

Review Article

Mechanisms of early brain injury after subarachnoid hemorrhage

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Apoptosis is the term given to programmed cell death, which has been widely connected to a number of intracranial pathologies including stroke, Alzheimer's disease, and more recently subarachnoid hemorrhage (SAH). Subarachnoid hemorrhage is a disease, without any form of effective treatment, that affects mainly the young and middle aged and as a result is responsible for severe disability in otherwise healthy and productive individuals. Despite intense research efforts in the field, we currently possess a very limited understanding of the underlying mechanisms that result in injury after SAH. However, a number of studies have recently indicated that apoptosis may be a major player in the pathogenesis of secondary brain injury after SAH. As a result, the apoptotic cascades present a number of potential therapeutic opportunities that may ameliorate secondary brain injury after SAH. Experimental data suggest that these cascades occur very early after the initial insult and may be related directly to physiologic sequela commonly associated with SAH. It is imperative, therefore, to obtain a thorough understanding of the early events that occur after SAH, which will enable future therapies to be developed.

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Introduction

Spontaneous subarachnoid hemorrhage (SAH) is an important cause of premature death and disability worldwide. Even though SAH accounts for only 5% of all strokes, it has been estimated in autopsy studies that up to 6% of the population may harbor an intracranial aneurysm (McCormick and Nofzinger, 1965) and that each year approximately 10/100,000 people suffer from an aneurysmal SAH (Schievink *et al*, 2004). Subarachnoid hemorrhage, therefore, is a devastating disease carrying with it a mortality of 12% before receiving medical attention (Schievink, 1997). With a further 40% dying within a month of admission to hospital and a 30% morbidity in the survivors, the severity of this disease cannot be underestimated (Sekhar *et al*, 1988; Grosset *et al*, 1992; Sobey and Faraci, 1998; Kaptain *et al*, 2000).

Despite major advances in surgical techniques, radiology, and anesthesiology, the mortality and morbidity rates after spontaneous SAH have not

changed in recent years (Schievink *et al*, 2004). In the past, research has concentrated primarily on vasospasm and its sequela, in an attempt to combat the high morbidity and mortality associated with SAH. To date, this has not resulted in a definitive treatment modality to prevent or ameliorate brain injury after SAH. Furthermore, it should be pointed out that early brain injury (EBI) is the primary cause of mortality in SAH patients (Broderick *et al*, 1994; Bederson *et al*, 1995). Therefore, it seems that EBI should be considered as a primary target for future research.

In this review, we intend to concentrate on EBI and the immediate pathophysiological events that occur after an SAH. We also intend to discuss the role of apoptosis in relation to SAH and the effects that these mechanisms have on the brain in the first 72 h after an SAH. We will also discuss the central role that apoptosis plays in relation to secondary brain injury in SAH. As a whole, apoptosis has been well described in the past and there are many fine reviews available (Vaux and Strasser, 1996; Cohen, 1997; Hetts, 1998; Thornberry and Lazebnik, 1998; Earnshaw *et al*, 1999; Reed, 2000; Strasser *et al*, 2000; Zamzami and Kroemer, 2001; Love, 2003). For this review, however, emphasis will be put on the apoptotic cascades as they relate to SAH. For an overall view of the schemata, see Figure 1.

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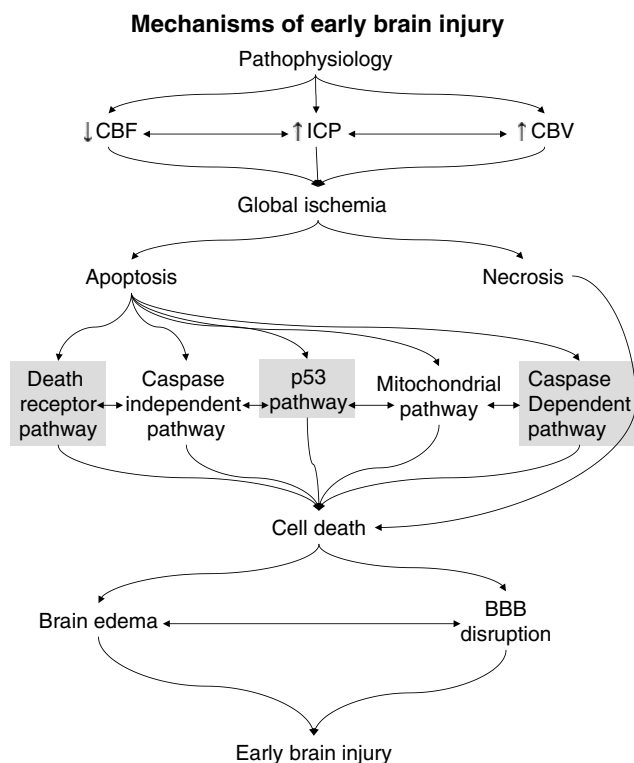


Figure 1 The overall scheme from subarachnoid hemorrhage to EBI. The apoptotic cascades highlighted are those that are known to be involved in SAH-induced apoptosis (Gules *et al*, 2003; Park *et al*, 2004; Zhou *et al*, 2005).

Early Brain Injury

To date, the majority of research performed worldwide has focused on vasospasm and has resulted in a cornucopia of theories (Sako *et al*, 1993; Cook, 1995; Butler WE, 1996; Macdonald *et al*, 1997; Barbosa *et al*, 2001; Zubkov *et al*, 2001; Macomson *et al*, 2002; Svirni *et al*, 2003; Hansen-Schwartz *et al*, 2003; Borel *et al*, 2003; Sasaki *et al*, 2004; Macdonald *et al*, 2004; Zhou *et al*, 2005; Badjatia *et al*, 2005; Kessler *et al*, 2005). Although some are more widely accepted than others, the full mechanisms of action have yet to be elucidated. One area that has evolved as a result of this research is the area of EBI. The term EBI has only recently been coined and refers to the immediate injury to the brain as a whole, within the first 72 h of the ictus, secondary to a SAH (Kusaka *et al*, 2004). Therefore, EBI refers to the events that occur in the brain before the development of vasospasm, although it can be suggested that the etiology of vasospasm may be linked to that of EBI, because they certainly share many of the same characteristics. The timing of this injury is immediate, although the sequela can be seen in the long term. There is currently substantial interest in EBI and the mechanisms of injury are only just being clarified (Doczi *et al*, 1986; Glenn *et al*, 2002; Gules *et al*, 2003; Kusaka *et al*, 2004;

Park *et al*, 2004; Claassen *et al*, 2004; Zhou *et al*, 2005). Therefore, EBI has great potential for the provision of treatment modalities, immediate and prophylactic, for patients with SAH, which may attenuate some of the devastating secondary injuries that SAH patients are prone to.

Pathophysiological Mechanisms of Early Brain Injury

The etiology of EBI lies with the initial bleed and the complex pathophysiological mechanisms that occur as a result, which predisposes the brain to secondary injury. Although these pathophysiological principles have only been shown in various animal models, they can be closely linked to clinical findings in humans; however, they have not been well studied to date (Claassen *et al*, 2004). Some evidence from patients with intracranial cerebral pressure (ICP) monitors already *in situ* at the time of a SAH has emerged, which follows the parameters found in experimental models (Nornes, 1978). At the moment of a SAH, blood is extravasated from a defect in an aneurysm, which under pressure leaks into the subarachnoid space (the bigger the defect, the bigger the blood volume) (Sobey, 2001). It has been shown both in clinical and experimental studies that there is an acute rise in the ICP, the extent of which reflects the severity of the bleed (Voldby and Enevoldsen, 1982) and a resultant decrease in the cerebral perfusion pressure (Fisher, 1975).

The precise mechanism behind this rise in ICP remains unknown, although the increase in volume secondary to the hemorrhage (Monroe–Kellie Hypothesis), vasoparalysis, and CSF drainage obstruction have been implicated (Grote and Hassler, 1988). In combination with the increase in ICP, cerebral blood flow (CBF) decreases (Bederson *et al*, 1995). The decrease in CBF and concomitant increase in ICP may also be a protective mechanism in an attempt to control blood loss from the aneurysm. Cerebral blood flow can decrease to almost zero in experimental models (Ostrowski *et al*, 2005). This is reflected by syncope in the clinical setting. Blood pressure also decreases, which again may be a protective mechanism to reduce blood loss, although this can be variable and clinically, by the time patients reach the hospital they tend to be hypertensive in an attempt to maintain cerebral perfusion pressure. Finally, the cerebral blood volume increases, perhaps as a result of vasodilatation.

Ultimately, these pathophysiological factors increase the ICP, which cannot be compensated for once it reaches critical levels. It is a combination of these factors that results in a global ischemic injury. This ischemic injury as a result of SAH can be seen in patients post mortem (Bederson *et al*, 1995) and is

seen as a leading cause of morbidity in patients with SAH (Frykholm *et al*, 2004). Although, the severity of the SAH is different from patient to patient, they all to a greater or lesser degree experience these changes in intracranial pathophysiology. It has been well established that patients with a World Federation of Neurological Surgeons (WFNS) defined grade one SAH, who do not exhibit clinical or radiologic vasospasm and who do not suffer any obvious complications with regard to surgery or postoperative course, still show long-term psychosocial difficulties (Hutter *et al*, 1999; Kreiter *et al*, 2002; Claassen *et al*, 2004). It has also been estimated that among the survivors of SAH, up to 50% experience some level of cognitive dysfunction in the long term (Bonita and Thomson, 1985; Kreiter *et al*, 2002). These injuries are not minor and yet are not given the same emphasis as more immediate and obvious disabilities. Up to 50% of SAH survivors never return to their previous employment, which further indicates the often underdiagnosed injuries sustained at the time of the initial bleed (Hutter *et al*, 1999; Kreiter *et al*, 2002).

