Survivin and gliomas: A literature review

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Abstract. Gliomas are the most common primary brain tumor, the diagnosis of which is challenging. In this respect, the use of immunohistochemical proliferation markers may aid diagnosis; survivin, also known as Baculoviral IAP Repeat Containing 5, is one such marker. Survivin is a unique member of the inhibitors of apoptosis protein gene family, and is known for its dual function as an apoptosis inhibitor and mitosis regulator. Furthermore, survivin has been demonstrated to be overexpressed in a number of malignancies. The purpose of the present literature review was to gain an overview of studies published on the diagnostic and/or prognostic use of survivin in gliomas. Using PubMed, 19 studies matching the inclusion criteria were ultimately included in the present review. The majority of the studies identified revealed that survivin was significantly associated with other proliferation markers, histological malignancy grade, and inversely associated with prognosis. However, there were a number of inconsistencies between studies, which suggests a requirement for standardization of immunohistochemical procedures.

Introduction

There is a multitude of histological types of brain tumors, which are classified according to the World Health Organization (WHO) (1). Gliomas are one such entity, and consist of astrocytomas, oligodendrogliomas, ependymomas and gangliogliomas. Overall, gliomas are the most common primary malignant intracerebral neoplasm with an incidence rate of 6.03 per 100,000 individuals every year (2). Classification and malignancy grading may be challenging, and there is constant investigation for improved methods to aid neuropathologists. In this respect, immunohistochemistry has received a lot of attention, and is a fundamental tool in the daily routine of the majority of pathology departments. Immunohistochemistry remains an important modality for glioma diagnosis, since it is a robust, convenient and affordable method, with which the majority of laboratories have considerable experience. Other relevant methods for diagnosing gliomas are molecular genetics, including fluorescence *in situ* hybridization and polymerase chain reaction. Indeed, all these techniques in combination are essential for classifying tumors in accordance with the newly published classification scheme of tumors of the central nervous system by the WHO (1).

Survivin, also known as Baculoviral IAP Repeat Containing 5, is a member of the inhibitors of apoptosis protein gene family. It is considered unique for its dual function as an apoptosis inhibitor and mitosis regulator (3,4). Survivin is generally only expressed during tissue development, and although it is observed in certain normal tissues with high proliferative activity, it is scarce in the majority of adult tissues (3). By contrast, survivin is overexpressed in numerous malignancies, including lung, pancreatic, breast, ovarian and colon cancer (3,5-8). Therefore, survivin has clinical potential; not only is it considered as an immunohistochemical diagnostic and prognostic marker, but it has also been identified as a potential target for therapy (3).

The aim of the present study is to provide a literature review on the use of survivin as an immunohistochemical marker in gliomas.

Materials and methods

Inclusion criteria. The present literature review was restricted to studies concerning human gliomas published in English during the last 10 years; therefore covering 2004-2014. Other inclusion criteria consisted of studies that focussed either on the prognostic and/or diagnostic value of survivin, and included a minimum of 15 patients.

Search terms. Studies were identified using the search engine PubMed (www.ncbi.nlm.nih.gov/pubmed). To identify relevant studies a meticulous set of search terms was constructed. The final search parameter included the terms 'survivin', 'gliomas', 'astrocytomas', 'oligodendrogliomas', 'ependymomas' and 'gangliogliomas'.

The full search code used is as follows and this yielded 202 studies: Survivin[All Fields] AND (("glioma"[MeSH

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Terms] OR "glioma"[All Fields] OR "gliomas"[All Fields]) OR ("astrocytoma"[MeSH Terms] OR "astrocytoma"[All Fields] OR "astrocytomas"[All Fields]) OR ("oligodendroglioma"[MeSH Terms] OR "oligodendroglioma"[All Fields] OR "oligodendrogliomas"[All Fields]) OR ("ependymoma"[MeSH Terms] OR "ependymoma"[All Fields] OR "ependymomas"[All Fields]) OR ("ganglioglioma"[MeSH Terms] OR "ganglioglioma"[All Fields] OR "gangliogliomas"[All Fields])) AND (("2004/01/01"[PDAT] : "2014/12/31"[PDAT]) AND English[lang]).

