and in-depth study of the pathogenetic mechanisms of various pathological conditions, especially in the case of neurodegenerative diseases. The goal of this mini-review is to provide a general survey of how neuropathologists can demonstrate and use apoptosis in the diagnosis, prognosis and management of brain tumors.

### Definition and Regulatory Circuits

Complete reviews on apoptosis are available which cover exhaustively a great amount of information (2-7). The first recognition of apoptosis in tissues is based on morphological criteria: chromatin compaction, splitting and leaning against the nuclear membrane, followed by its breaking into apoptotic bodies. These are later phagocytosed by macrophages. The whole process does not last more than few hours. Splitting of DNA into fragments of 180 bases by a DNase can be demonstrated with the insertion of digoxigenin-labeled nucleotides and by a polymerase or terminal nucleotidyl transferase (TdT). This represents the basis for the immunohistochemical demonstration of apoptosis which is called TUNEL. At the same time, splitting of DNA into regular fragments of 180 bases represents the rationale for detecting DNA "laddering" by gel electrophoresis, which is a biochemical method for demonstrating apoptosis (2).

More than one pathway to apoptosis, which has an extremely complicated regulation, have been described

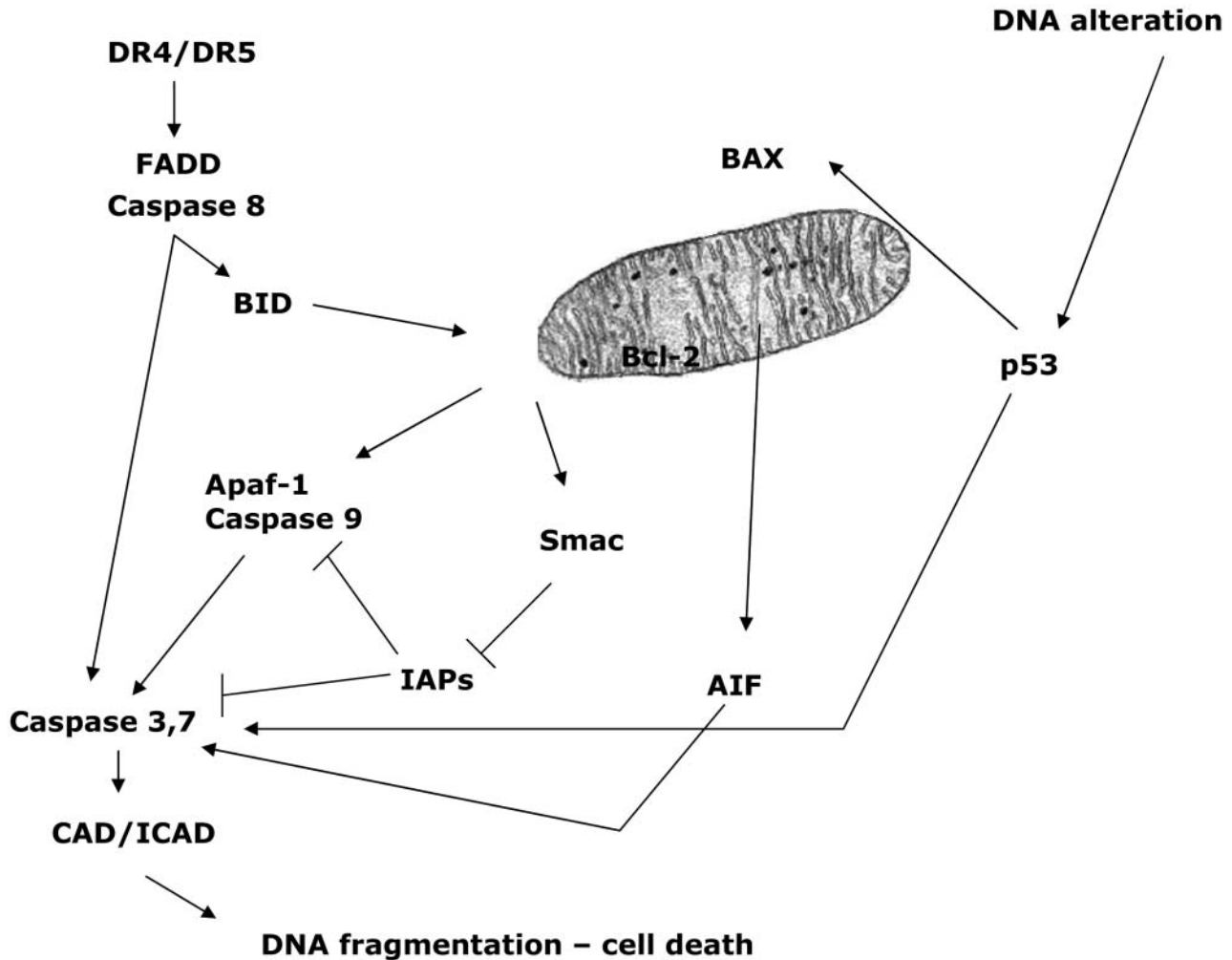


Figure 1. Pathways to apoptosis.

(Figure 1). There is an intrinsic (8) or transcriptional pathway *via* mitochondria, focused on *p53*. Once *p53* is activated by DNA damage, its signaling induces cell cycle arrest through *p21*, DNA repair through PARP-1 (4) and apoptosis through transport of *Bax* to the mitochondria. Mitochondria then release apoptosis inducing factor (AIF), Smac/DIABLO and cytochrome-c that in turn act through Apaf-1 and pro-caspase-9 leading to activation and cleavage of caspase-3. Caspase-3 cleaves the inhibitor of caspase-activated DNA (ICAD) activating CAD which then breaks DNA into fragments (3). In this pathway *Bcl-2* functions as an anti-apoptotic agent by activating the transcription factor NFκB (9) which is transferred to the nucleus, once released from IκBα, a protein that sequesters NFκB in the cytoplasm.

An extrinsic pathway is triggered by ligation of death receptors such as Fas/CD95, APO-1, TNFα or TRAIL receptors (10). Caspase-3 is activated through caspase-8 –