We believe that these subtle changes in behavior, memory, etc. are the result of EBI and represent long-term complications that cannot be explained by vasospasm alone. As a result of the global ischemic injury, secondary to raised ICP and decreased CBF as outlined above, apoptosis has been shown to be widespread in the brain after SAH. This has been shown in both animal models and in patients post mortem (Zubkov *et al*, 2000a, 2001; Park *et al*, 2004; Ostrowski *et al*, 2005; Zhou *et al*, 2005). Thus, it seems clear that an understanding of the apoptotic cascades in relation to SAH is vital to understand and treat SAH patients in the future.

Apoptosis in Early Brain Injury

Apoptosis was first described by Kerr *et al* (1972) and is essentially the term given to programmed cell death. It was first shown by Horvitz *et al* (1994) in the development of *Caenorhabditis elegans*. Since then, it has been shown to occur in many physiologic and pathologic states. To date, apoptosis has been extensively studied in stroke models and has been shown to be the dominant form of cell death in the penumbra (Li *et al*, 1997; Watanabe *et al*, 1999; Love, 2003). It has also been hypothesized that areas of tissue adjacent to dead or dying cells undergo apoptosis in an attempt to prevent overwhelming necrosis and inflammation. Although a core and penumbra cannot be identified *per se* in the SAH model, the brain as a whole is affected by global ischemia as discussed previously.

Following the global ischemia seen with SAH, apoptosis has been shown to occur in the hippocampus, blood-brain barrier (BBB), and vasculature with varying degrees of necrosis (Zubkov *et al*, 2001; Park *et al*, 2004; Ostrowski *et al*, 2005; Zhou *et al*,

2005). Apoptosis has been implicated in the development of vasospasm and smooth muscle cell proliferation in spastic arteries (Zubkov *et al*, 2000a). Apoptosis has even been demonstrated in aneurysms and has been implicated in aneurysmal formation and rupture both in humans and in animal models (Kondo *et al*, 1998; Hara *et al*, 1998). However, when the injury is global, the degree of apoptosis can be more devastating than the injury itself. There are a number of apoptotic pathways that are believed to play a role in SAH: the death receptor pathway, caspase-dependent and -independent pathways, as well as the mitochondrial pathway (Figure 2).

There are many similarities with regard to apoptosis and the many disease processes in which it is involved. To date, apoptosis has been studied extensively in stroke and to a limited degree in SAH. Therefore, the question remains as to what are the differences, if any, between the apoptotic cascades and the pathology. It is known that the initiating event whether it be stroke, SAH, neurotoxins, etc. can influence which apoptotic cascade will become dominant in that disease process, as can the type of

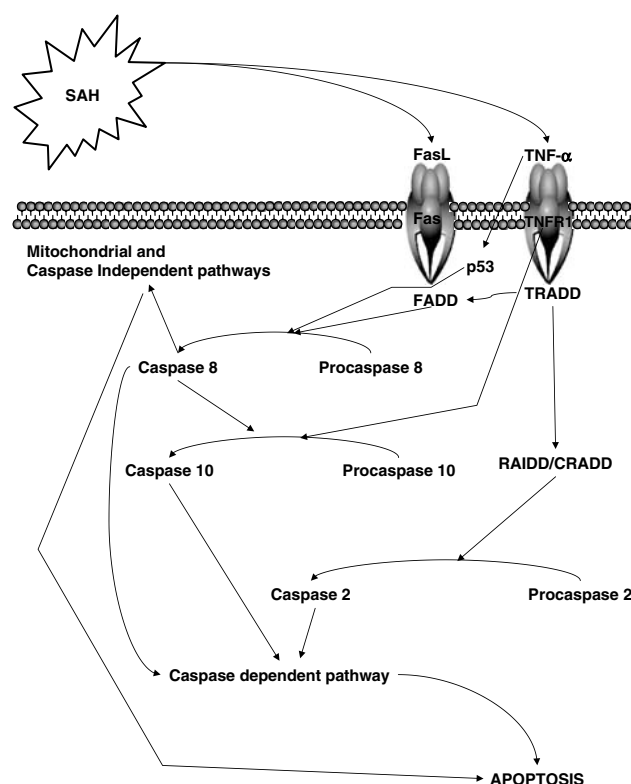


Figure 2 The overall apoptotic cascade in relation to SAH. Subarachnoid hemorrhage is considered to be an external stress event, which by a mechanism yet to be determined affects the death receptors. These in turn stabilize p53 and cleave procaspase 8, leading to apoptosis via the mitochondrial and caspase-independent pathways. Furthermore, the death ligands in conjunction with caspase 8 are also capable of triggering the caspase-dependent pathway leading to apoptosis.

cell that is influenced by the event. For example, it has been shown that the caspase-dependent cascade may be particularly important in relation to ischemia, whereas the caspase-independent cascade relates more to neurotoxin-induced apoptosis (Dawson and Dawson, 2004). With regard to SAH, to date, the death receptor/p53 pathway has been described as being particularly important (Zhou *et al*, 2005). Of course, once initiated, all of the cascades come into play as the relationships between these proteins is extensive and intricately interwoven.

The Death Receptor Pathway in Subarachnoid Hemorrhage

Subarachnoid hemorrhage can be considered to be an external stress event (Matz *et al*, 2000), which by a mechanism yet to be understood can, possibly through changes in the environment or physical structure of cells, lead to cellular suicide (Kidd, 1998). It has been shown in ischemic and hemorrhagic models that the injury if severe enough can result in DNA fragmentation (Matz *et al*, 2000, 2001; Matsushita *et al*, 2000). In the case of SAH, the cell membrane death receptors, for example, Fas, TNFR1, and DR3–5, are believed to be responsible for the translation of the signal across the cell membrane by activating the tumor necrosis factor receptor (TNFR) family, which appears to be the primary target in relation to SAH (Zhou *et al*, 2004, 2005). The members of the TNFR family are extensive and include tumor necrosis factor- α (TNF- α), TNF-related apoptosis-inducing ligand, and Fas ligand (Zheng *et al*, 1995; Ju *et al*, 1995; Kidd, 1998). We have shown in previous experimental investigations that TNF- α is upregulated after SAH (Zhou *et al*, 2004). It is these cytokines that probably through either an endocrine or paracrine mechanism provide the link between the external stress event and the internal apoptotic cascades. A second theory has also been suggested, which is that of an immune response acting through hematogenous cells resulting in activation of the death receptors (Jiang and Wang, 2004). However, further work is required in this area to determine which if any of these is correct.

All of the TNFR family members contain an intracellular death domain, which permits the transduction of the signal to the intracellular environment. In relation to the transduction of the SAH signal, the two cytokines identified as being the most influential are TNF- α and Fas ligand. Tumor necrosis factor- α activates TNFR1, whereas Fas ligand activates Fas. Tumor necrosis factor- α has been shown to induce p53 in U937 cells and has been identified as a significant downstream mediator of cell apoptosis (Yeung and Lau, 1998). Although experimental evidence exists showing the upregulation of TNF- α and p53 stabilization in SAH (Zhou *et al*, 2005), there is a paucity of

information with regard to events occurring after this. We know from ischemic models that the death receptors are also capable of independently recruiting and activating caspases (Earnshaw *et al*, 1999). Fas ligand binds to its adapter molecule FADD, which cleaves procaspase 8 to caspase 8 (Muzio *et al*, 1998). TNFR1 binds to TRADD, which in turn binds to FADD resulting in caspase 8 cleavage as well. TNFR1 is also independently capable of cleaving procaspase 10, resulting in the formation of caspase 10 (Vincenz and Dixit, 1997). Finally, ligated TNFR1 can also bind to RIP and TRADD, which recruits RAIDD and CRADD, which influences procaspase 2 (Bergeron *et al*, 1998). Caspase 8 in turn activates Bid, which translocates to the mitochondria inducing cytochrome *c* release (Li *et al*, 1998). Additional experimental studies examining the effects of pancaspase inhibitors have shown a favorable outcome with regard to SAH, suggesting that p53 may work through either the caspase-dependent or mitochondrial pathways in SAH-induced apoptosis (Park *et al*, 2004; Zhou *et al*, 2004). For an excellent review of apoptosis and the death receptors, see Earnshaw *et al* (1999).

p53—‘Guardian of the Genome’

p53 is a nuclear transcription factor that has been exhaustively studied over the past number of decades, particularly with regard to its role in tumorigenesis. Referred to as the ‘guardian of the genome’ (Lane, 1992) and designated ‘molecule of the year’, for the year 1998 (Mowat, 1998), it contains an amino-terminal transactivation domain, a carboxy-terminal oligomerization domain, and a central DNA binding domain (Sheikh and Fornace, 2000; for review, see Prives and Hall, 1999). p53-mediated apoptosis has been identified in a host of intracranial pathologies, including ischemia, Alzheimer’s, Parkinson’s, and SAH (Daily *et al*, 1999; Mattson, 2000; Leker *et al*, 2004). p53 genes are responsible for the expression of a host of proteins, which can be transcription dependent or independent, depending on the cell type. We will be dealing with those proteins specifically known to influence the apoptotic cascades with regard to SAH (Sheikh and Fornace, 2000; Gules *et al*, 2003; Love, 2003; Leker *et al*, 2004; Hammond and Giaccia, 2005; Zhou *et al*, 2005).