Exclusion criteria. Based on title and abstracts 183 studies were excluded, leaving 19 studies for further evaluation. Exclusion criteria were animal studies, meta-analyses, tumors that were not gliomas, such as medulloblastomas, and studies where the primary focus was the detection of survivin by means other than immunohistochemistry.

Results

Survivin expression. Table I presents a summary of the 19 studies included in the present review (9-27). The majority of studies observed nucleic and cytoplasmic immunoreactivity for survivin expression in gliomas; however, there were differences in which expression was used for analysis. Whereas certain studies included, and even separately considered, the two forms of expression (9-14), other studies focused solely on nuclear staining (15-21). Furthermore, certain studies did not explicitly state which form was assessed (22-27).

In general, the studies used one of three methods for quantifying survivin in gliomas: Labeling index (LI), staining intensity (SI) or immunoreactivity score (IRS). LI, defined as the percentage of immunoreactive tumor cells out of the total number of cells, was used by 10/19 (52.6%) studies (14-16,18-24). The majority of studies that used SI applied a four-tiered system as follows: 0, negative staining; 1, weak staining; 2, moderate staining; and 3, strong staining (10-14,17,25). Okada *et al* (26) and Yeung *et al* (27) used variations of this system, as detailed in Table I. Survivin IRS was determined by the multiplication of the percentage of survivin-positive cells (0, <1%; 1, 1-25%; 2, 26-50%; 3, 51-75%; 4, >75%) and the values for survivin SI (13,17,25). Saito *et al* (9) focused specifically on the localization of survivin as being cytoplasmic and/or nuclear. In addition, that study recorded the staining as positive or negative, defined as >5 and ≤5%, respectively.

Survivin and Ki67/MiB-1. In total, 10 studies (52.6%) investigated whether there was an association between Ki67/MiB-1 expression and survivin in gliomas. The results varied as certain studies identified a significant association (14,15,18-22), while others did not (14,24,27). Saito *et al* (9) investigated whether the subcellular localization of survivin was associated with Ki67/MiB-1 expression, and demonstrated that it was not. Liu *et al* (14) reported a significant and a non-significant association with Ki67/MiB-1 for nuclear-survivin and cytoplasmic-survivin, respectively.

Survivin and tumor grades. The expression of survivin across histological malignancy grades was evaluated by 12/19 (63.2%) studies. A total of 8 out of the 12 studies (66.7%) revealed that there was a greater survivin immunoreactivity with increasing tumor grade, and reported statistically significant differences in survivin expression across histological grades of gliomas (13,14,16,20-22,24,25). However, 4 studies (33.3%) identified no significant association between survivin and tumor grade (9,17,26,27).

Survivin and survival. Out of the 19 studies, 14 (73.7%) investigated the prognostic value of survivin. In total, 6 studies (42.9%) were unable to demonstrate that survivin was significant in survival analysis (15,18,19,21,25,26). By contrast, 8/14 studies (57.1%) reported that a high expression of survivin was inversely associated with prognosis (9,11,12,16,20,22-24).

Discussion

The purpose of the present literature review was to provide an overview of the diagnostic and prognostic findings of survivin as an immunohistochemical marker in gliomas. In the majority of studies reviewed by the present study, survivin was positively associated with other proliferation markers, including Ki67/MiB-1, and histological malignancy grade, and inversely associated with prognosis. However, the data was encumbered with several elements of uncertainty that illustrate the requirement for a standardization of immunohistochemical procedures.

Expression and quantification of survivin. Cellular immunoreactivity for survivin is observed in the cytoplasm and nuclei, and it is very likely these forms of expression are associated with the dual function of survivin as an apoptosis inhibitor and a mitosis regulator, respectively (9-11). It has also been suggested that the difference in the subcellular localization of survivin varies according to the antibody used (18). The different immunohistochemical antibodies and the working dilutions used by the respective studies are listed in Table I. Kogiku *et al* (22) and Uematsu *et al* (24) did not use a commercial antibody, but prepared their own.

