Intestinal mucositis: The role of the Bcl-2 family, p53 and caspases in chemotherapy-induced damage

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REVIEW ARTICLE

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Abstract Intestinal mucositis occurs as a consequence of cytotoxic treatment through multiple mechanisms including induction of crypt cell death (apoptosis) and cytostasis. The molecular control of these actions throughout the gastrointestinal tract has yet to be fully elucidated; however, they are known to involve p53, the Bcl-2 family and caspases. This review will provide an overview of current research as well as identify areas where gaps in knowledge exist.

Keywords Mucositis · p53 · Bcl-2 family · caspases

Introduction

Mucositis (mucosal barrier injury) is a common side-effect of cancer chemotherapy for which there is no current treatment. Cytotoxic agents used during chemotherapy are effective at killing cancerous cells; however, they also indiscriminately target certain healthy tissues. The mucosal surfaces of the body are especially sensitive to these cytotoxic drugs; hence, the term mucositis is used to describe the damage to these surfaces arising from treatment [79, 82]. Whilst all mucosal membranes of the body experience toxicity associated with cancer treatment, for the purpose of this review, mucositis will refer to the gastrointestinal (GI) damage only.

Mucositis is common, occurring in approximately 40% of patients after standard doses of chemotherapy and in 100% of patients undergoing high-dose chemotherapy with stem cell or bone marrow transplantation [81, 143]. Considerable morbidity is associated with mucositis, with symptoms affecting the entire GI tract including pain, ulceration, vomiting and diarrhoea. Occasionally, mucositis can be fatal, especially in the case of irinotecan use (as described later), but more commonly, it requires treatment to be ceased or doses reduced to alleviate symptoms. The net result is increased length of hospital stay, use of opioids for pain management and, importantly, increased costs [81, 143]. Gastrointestinal mucositis is a major oncological problem that is now one of the most significant dose-limiting toxicities. This review will examine current literature concerning mechanisms known to cause mucositis throughout the gastrointestinal tract, concentrating especially on the molecular pathways involved.

The intestinal epithelium

The simple columnar epithelium of the intestine is made up of highly specialised cells whose primary function is to aid in absorption of nutrients across the epithelial lining whilst maintaining a physical barrier to the external environment [77]. Efficient absorption is facilitated within the small intestine through finger-like projections called villi, which

greatly enhance the surface area and therefore contact with luminal contents. The large intestine does not contain villi. A barrier is maintained by the epithelial cells, which are joined by tight junctions on their lateral boarders, thus limiting the passage of luminal contents across the cell [171]. Throughout the intestine, flask-like structures around the base of villi called crypts contain proliferative units and are responsible for maintaining epithelial integrity through constant production of new cells [194].

Cell proliferation and loss

The intestinal epithelium is one of the fastest proliferating tissues in the body [145]. Epithelial cells are constantly being produced within the crypts of the small and large intestine and differentiating into specialised absorptive cells as they migrate towards the lumen. Cell proliferation is especially rapid in the small intestine, with a cell division occurring every 5 min in the mouse [146]. The entire lining of the small intestine is replaced every 2 to 3 days in the rodent and 5 to 6 days in humans [206]. The turnover rate is 2 to 4 times slower in the large intestine [146]. An equal rate of cell loss is required to maintain homeostasis within the tissue, and while it is accepted that there is extensive and constant cell loss from the epithelium, there remains some controversy as to the exact nature of cell loss from the villus tip and luminal border of colonic crypts. It is generally accepted that once epithelial cells complete their migration along the crypt–villous axis, they are sloughed off into the lumen. Once detached, they undergo a programmed form of cell death due to loss of adhesion to the basement membrane, called anoikis, and are subsequently removed by local macrophages [52, 153, 203]. This notion of cell loss by exfoliation has been refuted by Hall and colleagues, who have attempted to prove that cells instead undergo programmed cell death whilst still attached to the basement membrane and are phagocytosed by surrounding enterocytes. They found that spontaneous cell death is responsible for, and closely approximates to, the total cell loss required to balance cell production in the murine small intestine [54]. They did however concede that loss of cells from the villi is partially due to shedding, as some cellular mass was found to accumulate in the lumen. Further to this, cuffs of cells undergoing programmed cell death present at the villus tip have also since been shown in the rat small intestine [206]. Thus, it is likely a combination of both cell sloughing and death is responsible for normal epithelial cell loss. A diagram of both the small and large intestinal crypt is shown in Fig. 1.