One of the largest target groups of p53 is the BCL-2 family, named after B-cell lymphoma, which contains a multitude of pro- and antiapoptotic genes (for an excellent review, see Antonsson and Martinou, 2000). Some of these include Bax, Bid, Bik, Bak, Bcl-Xs, and Bim (proapoptotic) and Bcl-2, Bcl-X_L, and Mcl-1 (antiapoptotic). However, it has been shown that it is the overall ratio of pro- to antiapoptotic signals that finally determines whether or not the cell dies (Philchenkov, 2004). Therefore, one can hypothesize that it may be dependent on the

strength of the signal or extent of the injury. That said it is not known how exactly the Bcl-2 family influences apoptosis. One of the most widely accepted theories suggests it is the ability of Bcl-2 to inhibit caspases by binding to Apaf-1 and preventing cytochrome *c* release, thus preventing apoptosis (Reed, 1997; Hetts, 1998).

p53 is transported from the nucleus to the cytosol of the cell where it remains at very low concentrations, because it is unstable and quickly degraded, largely owing to murine double minute two (MDM2) or, in humans, HDM2 and CREB binding protein transcriptional cofactor/p300 (CBP/p300) (O'Brate and Giannakakou, 2003). The export of p53 from the nucleus is tightly regulated (Ryan *et al*, 2001) and occurs when MDM2 ubiquitinates p53 in the nucleus, which allows for its exportation from the cell (Stommel *et al*, 1999). Once in the cytosol, under normal conditions it is quickly degraded. However, the phosphorylation of p53 allows for its stabilization and accumulation within the nucleus, which can occur secondary to many stress events, including SAH (Giaccia and Kastan, 1998). N-terminal phosphorylation occurs at serines 15 and 20 (Shieh *et al*, 1997; Unger *et al*, 1999), which prevents MDM2 binding and subsequent export, thereby increasing nuclear concentration and increasing translational capacity (O'Brate and Giannakakou, 2003). Interestingly, the phosphorylation at these two sites typically causes cell cycle arrest and DNA repair, whereas severe DNA damage leads to phosphorylation of serine 46 in addition to serines 15 and 20, which causes apoptosis and cell death (Nakamura, 2004). Again, whether this phenomenon is a result of the strength of the signal or not remains unknown. It has been suggested that the accumulation of p53 in the nucleus leads to the translocation of p53 into the cytoplasm (Sansome *et al*, 2001; Chipuk *et al*, 2004; Erster *et al*, 2004), where it acts either directly or indirectly on the mitochondria resulting in cytochrome *c* release. The methods by which p53 translocates to the mitochondria is unknown; however, a Grp75-dependent mechanism has been suggested (Mihara *et al*, 2003). This laboratory has previously shown the presence of p53 in the cytoplasm in a canine double injection model of SAH (Zhou *et al*, 2005). With regard to SAH, we suspect that cytoplasmic p53 causes apoptosis by acting either directly on the mitochondria or through an intermediate such as Bax. The reason for this lies with the fact that SAH *per se* does not cause DNA damage, and therefore we speculate that apoptosis secondary to stress is orchestrated by cytosolic p53.

Recent work examining the role of p53 in hypoxic conditions has shown that p53 is stabilized in response to hypoxia (Hammond and Giaccia, 2005). It should be pointed out however that there is some dispute regarding this (Goda *et al*, 2003). It seems that MDM2 protein levels decrease in response to hypoxia, which of course leads to an

accumulation of p53 in the nucleus (Koumenis *et al*, 2001). It is likely that the dispute lies with the degree of hypoxia experienced, with many authors agreeing that severe hypoxia is required to stabilize p53 (Schmid *et al*, 2004). This degree of hypoxia may be experienced transiently in SAH perhaps at the time of global ischemia, which may be enough to stabilize p53 (see above). However, the stabilization of p53 is further emphasized by the fact that the apoptotic signal is reinforced by hypoxia-inducible factor-1 (HIF-1), which is upregulated as a result of the global ischemic injury (Ostrowski *et al*, 2005).

Certainly it seems likely that HIF-1 is involved either directly, as a result of ischemia, or as a result of the SAH itself. Hypoxia-inducible factor-1 has been shown to rise acutely with respect to SAH, ischemia, and intracerebral hemorrhage (Bergeron *et al*, 2000; Ostrowski *et al*, 2005). Hypoxia-inducible factor-1 is known to upregulate the proapoptotic genes BNIP3 (Schmid *et al*, 2004) as well as vascular endothelial growth factor. Hypoxia-inducible factor-1 is also responsible for the progression of many different cascades including that of apoptosis, possibly acting through p53 to upregulate the mitochondrial apoptotic pathway; therefore, it has been theorized that p53 stabilization in ischemic conditions is HIF-1 dependent (An *et al*, 1998). That said, there is currently considerable confusion in the literature concerning the link between p53 and HIF-1 (Wenger *et al*, 1998). There is mounting evidence that under certain hypoxic conditions HIF-1 is able to stabilize p53 (Hammond *et al*, 2002). The exact mechanism of this interaction is unknown; however, recently, the discovery of the Nucleophosmin gene (NPM), which has been found to interact with both HIF-1 and p53 (Li *et al*, 2004), may explain many of the confounding opinions currently in the literature regarding HIF-1 and p53 interaction and their role in apoptosis. Unfortunately, more work is required in this area to fully elucidate the relationship. This laboratory has shown that both p53 and HIF-1 are upregulated after SAH (Ostrowski *et al*, 2005; Zhou *et al*, 2005). To date, however, the interplay between the two, if any, has not been demonstrated.

Currently, it is difficult to foresee an animal model capable of testing many of these theories in relation to the strength of the signal or the severity of the injury. The monofilament puncture model does not allow the operator to consistently cause the same degree of injury and the injection model is not representative of a true SAH. Although attempts to score severity have been made, they have so far failed to be consistent. In the light of these facts, it is difficult to examine the link between severity and apoptosis, at least at the moment.

Apoptosis and the Mitochondria

As mentioned above, p53 activates the mitochondrial pathway through its actions on the Bcl-2

family. The Bcl-2 family are divided into pro- and anti-death members (Gross *et al*, 1999); examples of the pro-death proteins include Bax, Bid, Puma, Bim, Noxa, and Bak (Jiang and Wang, 2004; for review, see Adams and Cory, 1998). Of these, it is difficult to know which are important for SAH. It is known that Puma, Noxa, Bid, and Bax (Reed, 2000; van Loo *et al*, 2002) are upregulated by p53 after DNA damage.

It is also known that in neuronal cell death it is the upregulation of Bax that initiates the apoptotic cascade (Cregan *et al*, 1999). In fact, it has been shown that Bax is required for p53-induced caspase 3 activation in neuronal cell death (Cregan *et al*, 1999). Similar findings were observed in ischemic murine models subjected to focal ischemia (Benchoua *et al*, 2001). As a whole, the Bcl-2 family can either stimulate or inhibit cytochrome *c* release from the mitochondria depending on the dominant signal, that is, pro- or antiapoptotic dominance (Philchenkov, 2004). It is important to note that apoptosis is not an all or none mechanism (Vaux and Strasser, 1996). In fact, in situations of sublethal injury, an apoptotic cell can recover and necrotic cells have been shown to possess the ability to switch to apoptosis in certain conditions (Vaux and Strasser, 1996). In addition, p53 cleaves procaspase 8 to form caspase 8, which in turn cleaves Bid to form truncated Bid. Truncated Bid then permits the release of cytochrome *c* from the mitochondria, which is further regulated by Bcl-2 and Bcl-x_L (van Loo *et al*, 2002).

Cytochrome *c* is a transcription protein located in the mitochondrial intermembranous space. During apoptosis, this membrane becomes permeable to cytochrome *c*, possibly through pore formation or membrane destruction (Vander Heiden *et al*, 1997, 1999; for review, see Zamzami and Kroemer, 2001). As a result, cytochrome *c* is released into the cytosol where it binds to Apaf-1 (Yakovlev *et al*, 2004). The cytochrome *c*/Apaf-1 complex referred to as the apoptosome then recruits and cleaves procaspase 9, which activates the downstream caspase cascade (Nijhawan *et al*, 2000) (see Figure 3). The critical step in this process is that of cytochrome *c* release, which is mediated by the Bcl-2 family, which in turn is controlled by p53 (Yakovlev *et al*, 2004). Caspase 9 is a prerequisite for the cleavage of procaspase 3 to caspase 3, which is known to be involved in p53-mediated apoptosis (Cregan *et al*, 1999). Interestingly, the intrinsic pathway (mitochondrial pathway) is energy dependent and probably only occurs in areas where ATP is available, for example the penumbra (Benchoua *et al*, 2001). In areas where energy is not available, that is, ATP-depleted areas, the extrinsic pathway, that is, caspase 8, which is capable of self-cleavage, with direct activation of caspase 3, occurs. Therefore, in SAH brains, either of these cascades can occur depending on the severity of the insult and the area of the brain being examined. For example, the hippocampal cells are far more prone to injury than other areas because of