Although the majority of studies mentioned the localization pattern of the antibody, certain studies do not state this explicitly. Furthermore, as shown in Table I, there were differences as to which form of expression was registered (cytoplasmic or nuclear), how the expression of the marker was quantified and how this was considered statistically. Three primary quantification methods were used: LI, SI and IRS. Additionally, Saito *et al* (9) simply registered the subcellular localization of survivin as cytoplasmic, nucleic or both.

Notably, even between studies using LI there were certain discrepancies, such as the minimum number of cells that were evaluated ranged between 500 and 1,000. In addition, there are differences in the percentage of immunoreactive cells defined by the studies as 'high' or 'low' expression of survivin. As an example, one study used </≥3% as a low/high expression, while another used a cut-off of ≤/>80%; these studies were conducted on astrocytomas grades II-IV (16,24). In numerous studies, the chosen cut-off appeared to be an arbitrary value.

The differences alluded to here, regarding expression and quantification, demonstrate the requirement of a standardization of procedures. The lack of specific guidelines allows for a variation of methods, which consequently renders it

Table I. Summary of studies included in the present literature review.

Author, date	Total patients, n	Histological types	Antibody	Quantification of expression	Results	(Ref.)
Saito et al, 2007	51	AA, 19; GBM, 32	Goat polyclonal anti-survivin (Santa Cruz Biotechnology, Inc., Dallas, TX, USA; dilution 1:100)	Nuclear and cytoplasmic staining were evaluated. ≥1,000 tumor cells were counted/sample in randomly selected fields (magnification, x400). +ve, >5% cells immunopositive; -ve, ≤5% immunopositive. +ve samples classified as follows: Nuclear +ve; cytoplasmic +ve; and nuclear/cytoplasmic +ve.	Immunoreactive cases, 51/51 (100%): Cytoplasmic, 23 cases (45%); nuclear, 10 cases (20%); both, 18 cases (35%). Localization survivin vs. Ki67/MiB-1 expression, P=0.6798; survivin nuclear +ve vs. cytoplasmic +ve, P=0.796; nuclear/cytoplasmic group survival time was shorter vs. nuclear or cytoplasmic alone (P=0.0001); survivin localization (nuclear/cytoplasmic vs. nuclear or cytoplasmic alone) associated with OS (P<0.001).	(9)
Xie et al, 2006	56	Primary GBM, 30; secondary GBM, 26	Rabbit polyclonal anti-survivin (catalog no., NB-500-201 K3; Novus Biologicals LLC, Littleton, CO, USA; dilution, 1:300; cytoplasmic detection); rabbit polyclonal anti-survivin (catalog no., sc-10811; Santa Cruz Biotechnology, Inc.; dilution, 1:400; nuclear detection)	Staining was evaluated semi-quantitatively. Staining score was obtained as the proportion of immunopositive cells: -, negative; +, staining in <25% cells; ++, staining in 25-50%; +++, staining in 51-75%; ++++, staining >75%.	