Stem cells

The stem cells of the small intestine are located within the lower part of the epithelial crypts and possess an element of pluripotency. They are capable of producing all four differentiated cell lineages within the crypt, namely: enteroendocrine cells, absorptive enterocytes, Paneth cells and goblet cells [150]. Each divide without maturation approximately every 20 h in the murine small intestine, with daughter cells undergoing further divisions as they migrate up towards the villi [151].

A clonogenic assay was devised by Withers and Elkind in 1969 to estimate the number of cells with a clonogenic capacity and hence the potential number of stem cells per crypt [19, 90]. This assay has since been used extensively, and it has been determined that approximately 4–16 and 1–4 stem cells are located in the small and large intestine, respectively. The stem cells are located within the crypts at positions 4–6 in the small intestine and 1–2 in the large intestine in the crypt cell hierarchy [149]. There is thought to be a further population of cells capable of clonogenic division; however, these are recruited to act as stem cells only following extensive crypt damage and loss of true stem cells [148].

Apoptosis

Kerr was the first to discover a novel form of cell death observed during his studies of acute liver injury in rats in the late 1960s. The unique morphological changes seen in this type of cell death were initially called shrinkage necrosis, but later renamed apoptosis [86, 87]. The classic morphological characteristics of a cell undertaking apoptosis include shrinking of the cellular cytoplasm, detachment from neighbouring cells and condensation of chromatin around the nuclear membrane. This is followed by blebbing of the cellular membrane and fragmentation of the nucleus and cytoplasm into membrane-bound vesicles. These are called apoptotic bodies and are quickly phagocytosed and degraded by surrounding cells [40, 88]. In contrast to necrosis, apoptosis effects individual cells with no cellular leakage at any stage of the process. This avoids the inflammatory response and major tissue disturbance that is associated with necrosis [161].

Apoptosis is an important homeostatic mechanism with wide-ranging applications throughout the body, including foetal development, demolition of redundant tissue and maintenance of tissue structure. It is also important for removing senescent cells or those that have sustained genomic damage, which left unchecked could lead to neoplasia. Apoptosis is an active and genetically regulated process, in which an individual cell responds to an internal or external stimulus resulting in its death. Deregulation of apoptosis can lead to pathological states such as neurodegenerative disease (excess cell loss) or cancer and autoimmune disease (excess cell accumulation) [100, 162, 183]. The intestinal epithelium has constant cell production and well-defined mechanisms are needed to regulate cell removal. Apoptosis is critical and is possibly even more