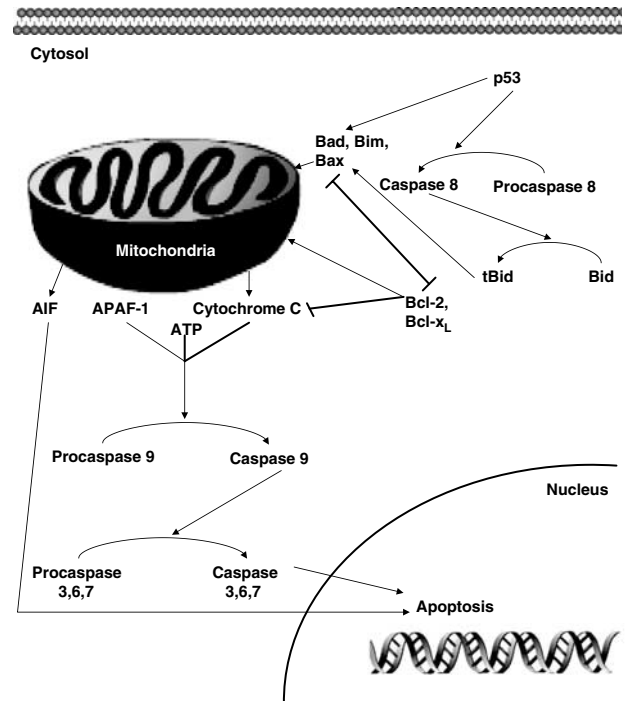


Figure 3 The mitochondrial and caspase-independent pathways. p53 cleaves procaspase 8 to form caspase 8, which cleaves Bid to form truncated Bid. Bax is required for p53 stimulation of apoptosis and truncated Bid is instrumental in the release of cytochrome *c*, which is under the control of the Bcl-2 family of proteins. Depending on the overall concentration of pro- versus antiapoptotic members of the Bcl-2 family, cytochrome *c* is released. Cytochrome *c* along with Apaf-1 and ATP cleaves the apoptosome, resulting in the activation of the caspase cascade. In addition, the caspase-independent cascade is represented here, as AIF can directly induce apoptosis. Other proteins in the independent cascade such as SMAC/DIABLO and endonuclease G are not represented.

their sensitivity to ischemia and ATP requirements (Park *et al*, 2004). Similarly, it has also been shown that cells undergoing either necrosis or apoptosis are capable of switching depending on the energy reserves available (Leist *et al*, 1997; Eguchi *et al*, 1997). It has been shown that the translocation of cytochrome *c* from the mitochondria can be arrested through the inhibition of p53. Similarly, caspase 8 was also shown to decrease in experimental models of SAH-induced apoptosis after the prevention of p53 stabilization in the cytosol, suggesting that the caspase-dependent pathway and mitochondrial release of cytochrome *c* are important in SAH (unpublished data). Further work in this area is required to elucidate the importance of these pathways in SAH.

As a result of the ischemic insult induced by SAH, we have shown that apoptosis occurs in the endothelial cells of vessels. Experimentally, caspase 3 can be inhibited, which ameliorates the degree of apoptosis. Of interest however is that the prevention of apoptosis in the cerebral vasculature also attenuated the degree of vasospasm (Zhou *et al*, 2004).

Endothelial cell death may result in thrombus formation, induce cell proliferation as well as migration (McGirt *et al*, 2002; Borel *et al*, 2003; Zhou *et al*, 2004), in both penetrating and major cerebral vessels (Zubkov *et al*, 2000*b*, 2002*b*). Furthermore, apoptosis was identified in a patient who died from an SAH (Zubkov *et al*, 2000*a*). For a full review of the activation of the caspase cascades and mechanisms of action, see Thornberry and Lazebnik (1998).

Caspase-Independent Pathways

As discussed above, p53 seems central to the apoptotic cascades in SAH. Recently, a new role for p53 has been found in the caspase-independent cascade (Cregan *et al*, 2002). In many experimental models of stroke and SAH, inhibition of caspases has been shown to afford some protection; however, apoptosis still occurs (Zhan *et al*, 2001; Park *et al*, 2004; Zhou *et al*, 2004). Therefore, it seems clear that another caspase-independent cascade may be involved. Apoptosis-inducing factor (AIF) is a mitochondrial intramembranous flavoprotein that has been shown to be released from the mitochondria and translocate to the nucleus in response to various death signals (Daugas *et al*, 2000; Cregan *et al*, 2002). p53 has been shown to trigger the release of AIF in the absence of Apaf-1, resulting in a caspase-independent apoptotic cascade (Cregan *et al*, 2002).

Interestingly, in a similar way to cytochrome *c*, AIF appears to be under the control of the Bcl-2 family and in fact the release of both AIF and cytochrome *c* is inhibited if Bcl-2 members are blocked, suggesting that the Bcl-2 family may be solely responsible for the caspase-dependent and -independent cascades (Xiang *et al*, 1996; Cregan *et al*, 2002). The Bcl-2 family is also responsible for the inhibition of second mitochondria-derived activator of caspase/direct IAP (inhibitor of apoptosis protein) binding protein with low pI (Smac/Diablo) (Du *et al*, 2000; Verhagen *et al*, 2000), yet another mitochondrial protein similar to cytochrome *c*, which depresses procaspase-9 through the inhibition of inhibitor of apoptosis protein (Adrain *et al*, 2001). This makes the Bcl-2 family a powerful target for future therapeutic intervention. The role of Smac/Diablo in SAH has not to date been identified nor has that of endonuclease G, yet another mitochondrial protein.

Consequences of Apoptosis

One of the first complications related to both the pathophysiological aspects of SAH and the apoptotic cascades discussed above is the disruption of the BBB (Germano *et al*, 2000; Kusaka *et al*, 2004; Park *et al*, 2004). It is likely that the immediate pathophysiological upsets manifest themselves as early

BBB disruption (Peterson and Cardoso, 1983), whereas late BBB disruption is caused by the apoptotic phenomena (Gules *et al*, 2003). The evidence for this is sparse, because there is very limited information available from human studies regarding the time course of BBB disruption (Germano *et al*, 2000). Even in animal models, the time course is dependent on the animal model used (Peterson and Cardoso, 1983; Sasaki *et al*, 1985; Davis *et al*, 1986; Doczi *et al*, 1986; Johshita *et al*, 1990; Germano *et al*, 1998). Results from experimental models have found BBB changes ranging in time from 1 h to 6 days. However, the overall pattern appears to be a biphasic response of the BBB to SAH in the short and long term (Doczi, 1985). Although one can suggest tentatively that a similar biphasic effect can be seen in humans, it is far from categorical.

Damage to the endothelial cells as well as leading to BBB disruption, may also lead to a decrease in the production of endothelial-dependent relaxing factors, and this has been speculated to aggravate vasospasm locally, if not generally (Kassell *et al*, 1985; Zhang *et al*, 1998). This is further aggravated by denuding the vessels of endothelial cells, which exposes the vessel to a host of vasoactive and toxic metabolites, which can also aggravate vasospasm (Miranda *et al*, 1996; Zhou *et al*, 2004). Clinically, it is probably a combination of these factors and others that results in BBB disruption and vasospasm. The destruction of the BBB and subsequent edema have been implicated as one of the major predictors of cognitive dysfunction in the long run after SAH (Kreiter *et al*, 2002).

Brain Edema

Brain edema is a major component of EBI as a direct consequence of the disruption to the BBB (Laszlo *et al*, 1995; Doczi, 2001) and not as a result of vasospasm (Claassen *et al*, 2002). Although brain edema secondary to SAH has been largely ignored in the literature, Classen and colleagues showed that 8% of patients had global cerebral edema detected by CT scan on admission and that an additional 12% developed appreciable edema over the first 6 days (Kassell *et al*, 1990*a,b*; Claassen *et al*, 2002). The destruction of the BBB after a SAH is not well understood although a number of different mechanisms have been proposed as outlined above.

In patients with SAH, classic vasogenic edema has been described, which is a direct result of BBB breakdown and which was also shown in experimental models (Doczi, 1985). However, recently, cytotoxic edema in combination with vasogenic edema has been described using MRI techniques (Sibon *et al*, 2004; Orakcioglu *et al*, 2005). The presence of cytotoxic edema indicates the global ischemic injury that occurs at the time of a SAH, as cytotoxic edema occurs largely because of the failure of energy-dependent Na⁺/K⁺ pumps. The role of

brain edema in relation to EBI has come to the forefront of research using MRI. MRI has been described as a powerful tool for the noninvasive detection of EBI (Busch *et al*, 1998). The use of apparent diffusion gradients demonstrates cellular swelling after a propagating wave of ischemia, which could be seen spreading throughout the ipsilateral and contralateral hemispheres. Experimental models using these MRI techniques have shown a fall of the apparent diffusion gradient as early as 2 mins after the SAH, confirming a global ischemic insult and demonstrating global cytotoxic edema (Busch *et al*, 1998).