DISC, which is normally inhibited by FLIP, and it becomes thus the common final step to apoptosis for both pathways. The triggers of the extrinsic pathway belong to the TNF super-family, such as APO-2 and TRAIL (TNF related apoptosis-inducing ligand) that are active on DR4 and DR5 through the death domains FADD and TRADD.

Cross-talk between the two pathways can be realized through cleavage of Bid (Bcl-2 inhibitory BH3-domain-containing protein) (11) by caspase-8. Truncated Bid translocates to mitochondria and activates the pathway through cytochrome-c, AIF, Apaf-1, Smac/DIABLO and IAP (12). In this regard, tumor cells have been divided into "type 1", in which caspase-8 directly processes downstream effector caspases including caspase-3 (13) and "type 2", in which mitochondrial amplification of the death receptor-induced signal is required for apoptosis through

translocation of Bid (14). The level of caspase-8 expression is important for the mitochondrial amplification of the death signal (15).

Other pathways lead to apoptosis or interfere with those leading to it. PI3 kinase-Akt pathway regulates apoptosis and in turn is controlled by Ras, PTEN, ceramide downstream CD95, c-Jun/JUNK, I $\kappa$ B $\alpha$  (16) and the granzyme B pathway (17). Another possibility is that AIF, found in the mitochondrial intermembrane space (18), inducing cell death by alkylation, translocates to the nucleus and activates caspase-dependent cell death; this can be prevented by *Bcl-2* (19). Cell death from PARP-1 activation is mediated by AIF (20).

Apoptosis has a complex regulation, because it is at the crossroad of different pathways. Apoptosis is mediated by different mechanisms involving, for example, proteolytic enzymes, including cathepsins which play a role once released from lysosomes (21, 22), regulatory circuits including Ras/MAPK that controls cell proliferation, mitochondrial IAPs (inhibitory of apoptosis proteins) that inhibit caspase-3, which is in turn inhibited by mitochondrial Smac/DIABLO (23), and survivin, particularly important in tumors (24). Besides p53, Bax, PARP-1 and caspases, a key regulatory mechanism is represented by NF $\kappa$ B (25). Moreover, apoptosis is regulated by proteasome inhibitors that induce it by Fas, by *c-myc* accumulation following induction of Fas-L in glioma cells (26), independently of p53-p21 or by mechanisms involving caspases and cytochrome-c release (27, 28). The ubiquitin-proteasome system plays a role in anti-apoptotic surveillance (29).