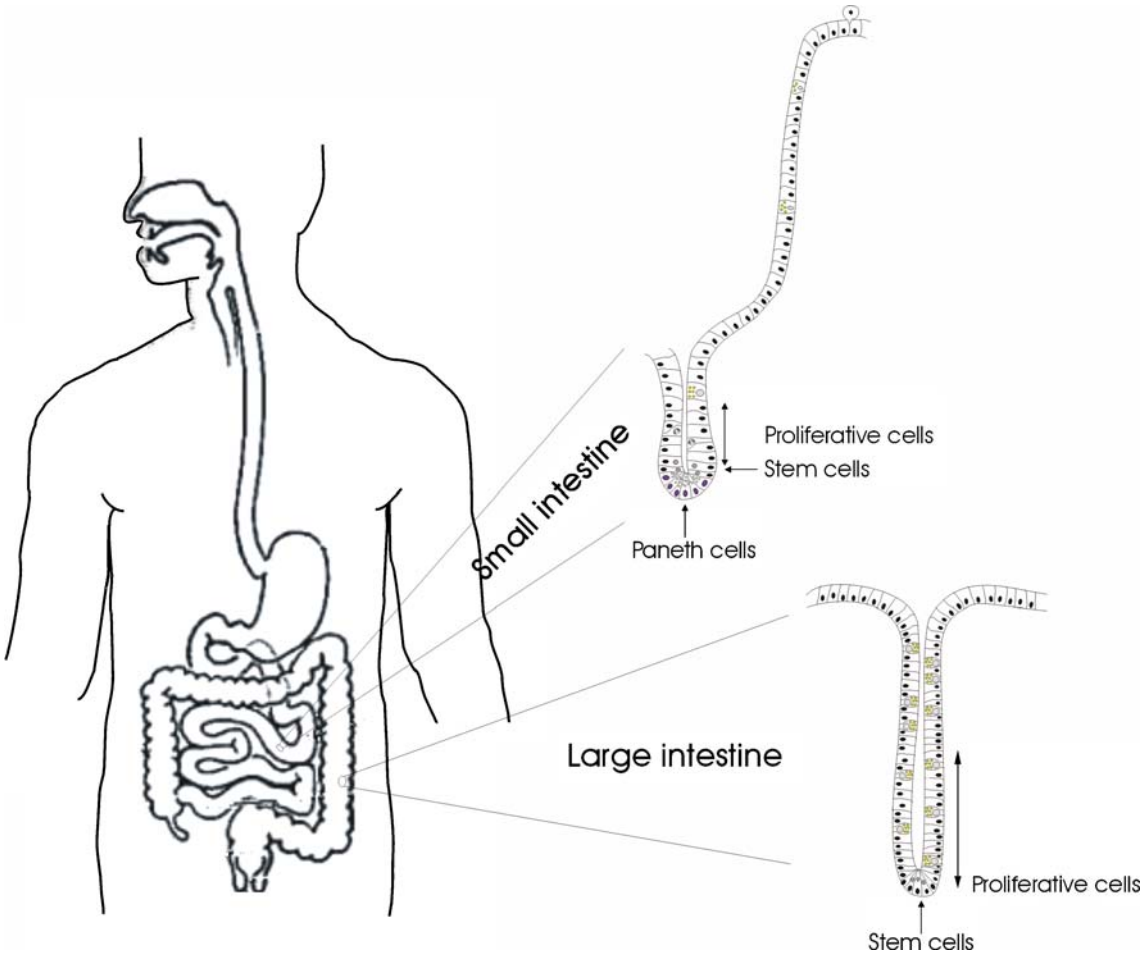

Fig. 1 Diagram of small and large intestinal epithelium showing crypts and villi. Stem cells located in the basal region of the crypt and daughter cells located directly above produce cells to migrate up the crypt axis and feed multiple villi in the small bowel and move towards the table region in the colon. Ageing cells differentiate as they move towards the lumen for extrusion

important than control of cell proliferation in maintaining correct tissue structure [163, 189]. Spontaneous apoptosis occurs in intestinal crypts, albeit not at a high frequency, removing what appears as normal, healthy cells [147]. This is thought to be removal of excess stem cells to help maintain a balance of proliferation [152].

Chemotherapy, apoptosis and mucositis

Cancer has usually been treated by surgery (if appropriate) or by use of cytotoxic chemotherapy and radiation therapy. These techniques have been successful in many haematological malignancies and a few solid tumours; however, the majority of malignancies have proven resistant to these interventions [63]. In all cases though, the effectiveness of cytotoxic treatments has been limited by the side-effects of these agents on normal tissues and cells. Various forms of supportive measures have been developed to reduce these toxicities, such as bone marrow rescue, antibiotics and growth factor support, but overall, the dose of treatment must still be limited [56]. The underlying problem is that chemotherapy drugs target healthy intestinal cells, causing apoptosis without being selective only to tumour cells [28].