Therefore, as mentioned above, the first arm of the biphasic response results in immediate brain edema. Through the mechanisms previously described, there is a resultant rise in the ICP, which further reduces CBF, leading to further ischemia (Fukuhara *et al*, 1998). As a result, there is more damage to the BBB and the apoptotic cascades are initiated, which leads to further breakdown in the BBB, suggesting a biphasic response. It is the disruption of the endothelial cells because of cell death that allows for the acute rise in both cerebral edema and ventricular volumes (Laszlo *et al*, 1995). Therefore, brain edema contributes to the rise in ICP seen immediately after a SAH (Hayashi *et al*, 1977). It is also believed to result in acute vasoconstriction, which combined with the edema leads to a further reduction in CBF, resulting in global ischemic injury (Doczi, 2001). The mechanism by which this occurs has not to date been fully elucidated. Unchecked, this cycle will repeat itself, leading to further edema and eventually death.

This laboratory has shown brain edema in SAH models after SAH in the monofilament rat model. We have also shown a reduction in edema and preservation of the BBB with the use of caspase 3 inhibitors (z-VAD-FMK) (Park *et al*, 2004) and p53 inhibitors (Pifithrin) (Zhou *et al*, 2005). Furthermore, delayed global edema has been shown to be an independent predictor of death (Claassen *et al*, 2002).

The psychological problems faced by SAH survivors have been well described in the past (Vilkkki *et al*, 1990, 2004; Jarvis, 2002; Powell *et al*, 2004; Jarvis and Talbot, 2004). Even patients with a relatively benign SAH and postoperative course describe long-term memory and concentration problems. The etiology behind this phenomenon remains to be clarified. Animal studies using SAH models have demonstrated profound hippocampal neuronal loss within a relatively short period of time. Histologic data confirm over 30% loss in some studies. There have been few if any long-term studies examining memory and learning capacity after SAH. It is believed that the loss of hippocampal neurons occurs secondary to the global ischemia, which occurs at the time of a SAH. This is probably exacerbated by BBB breakdown and brain edema. Although some degree of global ischemia occurs in

all patients who have had a SAH, it seems incredulous that grade one patients who complain of headache only with no syncope have experienced such a degree of ischemia as to lose hippocampal neurons. The hippocampal cell loss can be prevented using various apoptotic inhibitors to some degree, which appears to lessen the long-term neurologic sequelae in animal models at least (Park *et al*, 2004).

Necrosis and Subarachnoid Hemorrhage

Apoptotic and necrotic pathways often occur simultaneously and in fact it can be difficult to differentiate between the two. In general, apoptosis can be regarded as an energy-dependent process whereas necrosis is not. As a result, whether a cell becomes apoptotic or necrotic is dependent on the strength of the initial injury, which if severe will consume energy quickly, leading to necrosis. However, in SAH, if the initial bleed were severe enough to prevent blood flow to the brain as in a global stroke, it is unlikely that the brain tissue would survive. As a result, necrosis is not a major factor in SAH, unlike stroke. In addition, it is generally believed that necrosis is not a reversible entity, making it an undesirable target for therapeutic intervention.

However, necrosis has been implicated in two major areas, which are the vasculature and the circumventricular regions. Some studies have shown the presence of necrotic cells in the vasculature. Necrotic cells can be found in the major vessels, particularly in the smooth muscle layer, where they are believed to contribute to vasospasm (Hughes and Schianchi, 1978; Ogihara *et al*, 2001). Cell culture studies have suggested that oxyhemoglobin may play a leading role with regard to necrosis in the smooth muscle layer (Ogihara *et al*, 2001). However, what is not clear from the available data is whether necrosis in the vessel contributes to vasospasm or is in fact the result of vasospasm, as it has been well established that the energy metabolites within a vessel experiencing vasospasm are significantly diminished (Yoshimoto *et al*, 1993). Lastly, necrosis has been implicated in brain edema after SAH. It has been shown that necrosis occurs after SAH in circumventricular regions, which contribute to fluid and electrolyte balance (Akpınar *et al*, 2005). In particular, the subfornical organ and organum vasculosum lamina terminalis were found to be susceptible. Additional work is needed in this area to fully explore the consequences of these findings.

Conclusions

The future of this topic lies in a complete elucidation of the apoptotic cascades in relation to SAH.

To date, the apoptotic pathways believed to play a major role in SAH-induced apoptosis are the death receptor and TNF- α , p53, and the caspase-dependent cascades. Additional work is required to examine the caspase-independent and mitochondrial cascades in relation to SAH. In addition, several different inhibitors have been used such as caspase 3 and p53 inhibitors (Zubkov *et al*, 2002a; Park *et al*, 2004; Zhou *et al*, 2004, 2005). However, to the best of our knowledge, multiple level inhibitors have not been used. An example of this might be to selectively block the limiting steps of the caspase-independent and -dependent cascades and the Bcl-2 family proteins. This would inhibit all known apoptotic routes. Such an approach would be controversial and fewer numbers of inhibitors would be used initially. It must be remembered that such a degree of inhibition of such an important pathway could be disastrous for the model, as was shown in gene knockout mice, where Apaf-1, caspase 9, and caspase 3 knockouts resulted in severe developmental defects incompatible with life (Kuida *et al*, 1996, 1998; Yoshida *et al*, 1998).

All of these pathways however have the same result, which is of course the death of the cell. Cell death after SAH has important implications not only for vasospasm but also for the long-term sequelae of SAH. What is interesting and unexplained is why perimesencephalic bleeds are not associated with similar sequelae. Although many theories exist to explain this phenomenon, it could be suggested that a perimesencephalic hemorrhage is not a stress event severe enough to initiate the apoptotic cascade. Current research activities in this area are focused on the inhibition of various mediators of these cascades to determine if cell survival can be increased and to look at the outcomes in these models. Such inhibitors have had some success and there is certainly potential to inhibit some if not all of these cascades, which may improve patient outcome through their effects on brain tissue as well as the vascular supply.

In summary, it appears that EBI is a combination of physiologic insults to the brain, resulting in global ischemia, BBB breakdown, edema, and cellular death signaling. These changes occur acutely and chronically although after 72 h vasospasm becomes the main protagonist. The consequences of EBI can be seen immediately and in the long term. There are currently few studies examining the role of EBI in the short- and long-term outcome of SAH models. There has recently been renewed interest in cell death mechanisms regarding SAH and as these are innately tied in with SAH, research is continuing in these areas. It is also difficult to extrapolate a lot of this information to humans and this has not been carried out to date. The future of this area of research is clearly to fully elucidate the appropriate pathways, to relate these to the human condition, and finally to design potential pharmaceutical treatment options with single or multiple inhibitors, which

may be able to suppress many of the devastating secondary injuries associated with SAH.

References

- Adams JM, Cory S (1998) The Bcl-2 protein family: arbiters of cell survival. *Science* 281:1322–6
- Adrain C, Creagh EM, Martin SJ (2001) Apoptosis-associated release of Smac/DIABLO from mitochondria requires active caspases and is blocked by Bcl-2. *EMBO J* 20:6627–36
- Akpinar G, Acikgoz B, Surucu S, Celik HH, Cagavi F (2005) Ultrastructural changes in the circumventricular organs after experimental subarachnoid hemorrhage. *Neurol Res* 27:580–5
- An WG, Kanekal M, Simon MC, Maltepe E, Blagosklonny MV, Neckers LM (1998) Stabilization of wild-type p53 by hypoxia-inducible factor 1 α . *Nature* 392:405–8
- Antonsson B, Martinou JC (2000) The Bcl-2 protein family. *Exp Cell Res* 256:50–7
- Badjatia N, Topcuoglu MA, Buonanno FS, Smith EE, Nogueira RG, Rordorf GA *et al*. (2005) Relationship between hyperglycemia and symptomatic vasospasm after subarachnoid hemorrhage. *Crit Care Med* 33:1603–9
- Barbosa MD, Arthur AS, Louis RH, MacDonald T, Polin RS, Gazak C *et al*. (2001) The novel 5-lipoxygenase inhibitor ABT-761 attenuates cerebral vasospasm in a rabbit model of subarachnoid hemorrhage. *Neurosurgery* 49:1205–12
- Bederson JB, Germano IM, Guarino L (1995) Cortical blood flow and cerebral perfusion pressure in a new non-craniotomy model of subarachnoid hemorrhage in the rat. *Stroke* 26:1086–91
- Benchoua A, Guegan C, Couriaud C, Hosseini H, Sampaio N, Morin D *et al*. (2001) Specific caspase pathways are activated in the two stages of cerebral infarction. *J Neurosci* 21:7127–34
- Bergeron L, Perez GI, Macdonald G, Shi L, Sun Y, Jurisicova A *et al*. (1998) Defects in regulation of apoptosis in caspase-2-deficient mice. *Genes Dev* 12:1304–14
- Bergeron M, Gidday JM, Yu AY, Semenza GL, Ferriero DM, Sharp FR (2000) Role of hypoxia-inducible factor-1 in hypoxia-induced ischemic tolerance in neonatal rat brain. *Ann Neurol* 48:285–96
- Bonita R, Thomson S (1985) Subarachnoid hemorrhage: epidemiology, diagnosis, management, and outcome. *Stroke* 16:591–4
- Borel CO, McKee A, Parra A, Haglund MM, Solan A, Prabhakar V *et al*. (2003) Possible role for vascular cell proliferation in cerebral vasospasm after subarachnoid hemorrhage. *Stroke* 34:427–33
- Broderick JP, Brott TG, Duldner JE, Tomsick T, Leach A (1994) Initial and recurrent bleeding are the major causes of death following subarachnoid hemorrhage. *Stroke* 25:1342–7
- Busch E, Beaulieu C, de Crespigny A, Moseley ME (1998) Diffusion MR imaging during acute subarachnoid hemorrhage in rats. *Stroke* 29:2155–61
- Butler WE PJZNMK (1996) Intracellular calcium, myosin light chain phosphorylation and contractile force in experimental cerebral vasospasm. *Neurosurgery* 38:781–8