Of the many regulatory steps of the apoptotic pathways, some seem to be more evident in tumors while others are apparent in neurodegenerative diseases. PARP-1, located in the nucleus and activated for repair (30) when DNA is damaged by radio- or chemotherapy (31), plays an interesting role. The intact molecule prevents apoptosis by keeping endonucleases in an inactive state (32). When PARP-1 is cleaved into fragments of 24 and 89 kDa by caspases, it becomes a marker of early apoptosis (33). The 24 kDa fragment contains a DNA-binding domain that blocks access of DNA repair enzymes favoring apoptosis, whereas the second fragment is incapable of activation by DNA nicks, thus preventing energy depletion (34). The PARP-1 fragments can be recognized by monoclonal antibodies (35). When PARP-1 is activated, for example by NOS after a DNA strand break, it polymerizes ADP-riboses to poly-ADP-ribose (PAR) using NAD<sup>+</sup> and ribose which react with proteins for repair. The process of ribosylation is ATP-dependent and leads to energy depletion and cell death by necrosis (36). PARP-1 cleavage prevents its over-activation, so that PARP-1 activation leads to necrosis and PARP-1 cleavage leads to apoptosis. In medulloblastomas, for example, cleaved PARP-1 co-

localizes with cleaved caspase-3 (37). Interestingly, the extra-cellular levels of glutamate are elevated in gliomas. Glutamine is normally exchanged for cystine, and glioma cells lack glutamate transporter expression (38) with consequent elevated levels of glutamate. EAAT-2 glutamate transporter reduces cell proliferation leading to induction of apoptosis through caspase-3 activation and cleavage of PARP-1 (39).

It is important to consider that PARP-1 activation appears earlier than DNA nicks as monitored by TdT (40) and that modifications of the proteins to which ADP-riboses adhere after ribosylation are only transient for the quick intervention of an ADP-ribose-glycohydrolase (PARG) that catalyses the hydrolysis of PAR in free ADP-riboses. There is coordination between PARP-1 activation after DNA damage and the short occurrence of polymers mediated by PARG (34).

Survivin, an IAP, contains a single baculovirus repeat that regulates the G2/M phase of the cell cycle, is associated with mitotic spindle microtubules and inhibits caspase-3 and -7 activity (41-43). Once it is blocked by antisense oligonucleotides, PARP-1 is cleaved and apoptosis is induced through caspase-3 (44).

### Assessment of Apoptosis in Tissues

Typical chromatin morphology, positive TUNEL and DNA laddering by gel electrophoresis are the criteria for the determination of apoptosis in tissues. These criteria are more reliable if performed contemporarily. TUNEL is a good marker provided that it is critically applied (Figure 2). Setting aside whether apoptosis is due to single or double-stranded DNA breaks and the choice between using a polymerase or nucleotidyl transferase (45-51), there is no doubt that TUNEL demonstrates DNA breaks not only of apoptosis, but also of necrosis, DNA duplication, gene transcription and even post-mortem autolysis. Since it may show variable staining intensities, a cut-off has been used for distinguishing a more intense staining, due to the DNA breaks occurring during apoptosis, from a less intense one due to DNA duplication (52). This technique can even identify single DNA strand breaks (53) or cells which survived apoptosis (54). The most important consideration for the reliability of TUNEL staining is that apoptosis or its initial stages can escape detection, because of the short duration of the process. This is the most important confutation of the statement that apoptosis is not or is only rarely found in neurodegenerative diseases. Under appropriate conditions, early stages of apoptosis can be detected by single strand DNA antibodies (55, 56). Quantitative analysis of apoptosis with laser scanning microscopy has been proposed to solve this problem (57, 25).