Cytotoxic agents induce gastrointestinal toxicity to differing degrees, causing the severity of mucositis to vary between patients depending on their cancer and treatment regimen [72, 73]. This may be a factor of different classes of cytotoxic agents acting at particular cell positions within the crypt hierarchy. For example, drugs such as doxorubicin and bleomycin are known to act at cell positions 4–6, while actinomycin D and cyclophosphamide act at positions 6–8 and methotrexate and 5-fluorouracil (5-FU) act slightly higher at positions 8–11 [72, 73].

Classically, gastrointestinal mucositis has been attributed to both the high cell proliferation rate being interrupted and reduced by chemotherapy drugs and to the direct

killing of crypt cells [79]. While this is still true, recently a new paradigm for mucositis has arisen. The term alimentary mucositis (AM) has been coined to describe the damage that occurs to the whole alimentary tract, rather than looking at oral mucositis and gastrointestinal mucositis as separate pathologies [80]. This has developed since the realisation that the alimentary tract is all one structure embryonically and that the body has only limited ways in which it can respond to damage [80]. Therefore, it is possible that the mechanisms behind the pathobiology of mucositis are also similar regardless of region [83]. The five-phase model described for oral mucositis also must be applied to the intestine [180]. The five phases are: (1) initiation, (2) upregulation and message generation, (3) signalling and amplification, (4) ulceration and inflammation and (5) healing. Briefly, the initiating events of chemo- and radiotherapy are the generation of oxidative stress and reactive oxygen species (ROS) which act to directly damage cells, tissues and blood vessels. Secondly, the transcription factor nuclear factor kappa B (NF-κB) is activated and leads to the upregulation of many genes, including those responsible for the production of the pro-inflammatory cytokines, as well as adhesion molecules and cyclooxygenase-2. This results in further tissue injury and apoptosis. During the third phase, a feedback loop occurs, whereby tumour necrosis factor (TNF)-α acts on a number of pathways to reinforce NF-κB activation as well as the ceramide pathway. Following this, an ulcerative phase is observed, with bacterial colonisation, subsequent increased pro-inflammatory cytokine production and hence inflammation. Finally, healing occurs with the renewal of epithelial proliferation and differentiation and the re-establishment of the normal local microbial flora. These events are occurring in virtually all cells and tissues of the mucosa and also with considerable crossover of phases [179–181]. Thus, mucositis is not necessarily limited to just the epithelial layer of the mucosa, but could involve the mesenchyma and endothelium. This remains a hypothetical pathway in the intestine, and this review will continue to concentrate on the epithelial injury portion of mucositis research.

Both radiotherapy and chemotherapy damage the intestinal lining and cause apoptosis rather than necrosis of epithelial crypt cells, and crypt hypoplasia [3]. There is also increased permeability through loosening of tight junctions and an increased susceptibility to infection. Structural changes in the small intestine following treatment are also thought to be responsible for malabsorption and patient symptoms peak on days 3–5 [79]. Once established, there is no effective treatment for mucositis except limiting the dose of chemotherapy drugs administered per cycle, which may reduce the chance of remission. Mucositis has become a limiting factor in the effectiveness of cancer treatment since bone marrow toxicity can be managed by supportive care, blood transfusion and colony-stimulating factors [82].