Immunoreactive cases: Cytoplasmic, 37/56 cases (66%); nuclear, 43/56 cases (77%). Survivin-C expression in primary vs. secondary GBM, 83% vs. 46% (P<0.001); survivin-N expression primary vs. secondary GBM, 73% vs. 81% (P=0.51). Overall expression of cytoplasmic and nuclear survivin in 15 cases was concordant between precursor lesion vs. secondary GBM; expression level of survivin-C was higher in secondary GBM, no difference in survivin-N. Survivin-N vs. clinicopathological features, P>0.05; survivin-C vs. tumor size, P<0.01; survivin-C vs. other clinico-pathological features, P>0.05; mean progression time (months) between precursor lesion and secondary GBM was shorter in cytoplasmic +ve vs. -ve cases (P<0.05); survivin-C vs. AI, P<0.001; survivin-N vs. AI, P>0.05.	(10)
Shirai et al, 2009	66	GBM, 66	Anti-survivin antibody (Novus Biologicals LLC; dilution, 1:300)	Cell nuclei and cytoplasms of >500 cells evaluated. Nuclear and cytoplasmic survivin scores evaluated with cell positivity and SI: 0, no staining; 1, <50% of cell positivity and any intensity; 2, >50% of cell positivity and weak to moderate intensity; 3, >50% cell positivity and strong intensity.	Immunoreactive cases: Cytoplasmic, 58/66 (87.9%); nuclear, 47/66 (71.2%). Survivin-C score of 0, 8 patients (12.1%); 1, 47 patients (71.2%); 2, 6 patients (9.1%); 3, 5 patients (7.6%); survivin-C not associated with prognosis. Survivin-N score of 0,19 patients (28.8%); 1, 26 patients (39.4%); 2, 9 patients (13.6%); 3, 12 patients (18.2%); 3-year OS rate of survivin-N lower for score 3 vs. 2 (P=0.0003); survivin-N +ve predictor of OS (P=0.003; multivariate analysis).	(11)
Jung et al, 2012	62	GBM, 62	Anti-survivin (Lab Vision Corp, Fremont, CA, USA; catalog no., MS-1202; dilution, 1:25; microwave; cytoplasmic/nuclear detection)	SI score (0, -ve; 1, weak, but detectable; 2, moderate; 3, strong) and percentage of immunoreactive stains were automatically analyzed by Tissue Mine. H-score = Sum of percentages of positively stained cells x weighted staining intensity.	Immunoreactive cases: uncertain. Hierarchical clustering of the patients into two groups; no significant difference in clinical characteristics, but difference in survival rate. Survivin-N identified as 1/10 proteins whose expression was significantly different between the two groups (P=0.0295; Student's t-test).	(12)
Zhen et al, 2005	83	PA, 17; DA, 16; EPAII, 5; OAII, 4; AA, 11; OAIII, 4; EPA, 3; GBM, 12; MB, 9; NB, 2.	Goat polyclonal anti-survivin (Santa Cruz Biotechnology, Inc.; dilution, 1:100)	IRS = percentage of survivin +ve cells (0, <1%; 1, 1-25%; 2, 26-50%; 3, 51-75%; 4, >75%) x SI (0, no staining; 1, weak; 2, moderate; 3, strong).	Immunoreactive cases, 48/83 (57.8%); IRS in 83 cases, 3.75±3.89. IRS by WHO grade: Grade I, 1.29±2.62; grade II, 2.56±3.44; grade III, 4.78±3.89; grade IV 6.04±3.78. Survivin IRS grade I vs. grade III, P=0.023; grade II vs. grade IV, P=0.011; grade I vs. grade IV, P<0.001. Survivin IRS associated with PCNA PI (P<0.001). PCNA PI was higher in survivin +ve groups (P<0.001). AI expression in survivin +ve vs. -ve groups, P=0.108; survivin was inversely associated with AI (P<0.001). Overall daily growth significantly higher in survivin +ve vs. -ve group (P=0.001), with +ve association with survivin IRS (P<0.001).	(13)