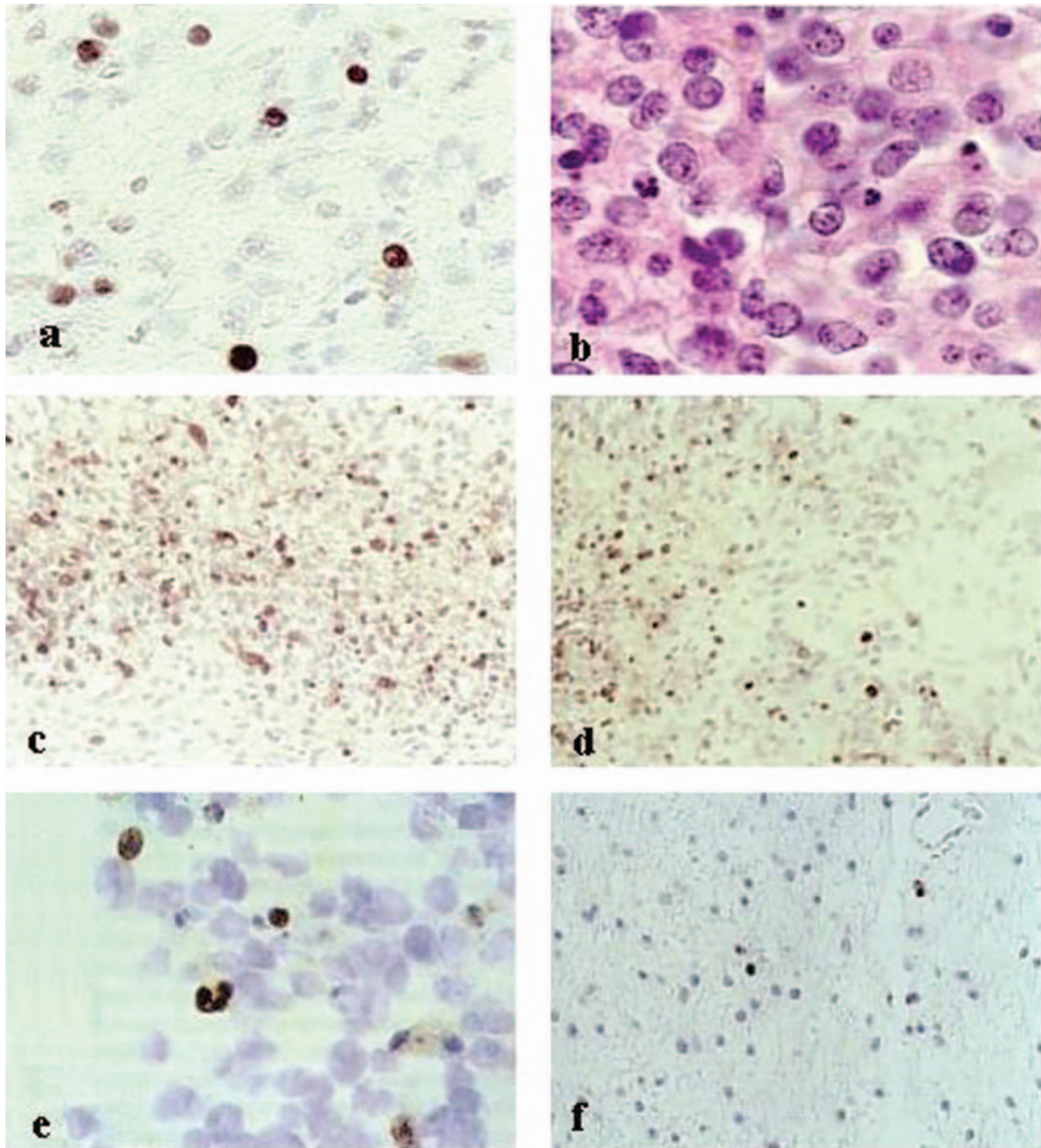


Figure 2. a) Apoptotic nuclei in a proliferating area of glioblastoma. TUNEL, x400; b) Apoptotic nuclei and bodies in the same area. H&E, x400; c) Apoptotic and irregular necrotic nuclei in a pseudo-palisade. TUNEL, x200; d) Apoptotic nuclei in the same area. TUNEL, x200; e) Apoptotic nuclei and bodies positive for caspase-3. DAB, x400; f) Apoptotic nucleus in an astrocytoma. TUNEL, x200.