Methotrexate and the gastrointestinal tract

Methotrexate (MTX) is a commonly used chemotherapy drug in the treatment of leukaemia and a variety of solid tumours. It is an anti-metabolite that attacks the cell in its S phase and disrupts DNA synthesis, and its mode of action is to inhibit dihydrofolate reductase (DHFR), a key enzyme in cell replication, leading to its sub-category as a folate antagonist [71, 190]. Methotrexate induces diarrhoea and anorexia, accompanied by malabsorption, malnutrition and dehydration in patients. It also inhibits epithelial proliferation and enterocyte function and increases the risk of gut-associated sepsis due to disruption of the mucosal barrier. Gastrointestinal toxicity is now the major dose-limiting factor for methotrexate administration [141, 198]. The toxicity of MTX to the gastrointestinal epithelium is dependent on the duration of cytotoxic exposure rather than the peak levels achieved by the drug. Clearance of MTX from plasma following intravenous injection is triphasic, and the terminal half-life, which begins around 24–36 h following treatment initiation, contributes the most to gastrointestinal toxicity [121].

The small intestine is the predominant site of damage after treatment with MTX. The most severe effect can be seen in the proximal small bowel, with pronounced crypt and villous ablation. Treatment with MTX causes an increase in apoptosis that reaches its peak approximately 6 h after administration in the rat [44]. The majority of apoptosis occurs in the crypt, removing the proliferative cells and leading to crypt hypolasia. The crypt is then unable to sufficiently supply the villus with new cells and atrophy occurs, reaching a nadir by 48 h. Once this initial injury to the mucosa is over, the epithelium enters a highly proliferative state to repair and regenerate the intestinal lining [66, 67, 188].

Irinotecan and the gastrointestinal tract

Irinotecan hydrochloride (or CPT-11) is a reasonably new chemotherapeutic drug, used primarily to treat colorectal carcinoma, as well as other solid tumours. Its action on cells is to inhibit the DNA enzyme topoisomerase I. The main side-effect of its use is severe and frequent gastrointestinal toxicities, most notably, diarrhoea [15, 74, 186]. Irinotecan damages both the small and large intestine [45]. Through laboratory investigations using a number of animal models, the response of the gastrointestinal tract to irinotecan has been somewhat elucidated; however, the exact mechanism of diarrhoea induction remains unknown [5, 15, 74, 186]. Changes seen in the gut following irinotecan administration include vacuolation of the epithelium, blood vessel dilatation and infiltration of polymorphic cells, and goblet cell metaplasia. It is suggested that these changes lead to malabsorption of water and elec-

trolytes and mucin hypersecretion, resulting in diarrhoea [74]. An opposing theory is that the diarrhoea is due to haemorrhagic enterocolitis. One of the mechanisms is reactivation of metabolic by-products of irinotecan by colonic bacteria. The active metabolite of irinotecan, SN-38, is retained within the intestine, and so high levels persist to cause damage [5]. Further to this, the microflora of the intestine contain β-glucuronidase activity which can convert SN-38 glucuronide (a detoxified form of SN-38) back to SN-38. Since it is SN-38 that causes intestinal cytotoxicity, inhibition of β-glucuronidase should improve irinotecan-induced diarrhoea. This has been shown, where administration of antibiotics reduced colonic β-glucuronidase activity and markedly ameliorated diarrhoea [186].

A better understanding of the mechanisms behind irinotecan-induced toxicities is important because of its widespread use. The antimetabolite fluorouracil has been the drug of choice in the treatment of metastatic colorectal cancer for several decades; however, patients who progress from this treatment are often given irinotecan [48]. However, its continued use will be questionable if the dose-limiting and often life-threatening diarrhoea associated with treatment cannot be prevented [47].

Molecular control of cell death

The nematode *Caenorhabditis elegans* contains the best defined genetic pathway of cell death, which consists of two autosomal-recessive death effector genes, CED-3 and CED-4, and one autosomal-dominant death repressor gene, CED-9. CED-3 and CED-4 are essential for programmed cell death during the worm's development, and CED-9 can prevent their action. It has since been found that CED-3 belongs to a family of cysteine proteases called caspases, the effectors of apoptosis. CED-4 has Apaf-1, a mammalian adaptor protein as its mammalian homologue. CED-9 and mammalian Bcl-2 have also been proven to be structural and functional homologues, both having a survival function within the cell [57, 58, 113].