- Chipuk JE, Kuwana T, Bouchier-Hayes L, Droin NM, Newmeyer DD, Schuler M *et al.* (2004) Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. *Science* 303:1010–4
- Claassen J, Carhuapoma JR, Kreiter KT, Du EY, Connolly ES, Mayer SA (2002) Global cerebral edema after subarachnoid hemorrhage: frequency, predictors, and impact on outcome. *Stroke* 33:1225–32
- Claassen J, Vu A, Kreiter KT, Kowalski RG, Du EY, Ostapkovich N *et al.* (2004) Effect of acute physiologic derangements on outcome after subarachnoid hemorrhage. *Crit Care Med* 32:832–8
- Cohen GM (1997) Caspases: the executioners of apoptosis. *Biochem J* 326(Part 1):1–16
- Cook DA (1995) Mechanisms of cerebral vasospasm in subarachnoid haemorrhage. *Pharmacol Ther* 66:259–84
- Cregan SP, Fortin A, MacLaurin JG, Callaghan SM, Cecconi F, Yu SW *et al.* (2002) Apoptosis-inducing factor is involved in the regulation of caspase-independent neuronal cell death. *J Cell Biol* 158:507–17
- Cregan SP, MacLaurin JG, Craig CG, Robertson GS, Nicholson DW, Park DS *et al.* (1999) Bax-dependent caspase-3 activation is a key determinant in p53-induced apoptosis in neurons. *J Neurosci* 19:7860–9
- Daily D, Barzilai A, Offen D, Kamsler A, Melamed E, Ziv I (1999) The involvement of p53 in dopamine-induced apoptosis of cerebellar granule neurons and leukemic cells overexpressing p53. *Cell Mol Neurobiol* 19:261–76
- Daugas E, Susin SA, Zamzami N, Ferri KF, Irinopoulou T, Larochette N *et al.* (2000) Mitochondrio-nuclear translocation of AIF in apoptosis and necrosis. *FASEB J* 14:729–39
- Davis RP, Zappulla RA, Spigelman MK, Feuer EJ, Malis LI, Holland JF (1986) The protective effect of experimental subarachnoid haemorrhage on sodium dehydrocholate-induced blood–brain barrier disruption. *Acta Neurochir (Wien)* 83:138–43
- Dawson VL, Dawson TM (2004) Deadly conversations: nuclear–mitochondrial cross-talk. *J Bioenerg Biomembr* 36:287–94
- Doczi T (1985) The pathogenetic and prognostic significance of blood–brain barrier damage at the acute stage of aneurysmal subarachnoid haemorrhage. Clinical and experimental studies. *Acta Neurochir (Wien)* 77:110–32
- Doczi T, Joo F, Adam G, Bozoky B, Szerdahelyi P (1986) Blood–brain barrier damage during the acute stage of subarachnoid hemorrhage, as exemplified by a new animal model. *Neurosurgery* 18:733–9
- Doczi TP (2001) Impact of cerebral microcirculatory changes on cerebral blood flow during cerebral vasospasm after aneurysmal subarachnoid hemorrhage. *Stroke* 32:817
- Du C, Fang M, Li Y, Li L, Wang X (2000) Smac, a mitochondrial protein that promotes cytochrome *c*-dependent caspase activation by eliminating IAP inhibition. *Cell* 102:33–42
- Earnshaw WC, Martins LM, Kaufmann SH (1999) Mammalian caspases: structure, activation, substrates, and functions during apoptosis. *Annu Rev Biochem* 68:383–424
- Eguchi Y, Shimizu S, Tsujimoto Y (1997) Intracellular ATP levels determine cell death fate by apoptosis or necrosis. *Cancer Res* 57:1835–40
- Erster S, Mihara M, Kim RH, Petrenko O, Moll UM (2004) *In vivo* mitochondrial p53 translocation triggers a rapid first wave of cell death in response to DNA damage that can precede p53 target gene activation. *Mol Cell Biol* 24:6728–41
- Fisher CM (1975) Clinical syndromes in cerebral thrombosis, hypertensive hemorrhage, and ruptured saccular aneurysm. *Clin Neurosurg* 22:117–47
- Frykholm P, Andersson JL, Langstrom B, Persson L, Enblad P (2004) Haemodynamic and metabolic disturbances in the acute stage of subarachnoid haemorrhage demonstrated by PET. *Acta Neurol Scand* 109:25–32
- Fukuhara T, Douville CM, Elliott JP, Newell DW, Winn HR (1998) Relationship between intracranial pressure and the development of vasospasm after aneurysmal subarachnoid hemorrhage. *Neurol Med Chir (Tokyo)* 38:710–5
- Germano A, d'Avella D, Imperatore C, Caruso G, Tomasello F (2000) Time-course of blood–brain barrier permeability changes after experimental subarachnoid haemorrhage. *Acta Neurochir (Wien)* 142:575–80
- Germano A, Imperatore C, d'Avella D, Costa G, Tomasello F (1998) Antivasospastic and brain-protective effects of a hydroxyl radical scavenger (AVS) after experimental subarachnoid hemorrhage. *J Neurosurg* 88:1075–81
- Giaccia AJ, Kastan MB (1998) The complexity of p53 modulation: emerging patterns from divergent signals. *Genes Dev* 12:2973–83
- Glenn TC, Patel AB, Martin NA, Samii A, De Jesus C, Hovda DA (2002) Subarachnoid hemorrhage induces dynamic changes in regional cerebral metabolism in rats. *J Neurotrauma* 19:449–66
- Goda N, Ryan HE, Khadivi B, McNulty W, Rickert RC, Johnson RS (2003) Hypoxia-inducible factor 1 α is essential for cell cycle arrest during hypoxia. *Mol Cell Biol* 23:359–69
- Gross A, McDonnell JM, Korsmeyer SJ (1999) BCL-2 family members and the mitochondria in apoptosis. *Genes Dev* 13:1899–911
- Grosset DG, Straiton J, du TM, Bullock R (1992) Prediction of symptomatic vasospasm after subarachnoid hemorrhage by rapidly increasing transcranial Doppler velocity and cerebral blood flow changes. *Stroke* 23:674–9
- Grote E, Hassler W (1988) The critical first minutes after subarachnoid hemorrhage. *Neurosurgery* 22:654–61
- Gules I, Satoh M, Nanda A, Zhang JH (2003) Apoptosis, blood–brain barrier, and subarachnoid hemorrhage. *Acta Neurochir Suppl* 86:483–7
- Hammond EM, Denko NC, Dorie MJ, Abraham RT, Giaccia AJ (2002) Hypoxia links ATR and p53 through replication arrest. *Mol Cell Biol* 22:1834–43
- Hammond EM, Giaccia AJ (2005) The role of p53 in hypoxia-induced apoptosis. *Biochem Biophys Res Commun* 331:718–25
- Hansen-Schwartz J, Hoel NL, Xu CB, Svendgaard NA, Edvinsson L (2003) Subarachnoid hemorrhage-induced upregulation of the 5-HT_{1B} receptor in cerebral arteries in rats. *J Neurosurg* 99:115–20
- Hara A, Yoshimi N, Mori H (1998) Evidence for apoptosis in human intracranial aneurysms. *Neurol Res* 20:127–130
- Hayashi M, Marukawa S, Fujii H, Kitano T, Kobayashi H, Yamamoto S (1977) Intracranial hypertension in patients with ruptured intracranial aneurysm. *J Neurosurg* 46:584–90
- Hetts SW (1998) To die or not to die: an overview of apoptosis and its role in disease. *JAMA* 279:300–7
- Horvitz HR, Shaham S, Hengartner MO (1994) The genetics of programmed cell death in the nematode