Table I. Continued.

Author, date	Total patients, n	Histological types	Antibody	Quantification of expression	Results	(Ref.)
Liu et al 2006	102	DA, 19; AA, 16; GBM, 43; NBT, 24	Rabbit polyclonal anti-survivin (catalog no., BIRC5; R&D Systems, Inc., Minneapolis, MN, USA; dilution, 1:500)	Cytoplasmic and nuclear staining were scored separately. Survivin-C, 0-3 scoring used (combined intensity and extent of cells stained). Survivin-N LI: +ve cells in 1,000 tumor cells starting from the highest labeling region.	Immunoreactive cases: uncertain. Mean value survivin-C: DA, 0.7 ± 0.9; AA, 0.7 ± 0.6; GBM, 1.4 ± 0.9. Mean value survivin-N: DA, 0.6 ± 0.7; AA, 2.5 ± 2.5; GBM, 7.2 ± 6.4. Survivin-C: DA vs. AA, $P=0.0678$; DA vs. GBM, $P=0.0233$; AA vs. GBM, $P=0.0216$. Survivin-N: DA vs. AA, $P=0.0030$; DA vs. GBM, $P=0.0001$; AA vs. GBM, $P=0.0241$. Survivin-C and survivin-N vs. tumor grade, $P=0.028$ and $P<0.0001$, respectively. Survivin-C vs. Ki67/MiB-1, $P=0.0298$; survivin-N vs. Ki67/MiB-1, $P<0.0001$.	(14)
Habberstad et al, 2011	27	AA, 27	Rabbit monoclonal anti-survivin (clone, EP2880Y; Abcam, Cambridge, MA, USA; dilution, 1:250)	LI = percentage of immunoreactive cells/total cells. Determined by counting ≥1000 tumor cells or 3 HPFs.	Immunoreactive cases: 27/27 (100%). Survivin located in cytoplasm and nucleus; only nuclear positivity was recorded. Survivin associated with mitotic activity ($P=0.010$), Ki67/MiB-1 ($P<0.001$) and other markers [pHH3, mitosin and DNA topoisomerase IIα ($P<0.05$)]. Survivin not associated with survival ($P>0.05$).	(15)
Huang et al, 2011	91	DA, 25; AA, 17; GBM, 31; NBT, 18	Rabbit polyclonal anti-survivin (R&D Systems, Inc.; dilution, 1:600)	Survivin-N LI: Positively stained nuclei in 1,000 cells. Survivin-N characterized into two groups: <3% stained nuclei or $\geq3\%$ stained nuclei.	Immunoreactive cases: DA, 10/25 (40.0%); AA, 9/17 (52.9%); GBM, 25/31 (80.7%); NBT, 0/18 (0%). Survivin-N significantly higher in GBM (10.3 ± 5.3) and AA (3.1 ± 2.2) vs. DA (1.6 ± 2.3) ($P<0.001$). Survivin-N was a prognostic factor in disease-specific survival (log rank, $P<0.001$; Cox regression analysis, $P=0.023$) and in progression-free survival (log rank, $P<0.001$; Cox regression analysis, $P=0.032$).	(16)
Medina Villaamil et al, 2011	26	PA, 1; DA, 5; GBM, 5; GC, 11; RG, 6	Mouse monoclonal IgG$_{2a}$ survivin (clone, D-8; Santa Cruz Biotechnology, Inc.; dilution, 1:100)	IRS = percentage of +ve cells (0, <1%; 1, 1-25%; 2, 26-50%; 3, 51-75%; 4, >75%) x SI (1, weak; 2, moderate; 3, strong).	Immunoreactive cases: PA, 0/1 (0%); DA, 0/5 (0%); GBM, 1/5 (20%); GC, 2/11 (18%); RG, 0/6 (0%). No significant difference in expression of survivin among the tissue samples ($P>0.05$).	(17)
Mellai et al, 2008	20	GBM, 20	Rabbit polyclonal anti-survivin (catalog no., NB-500-201 K3; dilution, 1:500; Novus Biologicals LLC)	LI (each area) = percentage of positively stained cells in $\geq1,000$ cells.	Immunoreactive cases: 20/20 (100%). Survivin variably stained nuclei in viable areas; cytoplasmic staining inconstant and irregular, so nuclear staining considered for comparative evaluation. SI lower for survivin expression vs. Ki67. Positive association survivin vs. Ki67/MiB-1 ($P=0.0001$). No inverse association with AI ($P=0.1547$). No association with survival.	(18)
Preusser et al, 2005	104	GBM, 104	Rabbit polyclonal anti-survivin (clone, FL-142; catalog no., sc-10811; Santa Cruz Biotechnology, Inc.; dilution, 1:300)	Fraction of labeled tumor cell nuclei expressed as a percentage; 500 tumor cell nuclei were evaluated per specimen in fields with the highest density of immunopositive nuclei. Cut-off for survivin, $\leq/>14$.	Immunoreactive cases: 104/104 (100%). No survivin expression in NBT; faint cytoplasmic expression in some cells. Survivin index range, 2.4-44.0%. Association survivin index vs. Ki67/MiB-1 and DNA topoisomerase IIα ($P<0.0001$). Survivin did not associate with AI ($P=0.498$) or OS ($P=0.6368$). In survivin expressing cells, 91.08% co-expressed Ki67, and 58.85% of Ki67/MiB-1 expressing cells co-expressed survivin.	(19)
Preusser et al, 2005	63	EPAII, 44; EPAIII, 19	Polyclonal rabbit anti-survivin (clone, FL-142; catalog no., sc-10811; dilution, 1:300; Santa Cruz Biotechnology, Inc.)	Fraction of labeled tumor cell nuclei expressed as a percentage; 500 tumor cell nuclei evaluated in fields with highest density of immunopositive nuclei. Median cut-off value: Low ($\leq6.4\%$) and high	Immunoreactive cases: 63/63 (100%). Faint cytoplasmic expression in a few tumor cells. Expression: Overall, 0.6-43.2%; grade II, 0.6-35.8%; grade III, 2.8-43.2%. Mean survivin-expressing nuclei co-expressing with Ki67, 92.2%; mean Ki67-expressing tumor cell	(20)