Even though caspase-3 is only an indirect marker of apoptosis, it is indicative of the phenomenon because of its position as the last common step toward apoptosis, therefore serving as the "point of no return" on this route. Caspase-3 can be analyzed immunohistochemically using the relevant antibodies and its expression has been found to correlate with survival and apoptosis in tumors (58). Caspase-3 expression in gliomas, determined by

immunohistochemical methods, gave roughly the same distribution as TUNEL, but with quantitative and qualitative differences. The number of positive nuclei was lower than that determined with TUNEL and the staining was nuclear, cytoplasmic or both (59). Since it is a cytosolic protein, cytoplasmic staining should precede apoptosis and, after activating ICAD/DFF, this caspase translocates to the nucleus, as does CAD once released from ICAD (60, 61).

This may explain the possible double location of caspase-3 as determined using immunohistochemistry. In the nucleus it co-localizes with TUNEL (62), but with contrasting observations (63, 64). All things considered, it is likely that TUNEL is better at demonstrating apoptosis than caspase-3 immunohistochemical analysis (65).

Whether caspase-3 activation is really the "point of no return" toward apoptosis is of paramount importance, because all demonstrations that activation of pathways leading to apoptosis may be equivalent to apoptosis are based on the conjecture that caspase-3 activation is undoubtedly the last step. This does not seem to be the case, since it has been shown that further proteolytic caspase activity can be inhibited by IAP (66), c-IAP-1 and survivin which are up-regulated in bFGF-rescued 423 cells (67). There are other demonstrations that caspase-3 activation is not necessarily followed by cell death (67). As a consequence, the "point-of-no-return" in death-induced cells should be moved downstream of activated caspase-3 into the execution phase of apoptosis.

### Apoptosis in Brain Tumors

*Direct demonstration.* Apoptosis has been studied from three aspects in brain tumors. As opposed to cell proliferation, it could represent cell loss and indicate a better prognosis; its failure may be responsible for tumor development, and its induction can be instrumental to therapy. Apoptosis was demonstrated in brain tumors by our group eleven years ago (68). It was detected in medulloblastomas and identified by the (previously called) lymphocyte-like nuclei, recognized as the remnants of pathological mitoses, bearing denaturated DNA, from which no nucleus recovery was possible (69). It was then demonstrated in astrocytic gliomas (70-72) with an increasing frequency from astrocytomas to glioblastomas (73-81). Apoptosis was demonstrated to be related to shorter survivals (52), with some exceptions (77). The ratio of apoptosis/cell proliferation was found associated with patient survival and used for stratification for the evaluation of treatments (82).

In glioblastomas, apoptotic nuclei can be found both in the perinecrotic pseudo-palisades, around the central necrosis where necrotic nuclei are located, and in proliferating areas where they show a linear correlation with mitoses (83). The rationale would suggest that apoptotic nuclei of peri-necrotic pseudo-palisades are produced through the receptorial or extrinsic pathway, triggered by hypoxia, and those of the proliferating areas are produced by the transcriptional or intrinsic pathway, focused on *p53*. A working hypothesis was that circumscribed necroses develop from proliferating centers with high cell density because of the imbalance between the quick tumor cell proliferation and the low proliferation rate of endothelial cells (84). Both pathways, therefore, could be involved in

apoptosis in this location. Data from other studies were in both support and contradiction of this hypothesis. For example, Fas/APO.1 was expressed in cells around large necroses (85), not linked to *p53* status (86) and Ras-Akt interaction served as a switch from apoptosis to necrosis through TNF-pro-coagulation activity (87). Conversely, the association of apoptosis with duplicating cells of the proliferating areas could be in favor of an origin from the intrinsic pathway which is linked to the cell cycle; however, the role of hypoxia in this point, as the product of the imbalance between the proliferation rate of tumor and of endothelial cells, could not be excluded, even though only single cells seem to be affected.