- Caenorhabditis elegans*. *Cold Spring Harb Symp Quant Biol* 59:377–85
- Hughes JT, Schianchi PM (1978) Cerebral artery spasm. A histological study at necropsy of the blood vessels in cases of subarachnoid hemorrhage. *J Neurosurg* 48: 515–25
- Hutter BO, Kreitschmann-Andermahr I, Mayfrank L, Rohde V, Spetzger U, Gilsbach JM (1999) Functional outcome after aneurysmal subarachnoid hemorrhage. *Acta Neurochir Suppl* 72:157–74
- Jarvis A (2002) Recovering from subarachnoid haemorrhage: patients' perspective. *Br J Nurs* 11:1430–7
- Jarvis A, Talbot L (2004) Multiprofessional follow up of patients after subarachnoid haemorrhage. *Br J Nurs* 13:1262–7
- Jiang X, Wang X (2004) Cytochrome *c*-mediated apoptosis. *Annu Rev Biochem* 73:87–106
- Johshita H, Kassell NF, Sasaki T (1990) Blood–brain barrier disturbance following subarachnoid hemorrhage in rabbits. *Stroke* 21:1051–8
- Ju ST, Panka DJ, Cui H, Ettinger R, el Khatib M, Sherr DH et al. (1995) Fas(CD95)/FasL interactions required for programmed cell death after T-cell activation. *Nature* 373:444–8
- Kaptain GJ, Lanzino G, Kassell NF (2000) Subarachnoid haemorrhage: epidemiology, risk factors, and treatment options. *Drugs Aging* 17:183–99
- Kassell NF, Sasaki T, Colohan AR, Nazar G (1985) Cerebral vasospasm following aneurysmal subarachnoid hemorrhage. *Stroke* 16:562–72
- Kassell NF, Torner JC, Haley EC, Jr, Jane JA, Adams HP, Kongable GL (1990a) The international cooperative study on the timing of aneurysm surgery. Part 1: overall management results. *J Neurosurg* 73:18–36
- Kassell NF, Torner JC, Jane JA, Haley EC, Jr, Adams HP (1990b) The international cooperative study on the timing of aneurysm surgery. Part 2: surgical results. *J Neurosurg* 73:37–47
- Kerr JF, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 26:239–57
- Kessler IM, Pacheco YG, Lozzi SP, de AA, Jr, Onishi FJ, de Mello PA (2005) Endothelin-1 levels in plasma and cerebrospinal fluid of patients with cerebral vasospasm after aneurysmal subarachnoid hemorrhage. *Surg Neurol* 64(Suppl 1):S1–5
- Kidd VJ (1998) Proteolytic activities that mediate apoptosis. *Annu Rev Physiol* 60:533–73
- Kondo S, Hashimoto N, Kikuchi H, Hazama F, Nagata I, Kataoka H (1998) Apoptosis of medial smooth muscle cells in the development of saccular cerebral aneurysms in rats. *Stroke* 29:181–8
- Koumenis C, Alarcon R, Hammond E, Sutphin P, Hoffman W, Murphy M et al. (2001) Regulation of p53 by hypoxia: dissociation of transcriptional repression and apoptosis from p53-dependent transactivation. *Mol Cell Biol* 21:1297–310
- Kreiter KT, Copeland D, Bernardini GL, Bates JE, Peery S, Claassen J et al. (2002) Predictors of cognitive dysfunction after subarachnoid hemorrhage. *Stroke* 33: 200–8
- Kuida K, Haydar TF, Kuan CY, Gu Y, Taya C, Karasuyama H et al. (1998) Reduced apoptosis and cytochrome *c*-mediated caspase activation in mice lacking caspase 9. *Cell* 94:325–37
- Kuida K, Zheng TS, Na S, Kuan C, Yang D, Karasuyama H et al. (1996) Decreased apoptosis in the brain and premature lethality in CPP32-deficient mice. *Nature* 384:368–72
- Kusaka G, Ishikawa M, Nanda A, Granger DN, Zhang JH (2004) Signaling pathways for early brain injury after subarachnoid hemorrhage. *J Cereb Blood Flow Metab* 24:916–25
- Lane DP (1992) Cancer. p53, guardian of the genome. *Nature* 358:15–6
- Laszlo FA, Varga C, Doczi T (1995) Cerebral oedema after subarachnoid haemorrhage. Pathogenetic significance of vasopressin. *Acta Neurochir (Wien)* 133:122–33
- Leist M, Single B, Castoldi AF, Kuhnle S, Nicotera P (1997) Intracellular adenosine triphosphate (ATP) concentration: a switch in the decision between apoptosis and necrosis. *J Exp Med* 185:1481–6
- Leker RR, Aharonowiz M, Greig NH, Ovidia H (2004) The role of p53-induced apoptosis in cerebral ischemia: effects of the p53 inhibitor pifithrin alpha. *Exp Neurol* 187:478–86
- Li H, Zhu H, Xu CJ, Yuan J (1998) Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. *Cell* 94:491–501
- Li J, Zhang X, Sejas DP, Bagby GC, Pang Q (2004) Hypoxia-induced nucleophosmin protects cell death through inhibition of p53. *J Biol Chem* 279:41275–9
- Li Y, Chopp M, Powers C, Jiang N (1997) Apoptosis and protein expression after focal cerebral ischemia in rat. *Brain Res* 765:301–12
- Love S (2003) Apoptosis and brain ischaemia. *Prog Neuropsychopharmacol Biol Psychiatry* 27:267–82
- Macdonald RL, Curry DJ, Aihara Y, Zhang ZD, Jahromi BS, Yassari R (2004) Magnesium and experimental vasospasm. *J Neurosurg* 100:106–10
- Macdonald RL, Weir B, Zhang J, Marton LS, Sajdak M, Johns LM (1997) Adenosine triphosphate and hemoglobin in vasospastic monkeys. *Neurosurg Focus* 3:e3
- Macomson SD, Brophy CM, Miller W, Harris VA, Shaver EG (2002) Heat shock protein expression in cerebral vessels after subarachnoid hemorrhage. *Neurosurgery* 51:204–10
- Matsushita K, Meng W, Wang X, Asahi M, Asahi K, Moskowitz MA et al. (2000) Evidence for apoptosis after intercerebral hemorrhage in rat striatum. *J Cereb Blood Flow Metab* 20:396–404
- Mattson MP (2000) Apoptosis in neurodegenerative disorders. *Nat Rev Mol Cell Biol* 1:120–9
- Matz PG, Fujimura M, Chan PH (2000) Subarachnoid hemolysate produces DNA fragmentation in a pattern similar to apoptosis in mouse brain. *Brain Res* 858: 312–319
- Matz PG, Fujimura M, Lewen A, Morita-Fujimura Y, Chan PH (2001) Increased cytochrome *c*-mediated DNA fragmentation and cell death in manganese-superoxide dismutase-deficient mice after exposure to subarachnoid hemolysate. *Stroke* 32:506–15
- McCormick WF, Nofzinger JD (1965) Saccular intracranial aneurysms: an autopsy study. *J Neurosurg* 22: 155–9
- McGirt MJ, Lynch JR, Blessing R, Warner DS, Friedman AH, Laskowitz DT (2002) Serum von Willebrand factor, matrix metalloproteinase-9, and vascular endothelial growth factor levels predict the onset of cerebral vasospasm after aneurysmal subarachnoid hemorrhage. *Neurosurgery* 51:1128–34
- Mihara M, Erster S, Zaika A, Petrenko O, Chittenden T, Pancoska P et al. (2003) p53 has a direct apoptogenic role at the mitochondria. *Mol Cell* 11:577–90