The Bcl-2 family

Bcl-2 is the mammalian homologue of Ced-9. It is a proto-oncogene that was first discovered owing to the characteristic t(14;18) translocation found in the majority of follicular lymphomas. In this translocation, Bcl-2 at chromosome segment 18q21 is juxtaposed with the immunoglobulin heavy chain locus at 14q32, resulting in deregulated and subsequent overexpression of the gene's protein product. It is the first example found of an oncogene that aids tumour genesis via lack of cell death rather than by increasing proliferation [1, 208]. Bcl-2 holds cells in G_0/G_1 phase of the cell cycle, and this accounts for the usual early appearance of low-grade disease comprising small resting B cells in follicular lymphomas which overexpress Bcl-2 [142]. In T cells, Bcl-2 maintains G_0 phase by delaying the loss of the cyclin-dependent kinase inhibitor, p27, by inhibiting interleukin (IL)-2 production by the transcription factor nuclear factor of activated T cells (NFAT) [110]. Activation of NFAT requires calcineurin, a calcium-dependent phosphatase, which Bcl-2 is able to bind effectively [33]. Bcl-xL has also been shown to delay cell cycle entry by elevating p27 levels in fibroblasts [50]. More pertinent to this review though is that Bcl-2 has been shown to have the ability to rescue cells from apoptosis in response to a wide range of stimuli, including radiation, heat, chemotherapy agents and granzyme B. The exact way in which Bcl-2 inhibits apoptosis has been difficult to elucidate due to its lack of conformational similarity to other proteins which have a known function [122, 210], but possible mechanisms will be discussed below.

Bcl-2 is an integral membrane protein and is the founding member of a family that now has at least 20 members identified in mammalian species (Fig. 2) [60]. The Bcl-2 family consists of both pro-apoptotic and anti-apoptotic members, and their main action appears to be regulation of cellular protease activation. Anti-apoptotic family members include Bcl-2, Bcl-xL, Bcl-w, Mcl-1 and A1. Pro-apoptotic family members belong to one of two groups, the Bcl-2-like proteins including Bax and Bak, and the single domain proteins including Bcl-xS, Bad, Bim, Bid, Noxa and Puma (Fig. 2) [17, 118, 131, 135].

The protein structure of the Bcl-2 family members consists of conserved regions of amino acids called Bcl-2 homology (BH) domains. The BH domains allow interaction between the family members, which form homodimers and heterodimers with each other. There are four domains identified, BH-1 to BH-4, and family members can contain from only one up to all four of the domains. The BH-1 and BH-2 domains are thought to be critical for the anti-apoptotic action of Bcl-2 and Bcl-xL, whilst the BH-3 domain is primarily associated with inducing death in the pro-apoptotic family members [17, 84, 210]. In the pro-survival members, the central BH-1, 2 and 3 domains form a hydrophobic cleft which is stabilised by the N-terminal BH-4 domain, while the BH-3 motif of pro-apoptotic family members consists of an amphipathic helix. This allows the α-helix of antagonising pro-apoptotic Bcl-2 family members to dock within the hydrophobic groove of the anti-apoptotic protein, in a similar fashion to a receptor ligand interaction, and regulate its action within the cell [12, 23, 35].

The ratio of pro-apoptotic to anti-apoptotic family members within each cell is important for determining protein–protein interactions and ultimately the cellular sensitivity to an apoptotic stimulus [137, 160, 170]. There have been two main models proposed for how this ratio determines a cell's propensity for apoptosis. The first is that Bcl-2 and Bcl-xL are passive receptors of the apoptosis-inducing BH-3 domain of pro-apoptotic members, so that