The association of apoptotic nuclei with circumscribed necroses is in favor of their regressive significance and, therefore, of their favorable prognostic meaning, but this is contradicted by the ominous significance that circumscribed necroses bear in general in astrocytic gliomas. Importantly, apoptotic index (AI) does not predict the interval to recurrence in astrocytic gliomas (88).

In oligodendrogliomas, AI is generally higher than in astrocytomas and it increases with the degree of malignancy, correlating with topoisomerase II $\alpha$  (89), possibly serving as a prognostic factor after multivariate analysis (90). AI is also high in medulloblastoma, especially in nodules (91), with (92) or without correlation with shorter survival, as well as in ependymomas, central neuroblastomas, PNETs and metastases. AI correlates with cell proliferation, but not with a specific relevant factor (93). It may also appear in relation with therapies (94).

In meningiomas, as with other benign tumors, the AI is very low (95, 96) even though higher in malignant tumors (95). However, using antibodies to single stranded DNA, the frequency increases and the AI correlates with malignancy grade (97).

In conclusion, apoptosis increases with malignancy, because either the proliferation rate or necroses due to hypoxia increase, but it cannot be used for specific prognosis (98).

A role of apoptosis in tumor development has been suggested, but there are very few observations in favor of the hypothesis that this might be due to apoptotic failure as a consequence of *p53* inactivation (99). A higher AI was found in astrocytomas that did not transform than in those that did transform into anaplastic tumors after a second operation (5). In this regard, the occurrence of apoptosis and caspase-3 activation is observed in neuroepithelial precursor cells exposed to transplacental ethylnitrosourea, as well as in the development of more malignant tumors when *p53* is inactivated (100). In the same line of thought, tumor cells escaping spontaneous or induced apoptosis have broken DNA leading to an accumulation of mutations and, therefore, tumor progression. On the other hand, this is what occurs in cells which repair themselves after radio- or chemotherapy

(25). *Indirect demonstration.* Many observations focus on the activation of pro-apoptotic pathways or deactivation of anti-apoptotic pathways in brain tumors. This is exemplified by the demonstrations of increased expression of Bax and of the caspase cascade, as the executors of apoptosis and markers of favorable prognosis, as well as of Bcl-2 as a predictor of unfavorable prognosis (101). However, all indirect indices of apoptosis do not show the same efficacy as the direct indices, because all of the pathways leading to apoptosis, up until the point of no return, can be blocked or they may intersect with other contrary pathways. In spite of this, there are numerous observations that either support or refuse the use of indirect demonstrations of apoptosis in tissues both *in vivo* and *in vitro*. For example, in cell lines stimulated by macrophage extracts containing TNF $\alpha$  and IFN $\gamma$  the number of apoptotic nuclei and factors involved in the receptorial pathway, such as Fas/FasL, caspase-8 and Bax, increase, whereas the growth of the treated tumor transplants decrease (102, 103). There are also numerous demonstrations of the effects of the most important anti-apoptotic factor, Bcl-2. As a matter of fact, in many instances it is difficult to state whether a factor is pro- or anti-apoptotic, because of its involvement in more than one molecular pathways, and because of frequently contrasting results. No correlation of Bcl-2 with malignancy was found in gliomas in a series of studies (104-106) or with apoptosis in glioblastomas (107). On the other hand, Bcl-2 together with TP53, p21 and CD95 did not correlate with survival in glioblastomas (108), whereas other studies showed a correlation of Bcl-2 with survival in anaplastic astrocytomas, but not in glioblastomas (109). In low-grade gliomas, Bcl-2 and Bax were poorly expressed as determined using immunoblots and *vice-versa* using by immunohistochemistry. The opposite was found in high-grade gliomas, such that the two proteins seem to be regulated at different levels (109). In glioma cell lines, over-expression of Bcl-2 causes decrease of TRAIL-induced cleavage of caspase-8 and Bid and blockage of cleavage of caspase-9, -7 and -3 and of XIAP (110).

Great attention has been focused to the pro-apoptotic family, including Bax and Bak. Patients with gliomas that contain the N-terminal truncated form of Bax, Bax $\psi$ , which is a powerful inducer of apoptosis, have a longer survival (111). Moreover, Bax-deficient glioblastomas are resistant to apoptotic stimuli; Bax deficiency is counter-acted by Bak; Bak and Bax deficiency can impair the apoptotic program (112).