Table I. Continued.

Author, date	Total patients, n	Histological types	Antibody	Quantification of expression	Results	(Ref.)
				(>6.4%) survivin expression.	nuclei co-expressing survivin, 62.9%. Ki67/MiB-1 index higher vs. survivin index ($P<0.001$). Increased survivin associated with grade III ($P=0.003$). Survivin index associated with Ki67/MiB-1 and DNA topoisomerase IIα ($P<0.001$). Survivin index associated with OS ($P=0.0032$; univariate analysis, but not independent prognostic factor in multivariate analysis).	
Ridley et al, 2008	65	EPAII/III, 65	Rabbit polyclonal anti-survivin (catalog no., sc-10811; Santa Cruz Biotechnology, Inc.; dilution, 1:1,000)	LI = percentage of immunopositive tumor cell nuclei exhibiting nuclear staining / total number cells evaluated. Tumors were categorized into low (≤1%), intermediate (2-4%) and high (≥5%) survivin expression levels.	Immunoreactive cases: 65/65 (100%). Mean LI, 1.1%; median LI, 0.5% (range, <1-6%). Low expression, 51 tumors (79%); intermediate expression, 11 (17%); high expression, 3 (4%). Survivin clearly associated with Ki67/MiB-1 ($P<0.001$). Survivin had no prognostic value (OS, $P=0.110$; EFS, $P=0.360$). Increased LI survivin associated with grade III tumors ($P<0.001$).	(21)
Kogiku et al, 2008	99	LGA, 18; AA, 34; GBM, 47	Produced own antibody; see Uematsu et al (17)	Survivin index = percentage immunostained cells per 200 cells in 5 fields of view. Low index, ≤50% cells stained; high index, >50% cells stained.	Immunoreactive cases: uncertain. Antiserum detected cytoplasmic and nuclear survivin. Survivin associated with prognosis ($P<0.0001$; univariate analysis). Median survival shorter for high vs. low index ($P<0.0001$). Survivin was a predictor of survival in high- (grade IV, $P=0.0207$) and low-grade (grades II and III, $P=0.0004$) glioma. Survivin remained significant in the multivariate analysis ($P<0.0269$). Survivin index associated with age ($P=0.0017$), KPS score ($P=0.0006$), tumor grade ($P=0.0002$) and Ki67/MiB-1 ($P=0.0002$). Survivin was not associated with EGFR ($P=0.0573$).	(22)
Rousseau et al, 2006	15	Gangliogliomas, 15	Polyclonal anti-survivin (Novus Biologicals LLC; dilution, 1:500)	Evaluated using LI. Proportion of immunoreactive cells for survivin was quantified as a percentage of the neoplastic glial component.	Immunoreactive cases: 6/15 (40%). Survivin detected only in glial cells. Relapsing lesions were all (5/5) immunopositive (2 originally not immunoreactive). No detectable survivin/<1% immunoreactivity in 11/14 tumors (all low-grade). Gangliogliomas (n=5), >5% immunopositive cells: 4, malignant; 1, relapsing low-grade. Immunopositivity for survivin increased with the Ki67/MiB-1 LI.	(23)
Uematsu et al, 2005	29	DA, 9; AA, 12; GBM, 8	Prepared own antiserum: Polyclonal anti-survivin (dilution, 1:250). Obtained from human neuroblastoma using RT-PCR with specific primers	Survivin index = percentage of immunostained cells per 200 cells in 5 fields per section. Low survivin, ≤80%; high survivin, >80%.	Immunoreactive cases: 29/29 (100%). Survivin undetectable around gliosis and in NBT. Mean % immunoreactive cells: DA, 70.0%; AA, 81.3%; GBM, 85.0%. Increase in survivin +ve cells inversely associated with OS time ($P=0.049$). Survival time shorter for patients with a high expression vs. low expression ($P=0.003$). No association between survivin and Ki67/MiB-1 ($P=0.065$). Difference between survival time in DA and AA patients with high vs. low survivin index ($P=0.007$). Survivin associated with survival ($P=0.036$; multivariate analysis).	(24)
Lin et al, 2012	154	PA, 23; DA, 21; AA, 48; GBM, 62	Goat polyclonal anti-survivin (clone, c-19; Santa Cruz Biotechnology, Inc.; diluted in 0.1M PBS, 1:200)	For each slide, 10 HPFs were randomly picked for quantification. Survivin IRS (0, -ve; 1-3, weak; 4-12, moderate-strong) was determined by multiplication of SI (0, no staining; 1, weak staining; 2, moderate staining; 3, strong staining) and percentage	Immunoreactive cases: uncertain. Survivin IRS: Non-neoplastic brain parenchyma, 0.15±0.03; PA, 0.54±0.11; DA, 0.66±0.08; AA, 2.56±0.17; GBM, 4.78±0.26. Median IRS, 3.02±0.48. Survivin expression increased with pathological grade. Survivin was not a significant predictor of survival ($P=0.089$; multivariate analysis).	(25)