- Miranda FJ, Alabadi JA, Torregrosa G, Salom JB, Jover T, Barbera MD *et al.* (1996) Modulatory role of endothelial and nonendothelial nitric oxide in 5-hydroxytryptamine-induced contraction in cerebral arteries after subarachnoid hemorrhage. *Neurosurgery* 39:998–1003
- Mowat MR (1998) p53 in tumor progression: life, death, and everything. *Adv Cancer Res* 74:25–48
- Muzio M, Stockwell BR, Stennicke HR, Salvesen GS, Dixit VM (1998) An induced proximity model for caspase-8 activation. *J Biol Chem* 273:2926–30
- Nakamura Y (2004) Isolation of p53-target genes and their functional analysis. *Cancer Sci* 95:7–11
- Nijhawan D, Honarpour N, Wang X (2000) Apoptosis in neural development and disease. *Annu Rev Neurosci* 23:73–87
- Nornes H (1978) Cerebral arterial flow dynamics during aneurysm haemorrhage. *Acta Neurochir (Wien)* 41:39–48
- O'Brate A, Giannakakou P (2003) The importance of p53 location: nuclear or cytoplasmic zip code? *Drug Resist Updat* 6:313–22
- Ogihara K, Aoki K, Zubkov AY, Zhang JH (2001) Oxyhemoglobin produces apoptosis and necrosis in cultured smooth muscle cells. *Brain Res* 889:89–97
- Orakcioglu B, Fiebach JB, Steiner T, Kollmar R, Juttler E, Becker K *et al.* (2005) Evolution of early perihemorrhagic changes—ischemia versus edema: an MRI study in rats. *Exp Neurol* 193:369–76
- Ostrowski RP, Colohan AR, Zhang JH (2005) Mechanisms of hyperbaric oxygen-induced neuroprotection in a rat model of subarachnoid hemorrhage. *J Cereb Blood Flow Metab* 25:554–71
- Park S, Yamaguchi M, Zhou C, Calvert JW, Tang J, Zhang JH (2004) Neurovascular protection reduces early brain injury after subarachnoid hemorrhage. *Stroke* 35:2412–2417
- Peterson EW, Cardoso ER (1983) The blood–brain barrier following experimental subarachnoid hemorrhage. Part 1: response to insult caused by arterial hypertension. *J Neurosurg* 58:338–44
- Philchenkov A (2004) Caspases: potential targets for regulating cell death. *J Cell Mol Med* 8:432–44
- Powell J, Kitchen N, Heslin J, Greenwood R (2004) Psychosocial outcomes at 18 months after good neurological recovery from aneurysmal subarachnoid haemorrhage. *J Neurol Neurosurg Psychiatry* 75:1119–24
- Prives C, Hall PA (1999) The p53 pathway. *J Pathol* 187:112–26
- Reed JC (1997) Double identity for proteins of the Bcl-2 family. *Nature* 387:773–6
- Reed JC (2000) Mechanisms of apoptosis. *Am J Pathol* 157:1415–30
- Ryan KM, Phillips AC, Vousden KH (2001) Regulation and function of the p53 tumor suppressor protein. *Curr Opin Cell Biol* 13:332–7
- Sako M, Nishihara J, Ohta S, Wang J, Sakaki S (1993) Role of protein kinase C in the pathogenesis of cerebral vasospasm after subarachnoid hemorrhage. *J Cereb Blood Flow Metab* 13:247–54
- Sansome C, Zaika A, Marchenko ND, Moll UM (2001) Hypoxia death stimulus induces translocation of p53 protein to mitochondria. Detection by immunofluorescence on whole cells. *FEBS Lett* 488:110–5
- Sasaki T, Kassell NF, Yamashita M, Fujiwara S, Zuccarello M (1985) Barrier disruption in the major cerebral arteries following experimental subarachnoid hemorrhage. *J Neurosurg* 63:433–40
- Sasaki T, Kasuya H, Onda H, Sasahara A, Goto S, Hori T *et al.* (2004) Role of p38 mitogen-activated protein kinase on cerebral vasospasm after subarachnoid hemorrhage. *Stroke* 35:1466–70
- Schievink WI (1997) Intracranial aneurysms. *N Engl J Med* 336:28–40
- Schievink WI, Riedinger M, Jhutti TK, Simon P (2004) Racial disparities in subarachnoid hemorrhage mortality: Los Angeles County, California, 1985–1998. *Neuroepidemiology* 23:299–305
- Schmid T, Zhou J, Brune B (2004) HIF-1 and p53: communication of transcription factors under hypoxia. *J Cell Mol Med* 8:423–31
- Sekhar LN, Wechsler LR, Yonas H, Luyckx K, Obrist W (1988) Value of transcranial Doppler examination in the diagnosis of cerebral vasospasm after subarachnoid hemorrhage. *Neurosurgery* 22:813–21
- Sheikh MS, Fornace AJ, Jr (2000) Role of p53 family members in apoptosis. *J Cell Physiol* 182:171–81
- Shieh SY, Ikeda M, Taya Y, Prives C (1997) DNA damage-induced phosphorylation of p53 alleviates inhibition by MDM2. *Cell* 91:325–34
- Sibon I, Menegon P, Rouanet F, Dousset V, Orgogozo JM (2004) MRI of acute brainstem ischaemia: cytotoxic versus vasogenic oedema? *Eur J Neurol* 11:497–8
- Sobey CG (2001) Cerebrovascular dysfunction after subarachnoid haemorrhage: novel mechanisms and directions for therapy. *Clin Exp Pharmacol Physiol* 28:926–9
- Sobey CG, Faraci FM (1998) Subarachnoid haemorrhage: what happens to the cerebral arteries? *Clin Exp Pharmacol Physiol* 25:867–76
- Stommel JM, Marchenko ND, Jimenez GS, Moll UM, Hope TJ, Wahl GM (1999) A leucine-rich nuclear export signal in the p53 tetramerization domain: regulation of subcellular localization and p53 activity by NES masking. *EMBO J* 18:1660–72
- Strasser A, O'Connor L, Dixit VM (2000) Apoptosis signaling. *Annu Rev Biochem* 69:217–45
- Sviri GE, Shik V, Raz B, Soustiel JF (2003) Role of brain natriuretic peptide in cerebral vasospasm. *Acta Neurochir (Wien)* 145:851–60
- Thornberry NA, Lazebnik Y (1998) Caspases: enemies within. *Science* 281:1312–6
- Unger T, Juven-Gershon T, Moallem E, Berger M, Vogt SR, Lozano G *et al.* (1999) Critical role for Ser20 of human p53 in the negative regulation of p53 by Mdm2. *EMBO J* 18:1805–14
- van Loo G, Saels X, van Gurp M, MacFarlane M, Martin SJ, Vandenabeele P (2002) The role of mitochondrial factors in apoptosis: a Russian roulette with more than one bullet. *Cell Death Differ* 9:1031–42
- Vander Heiden MG, Chandel NS, Schumacker PT, Thompson CB (1999) Bcl-xL prevents cell death following growth factor withdrawal by facilitating mitochondrial ATP/ADP exchange. *Mol Cell* 3:159–67
- Vander Heiden MG, Chandel NS, Williamson EK, Schumacker PT, Thompson CB (1997) Bcl-xL regulates the membrane potential and volume homeostasis of mitochondria. *Cell* 91:627–37
- Vaux DL, Strasser A (1996) The molecular biology of apoptosis. *Proc Natl Acad Sci USA* 93:2239–44
- Verhagen AM, Ekert PG, Pakusch M, Silke J, Connolly LM, Reid GE *et al.* (2000) Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. *Cell* 102:43–53
- Vilkkilä J, Holst P, Ohman J, Servo A, Heiskanen O (1990) Social outcome related to cognitive performance and

- computed tomographic findings after surgery for a ruptured intracranial aneurysm. *Neurosurgery* 26: 579–584
- Vilkkki JS, Juvela S, Siironen J, Ilvonen T, Varis J, Porras M (2004) Relationship of local infarctions to cognitive and psychosocial impairments after aneurysmal subarachnoid hemorrhage. *Neurosurgery* 55:790–802
- Vincenz C, Dixit VM (1997) Fas-associated death domain protein interleukin-1beta-converting enzyme 2 (FLICE2), an ICE/Ced-3 homologue, is proximally involved in. *J Biol Chem* 272:6578–83
- Voldby B, Enevoldsen EM (1982) Intracranial pressure changes following aneurysm rupture. Part 1: clinical and angiographic correlations. *J Neurosurg* 56:186–96
- Watanabe H, Ohta S, Kumon Y, Sakaki S, Sakanaka M (1999) Increase in p53 protein expression following cortical infarction in the spontaneously hypertensive rat. *Brain Res* 837:38–45
- Wenger RH, Camenisch G, Desbaillets I, Chilov D, Gassmann M (1998) Up-regulation of hypoxia-inducible factor-1alpha is not sufficient for hypoxic/anoxic p53 induction. *Cancer Res* 58:5678–80
- Xiang H, Hochman DW, Saya H, Fujiwara T, Schwartzkroin PA, Morrison RS (1996) Evidence for p53-mediated modulation of neuronal viability. *J Neurosci* 16: 6753–6765
- Yakovlev AG, Di Giovanni S, Wang G, Liu W, Stoica B, Faden AI (2004) BOK and NOXA are essential mediators of p53-dependent apoptosis. *J Biol Chem* 279: 28367–74
- Yeung MC, Lau AS (1998) Tumor suppressor p53 as a component of the tumor necrosis factor-induced, protein kinase PKR-mediated apoptotic pathway in human promonocytic U937 cells. *J Biol Chem* 273:25198–202
- Yoshida H, Kong YY, Yoshida R, Elia AJ, Hakem A, Hakem R et al. (1998) Apaf1 is required for mitochondrial pathways of apoptosis and brain development. *Cell* 94:739–50
- Yoshimoto Y, Kim P, Sasaki T, Takakura K (1993) Temporal profile and significance of metabolic failure and trophic changes in the canine cerebral arteries during chronic vasospasm after subarachnoid hemorrhage. *J Neurosurg* 78:807–12
- Zamzami N, Kroemer G (2001) The mitochondrion in apoptosis: how Pandora's box opens. *Nat Rev Mol Cell Biol* 2:67–71
- Zhan RZ, Wu C, Fujihara H, Taga K, Qi S, Naito M et al. (2001) Both caspase-dependent and caspase-independent pathways may be involved in hippocampal CA1 neuronal death because of loss of cytochrome c From mitochondria in a rat forebrain ischemia model. *J Cereb Blood Flow Metab* 21:529–40
- Zhang J, Lewis A, Bernanke D, Zubkov A, Clower B (1998) Stroke: anatomy of a catastrophic event. *Anat Rec* 253: 58–63
- Zheng L, Fisher G, Miller RE, Peschon J, Lynch DH, Lenardo MJ (1995) Induction of apoptosis in mature T cells by tumour necrosis factor. *Nature* 377:348–51
- Zhou C, Yamaguchi M, Colohan AR, Zhang JH (2005) Role of p53 and apoptosis in cerebral vasospasm after experimental subarachnoid hemorrhage. *J Cereb Blood Flow Metab* 25:572–82
- Zhou C, Yamaguchi M, Kusaka G, Schonholz C, Nanda A, Zhang JH (2004) Caspase inhibitors prevent endothelial apoptosis and cerebral vasospasm in dog model of experimental subarachnoid hemorrhage. *J Cereb Blood Flow Metab* 24:419–31
- Zubkov AY, Aoki K, Parent AD, Zhang JH (2002a) Preliminary study of the effects of caspase inhibitors on vasospasm in dog penetrating arteries. *Life Sci* 70: 3007–18
- Zubkov AY, Ogihara K, Bernanke DH, Parent AD, Zhang J (2000a) Apoptosis of endothelial cells in vessels affected by cerebral vasospasm. *Surg Neurol* 53:260–6
- Zubkov AY, Tibbs RE, Aoki K, Zhang JH (2000b) Morphological changes of cerebral penetrating arteries in a canine double hemorrhage model. *Surg Neurol* 54: 212–9
- Zubkov AY, Tibbs RE, Clower B, Ogihara K, Aoki K, Zhang JH (2001) Apoptosis in basilar endothelial cells in a canine double hemorrhage model. *Acta Neurochir Suppl* 77:29–31
- Zubkov AY, Tibbs RE, Clower B, Ogihara K, Aoki K, Zhang JH (2002b) Morphological changes of cerebral arteries in a canine double hemorrhage model. *Neurosci Lett* 326:137–41