In malignant gliomas, an increase of calpain mRNA was observed together with caspases-3 and -9 and PARP-1 (113). In oligodendrogliomas, Bcl-2 was found to be increased with anaplasia (114), whereas it did not show any correlation with AI (115, 116) or Fas/FasL (117). In medulloblastomas, MDM2 amplification correlated with loss of caspases (118) and short survival, probably due to p53 inactivation (119). Apaf-1, the major intrinsic apoptosis activator, is of

particular interest since it is involved in the tumorigenesis of glioblastoma. It is located at chromosome 12q22-23 and it was found to be inactivated alternatively to the TP53 mutation in 70% of glioblastomas with 12q22-23 LOH (120).

APO2/TRAIL showed a distribution pattern similar to that of GFAP, with negative staining in tumor oligodendrocytes (121).

Recently it has been demonstrated that the pro-apoptotic protein ARTS, localized in the mitochondria, functions as a XIAP antagonist and activates caspase-3. It increases with malignancy in astrocytic tumors and parallels the apoptotic rate (122).

Very few observations are available in meningiomas. Caspase-3 increases with malignancy grade, proliferation index and apoptosis (123).

### Observations on *In Vitro* Cultures and Apoptosis in Therapies

It is very easy to induce apoptosis in cell cultures where the process can be followed and studied in detail. Proteins and genes involved in apoptotic regulation have been tested and the information obtained has contributed enormously to our knowledge of apoptosis in brain tumors and to the construction of its theoretical schemes. Unfortunately, the findings cannot be transferred directly to the *in vivo* observations, because of the different biological conditions between *in vitro* and *in vivo* studies. The micro-environment of the tumor mass cannot be reproduced and no nutrient gradient or necrosis model can be realized *in vitro*. Spheroid systems are the only type of culture available that allows to observe a central area of cell death with peripheral apoptotic nuclei useful for the study of the relationship between apoptosis, the development of necrosis and the role of the energy status in the cell (124). A large number of experiments have been performed (5) which contributed to a great part of our knowledge on apoptosis and demonstrated that the anti-tumor effects of radio- and chemotherapy are mostly mediated by apoptosis.

The second most important interest of *in vitro* studies on apoptosis is that apoptotic induction appears to parallel the expected response of tumors to therapies. Apoptosis can be induced by different stimuli including ligation of death receptors, cell stress, DNA damage and growth factor withdrawal. Many agents can be used to treat tumors such as nitric oxide, ceramide, cycloheximide, cisplatin, alkylating agents, proteasome inhibitors, interleukins (125-129, 26) and apoptosis can be reached by one of the multiple known pathways.

Many drugs showing anti-tumor properties are capable of inducing apoptosis *in vitro*. Drugs capable of producing this effect have been tested as chemotherapeutic agents. TRAIL/Apo2L, a member of the TNF family, demonstrated

selectivity against malignant tumor cells. It is known to interact with death receptors DR4 and DR5 and activate the caspase cascade through the extrinsic and intrinsic pathways. After pre-clinical experiments on its toxicity and efficacy, it was classified as a potential anti-tumor drug. TRAIL/CD95 does not cause toxicity in animals (130), even under the modulatory effect of EGFR (131), and it is inhibited by Bcl-2 over-expression (132). Important for a therapeutic application, hypoxia (133) and Resveratrol (134) each sensitize tumor cells to CD95L-induced cell death and apoptotic DNA endonuclease (DNase- $\gamma$ ) can be transferred into human glioma cell lines inducing apoptosis with DNA fragmentation (135). In glioblastoma cell lines, TRAIL was shown to trigger apoptosis along the extrinsic and the intrinsic pathways through DR5, caspase-8 and Bid. This was also possible in resistant cells, but only if they were pretreated with chemotherapeutic agents (136). Its direct or indirect activation of apoptosis in glioma cell lines is independent of DR5 and TP53 status. It down-regulates Akt by caspase-dependent cleavage and, conversely, inhibition of Akt enhances susceptibility to TRAIL and induces JNK activation that is not correlated with apoptotic induction (137).