Table I. Continued.

Author, date	Total patients, n	Histological types	Antibody	Quantification of expression	Results	(Ref.)
Okada et al, 2008	27	BSG, 15; NBSG, 12	Goat polyclonal anti-survivin (clone, c-19; Santa Cruz Biotechnology, Inc.; dilution, 1:100 in 1% BSA)	of +ve tumor cells (0, <1%; 1, 1-25%; 2, 26-50%; 3, 51-75%; 4, >75%). Survivin expression divided into two groups: 1, IRS ≥3; 2, IRS ≤2. +ve staining (+) = definite, but moderate, staining in the tumor. Strong +ve staining (2+), intense immunoreactivity.	Immunoreactive cases: BSG, 8/15 (53.3%); NBSG, 8/12 (66.6%). No association tumor grade vs. survivin expression.	(26)
Yeung et al, 2013	32	Pediatric EPA, 19; adult EPA, 13	Rabbit polyclonal anti-survivin (clone, FL-142, Santa Cruz Biotechnology, Inc.; dilution, 1:200)	Negative (0), no staining or staining equaling background intensity in NBT; moderate (1), definite staining above background intensity in tumor; strong (2), intense immunoreactivity.	Immunoreactive cases: Pediatric, 18/19 (95%); adult, 13/13 (100%). Pediatric results: WHO grade I, 3/19 cases had an SI of 1; grade II, 5/19 and 3/19 cases had SI of 1 and 2, respectively; grade III, 1/19, 3/19 and 4/19 cases had SI of 0, 1 and 2, respectively. Adult results: WHO grade I, 1/13 and 2/13 cases had SI of 1 and 2, respectively; grade II, 7/13 and 3/13 cases had SI of 1 and 2, respectively. No association survivin expression vs. age, gender, location or Ki67.	(27)

AA, anaplastic astrocytoma; AI, apoptotic index; BSG, brainstem glioma; DA, diffuse astrocytoma WHO grade II; EGFR, epidermal growth factor receptor; EPA, ependymomas; GBM, glioblastoma; GC, gliomatosis cerebri; HPF, high-power field; IRS, immunoreactivity scores; KPS, Karnofsky Performance Scale; LGA, low-grade astrocytoma; LI, labeling index; MB, medulloblastomas; NB, neuroblastomas; NBSG, non-brainstem glioma; NBT, normal brain tissue; OA, oligodendroglioma; OS, overall survival; PA, pilocytic astrocytoma; PCNA PI, proliferating cell nuclear antigen proliferation index; pHH3, mitosis-specific antibody anti-phosphohistone-H3; RG, reactive gliosis; RT-PCR, reverse transcription-polymerase chain reaction; SI, staining intensity; Survivin-C, cytoplasmic survivin; Survivin-N, nuclear survivin; WHO, World Health Organization; +ve, positive; -ve, negative.

challenging to compare different studies and interpret the results in a practical and translatable manner. That said, this is one of the well-known shortcomings of immunohistochemistry; the same antibody used by different laboratories may often lead to a variety of results, making it challenging to set any definite parameters. This can be paralleled to studies using with Ki67/MiB-1 (28,29).