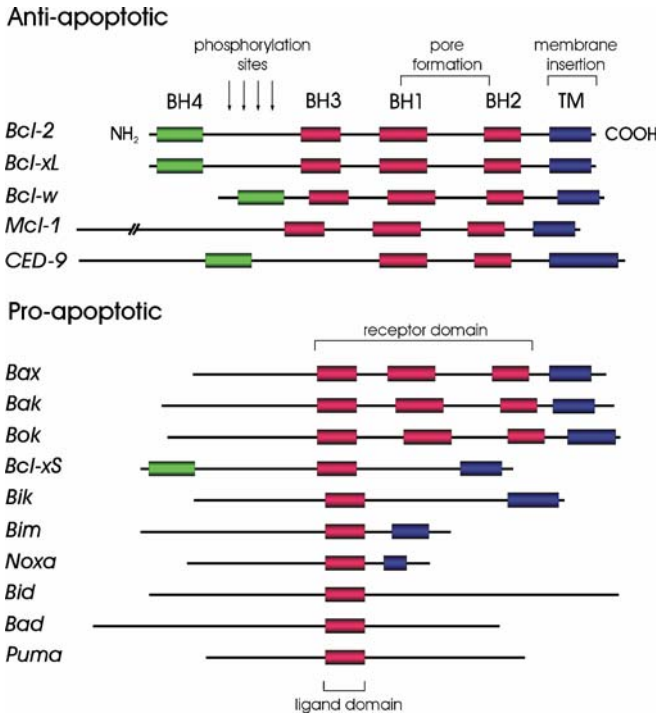

Fig. 2 Domain arrangements of various Bcl-2 family proteins, showing the 1 to 4 Bcl-2 homology (BH) domains and transmembrane domain at the carboxy terminus. Also indicated is the proposed function of each of the areas or domains

when they are abundant in the cell, heterodimerisation with Bax prevents the formation of toxic Bax homodimers simply by sequestration, resulting in cell survival. The second theory is that Bcl-2 and Bcl-xL are active repressors of cell death, and heterodimerisation with Bax blocks their ability to prevent apoptosis [84, 100]. Studies using mutant forms of Bcl-2 expressed in mammalian tissue found that mutated Bcl-2 that failed to bind Bax lost it's death-sparing function. This suggests that anti-apoptotic members of the Bcl-2 family require heterodimerisation to protect the cell from apoptosis [55]. It has also been shown that certain pro-apoptotic family members require heterodimerisation with the anti-apoptotic members of the family to exert their death-promoting activity. Bad is a BH-3-only domain protein that requires its ability to dimerise with Bcl-xL to induce cell death. Kelekar et al. stated that Bad induces cell death by dimerising with and deactivating Bcl-xL [85], whereas Yang et al. reported that Bad's mode of action is simply to sequester Bcl-xL from Bax, leaving Bax to form toxic homodimers and induce cell death [209]. It has also been shown that Bax and Bak are critical for BH-3-only protein-induced cell death. In experiments using Bax-/-Bak-/- cells, Bim, Bid and Bad failed to induce apoptosis despite heterodimerisation with anti-apoptotic Bcl-2 family members [204, 213]. Thus, there remains some controversy as to whether the pro-apoptotic or anti-apoptotic proteins play the dominant role in regulating cell death. Further to this, new hypothetical models of the way Bcl-2 family members interact in the cell to control apoptosis have been proposed. These include the suggestion that an intermediary protein, Bid, is responsible for Bax activation once all cellular Bcl-2 are saturated with BH-3-only proteins. It has also been postulated that heterodimerisation does not play a role at all and instead Bcl-2 prevents Bax activation through an indirect mechanism such as sequestering an as-yet-unknown protein or the small molecules released during early apoptosis that drive Bax activation. It also seems possible that in accordance with the *C. elegans* model, Bcl-2 acts to sequester a CED-4-like molecule to prevent it from causing downstream activation of Bax via caspases (explained in later sections of this review).