In glioblastoma cultures and cell lines, the proteasome inhibitor PS-341, which has an anti-tumor activity due to its ability to inactivate NF $\kappa$ B, arrests the cell cycle in G2/M with an increase in the expression of p21, p27 and B1 and a reduction in the expression of CDK2, CDK4 and E2F4; 35-40% of the cells become apoptotic and, in addition, the 85kDa fragment of PARP-1 appears and Bcl-2 is decreased. Moreover, JNK/cJun signaling is activated, NF $\kappa$ B is reduced and TRAIL and TNF $\alpha$ -induced apoptosis is enhanced (138). Interestingly, if NF $\kappa$ B is blocked by sulfasalazine, an anti-inflammatory drug, apoptosis is induced in glioblastomas *in vivo* and *in vitro* (139). In TRAIL-resistant human malignant glioma cell lines, co-treatment with non-toxic doses of sodium butyrate and TRAIL produced a strong increase in TRAIL-induced apoptosis. This also happened in glioma cells over-expressing Bcl-2, but not in normal human astrocytes. This might be used clinically for targeting tumors and sparing normal tissue (140). In the same experiment it was observed that treatment with butyrate decreased survivin and XIAP protein levels.

There are many other examples (141), including apoptosis induced by suppression of Rac1, a small GTP-binding protein (142) or suppressed by activation of EGFR (143, 144) through activation of Akt (145). However, it has been suggested that inhibition of EGFR may protect cells from hypoxia through a "starvation signal" (146). Other important experiments include those involving PI3K/Akt/mTOR and PTEN (147, 148).

Another apoptosis inducer is Tamoxifen, a PKC inhibitor. In rat glioma cell lines it was shown to activate phospho-

specific JNK1 and caspase-3 (149). It is worth noting that dexamethazone antagonizes the apoptosis induction activity of Tamoxifen (150). Temozolomide has also been tested as an apoptosis inducer in glioblastoma cell lines, where cell death initiates with activation of calpain and is accomplished through an increase of caspase-3 (151).

Over-expression of caspase-9 coupled with radiation inhibited cell invasion and associated with apoptosis in human glioma cell lines (152), in line with the repeatedly reported involvement of caspases in radiation-induced apoptosis (153, 154). Even when radiation was given at a low-dose, cells showed hypersensitivity (155). Apoptosis was induced through activation of caspase-8, -9 and -3 in established glioma cell lines infected with a recombinant adenoviral vector encoding the human Bax gene under the control of VEGF promoter element, in combination with the anti-human DR5 monoclonal antibody (156). Injection of dendritic cells and adenovirus expressing IFN $\alpha$  into mouse glioma cell lines induced apoptosis and an anti-tumor response (157). Also, these results can be utilized in the development of treatments for human malignant gliomas. Fenretinide, a synthetic retinoid that induces apoptosis in tumor cells *in vitro* is now under evaluation in clinical trials as a chemotherapeutic agent against several malignancies. It has been tested on primary cultures of meningioma cells and induced apoptosis through PARP-1 cleavage, up-regulating DR5 and abolishing IGF-I-induced proliferation (158).

## Conclusion

Apoptosis represents the major cause of cell loss in gliomas and balances with cell proliferation to determine the speed of growth. This is a matter of debate in tumor such as glioblastoma where necrosis are an important phenomenon and necrosis associated genes have been identified using DNA microarray analysis (159). Apoptosis parallels tumor malignancy at the phenotypic level, even though it does not reach the dignity of a prognostic factor, except in specific conditions. In every day practice, it is difficult to use AI for the assessment of tumor malignancy grade, even though its increase has been associated with genetic changes of TP53 that mark adverse outcomes (160). The regulation of apoptosis is very complicated, but without a doubt it can serve as a target for the development of new therapies.

The great amount of information concerning the machinery of apoptotic regulation *in vitro*, is difficult to transfer to the clinic. Up to now, only p53 intervention/manipulation has been used in clinical trials (161). Finally, the importance of escape of tumor cells from apoptosis, including that induced by death-receptors (162) which is related to drug resistance, should be considered.

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