Survivin and Ki67/MiB-1. Ki67/MiB-1 is a proliferation marker frequently used by pathologists to determine a tumor's proliferative activity. It is used in the diagnosis of various human malignancies, including breast cancer, neuroendocrine tumors and lymphomas. However, it is not officially recognized in the World Health Organization classification scheme of gliomas, since there is a considerable overlap in the proliferative indices of the marker between malignancy grades (28,30).

The studies reviewed by the present study report positive (14,15,18-22) and negative (9,24,27) findings regarding an association between Ki67/MiB-1 and survivin. The majority of studies (70%) suggest that there is a positive association (14,15,18-22). A few studies report co-expression of the two markers, and reveal that Ki67/MiB-1 stained a significantly larger fraction of tumor cell nuclei compared with survivin (14,18-20). This is understandable, since survivin is only expressed in the G2/M phase, whereas Ki67/MiB-1 is expressed in all phases of the cell cycle (20).

Uematsu *et al* (24) demonstrated that although the Ki67/MiB-1 index was significantly different between low-grade astrocytomas and glioblastomas, there was no difference in the LI between anaplastic astrocytomas and low-grade astrocytomas. By contrast, the survivin index was significantly different between anaplastic astrocytomas and low-grade astrocytomas, but not between glioblastomas and anaplastic astrocytomas. Based on these results the authors concluded that perhaps survivin may be a more sensitive marker compared with Ki67/MiB-1 in low-grade gliomas (24). However, Liu *et al* (14) reported that Ki67/MiB-1 LI and nuclear-survivin LI are significantly different between all grades of astrocytomas, and the differences observed with Ki67/MiB-1 are more significant compared with survivin. It is a point worth considering that Liu *et al* had 102 patients, while Uematsu *et al* had 29.

Survivin and tumor grades. Histological malignancy grade was considered in 12 studies, of which 8 reported that expression of survivin was associated with tumor grade (13,14,16,20-22,24), and 4 identified no significant association (9,17,26,27). Notably, all studies that report a significant association between survivin and grade use LI, except for Lin *et al* (25), who used IRS, and Liu *et al* (14), who used LI and SI. By contrast, none of the studies that reported negative findings used LI, but rather used variations of SI, IRS or simply a registration of the subcellular localization of immunoreactivity. Therefore, the findings indicate that survivin LI may indeed have some diagnostic potential. Future studies should take this into account as it demonstrates that objective measures of expression, including LI, are perhaps superior in a diagnostic setting. An important point is that, as with Ki67/MiB-1, survivin LI alone cannot and should not determine histological malignancy grade; the marker should always be used in combination with clinical information, which may aid pathologists in borderline cases (29).

Survivin and survival. The results regarding survivin and survival are ambiguous, and the previously mentioned heterogeneity of methods render it challenging to draw any clear conclusions. There is a slight majority of studies that report that a higher expression of survivin was significantly associated with poorer survival; however, these studies have various cut-off values for high and low expression of survivin (9,11,12,16,20,22-24). Therefore, it is not entirely straightforward to interpret the results at face value.

Concluding remarks. Survivin is an immunohistochemical marker with potential in tumor diagnostics and prognostics, as well as a prospective therapeutic target (3). However, the challenges encountered using survivin in gliomas are largely the same as those met when utilizing other immunohistochemical markers, including Ki67/MiB-1 (28,29). For this reason, it would be beneficial to standardize immunohistochemical analysis and counting procedures, and establish standardized cut-off values. Ultimately, the end goal is to identify markers that may be used in combination with clinical information to optimize therapy for each individual patient.

Acknowledgements

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