There are a considerable number of ways that the Bcl-2 family can be regulated to effect cellular survival or apoptosis, including transcription, translation, post-translational control, alternative splicing and intracellular redistribution [2]. In viable cells, the majority of Bax is monomeric and found either in cytoplasm or loosely attached to intracellular membranes. After an apoptotic stimulus, Bax translocates to the mitochondria, where it becomes an integral membrane protein and cross-linkable as a homodimer. Therefore, activation of Bax appears to involve subcellular translocation and dimerisation. It has been suggested that this translocation is affected through exposure of its amino terminal domain, which is concealed under normal conditions to keep the molecule in a closed configuration. The pro-apoptotic family member Bid has been proposed to be involved in exerting the conformational change to Bax after an apoptotic stimulus [32, 51]. Bcl-2 activity can be regulated by phosphorylation, which occurs at serine residues 70 and 87. Phosphorylation has been associated with loss of Bcl-2's anti-apoptotic action and can be induced by microtubule-damaging chemotherapy agents such as Taxol. Bcl-2 phosphorylation has been shown to inhibit heterodimerisation with Bax [53, 154, 178, 182].

Pro-apoptotic family members Bad, Bid and Bim are also controlled by distinct post-translation modifications. In the presence of IL-3, Bad is hyperphosphorylated and sequestered by the cytosolic phosphoserine-binding protein 14-3-3. Thus, it is unable to dimerise with anti-apoptotic Bcl-xL and is essentially inactive. Following IL-3 deprivation, Bad is dephosphorylated, allowing it to associate with Bcl-xL. The ability of Bad to induce cell death is dependent on it being able to dimerise with anti-apoptotic members of the Bcl-2 family, but requires dephosphorylation after an apoptotic stimulus for this to occur [85]. Bid is activated through caspase-mediated proteolysis. This activation allows Bid to induce the conformation change in Bax and Bak required for mitochondrial membrane insertion [32, 183]. Finally, Bim is regulated by binding to the dynein motor complex. In healthy cells, Bim is bound to LC8 cytoplasmic dynein light chain and therefore sequestered to the microtubule-associated dynein motor

complex. Apoptotic stimuli disrupt the interaction between LC8 and the dynein motor complex, thus freeing Bim. This allows Bim to translocate to the mitochondrial membrane where it interacts with Bcl-2, neutralising its anti-apoptotic activity [158, 159, 184].

Little is known about the transcriptional regulation of Bcl-2 family proteins in normal cells, although a number of transcription factors are slowly becoming evident. Firstly, it is known that p53 has both positive and negative regulatory effects on Bax and Bcl-2, respectively. In most settings, an apoptotic stimulus causes a p53-dependent increase in Bax transcription and a decrease in Bcl-2 transcription to alter the Bax/Bcl-2 ratio and induce cell death [123, 124, 205]. Control is through a p53-responsive binding site in the promotor of the Bax gene and Bcl-2 containing a p53-dependent negative response element in its 5′ untranslated region [10, 124]. Other Bcl-2 family members known to be p53-responsive include Bak, Bid, Bcl-xL, Noxa and Puma [78, 131, 166, 172]. NF-κB has also been investigated for its role in modulating transcription of anti-apoptotic Bcl-2 family members, in particular A1 [202]. In resting cells, NF-κB is sequestered in the cytoplasm in an inactive form which is bound to its inhibitor protein IκB. Upon stimulation (such as during chemotherapy treatment), IκB is phosphorylated, dissociates from NF-κB and is degraded by proteases. This allows NF-κB to become activated, whereby it translocates to the nucleus and binds to and upregulates target genes, including Bcl-2, Bcl-x and A1 [16, 18]. The retinoblastoma protein (pRB) has also been shown to transcriptionally activate the Bcl-2 gene in epithelial cells in conjunction with the transcription factor AP-2, which both bind to the same promoter sequence [27]. Further to this, the E2F1 transcription factor, which is a downstream target of pRB, has been shown to upregulate the expression of pro-apoptotic Bcl-2 family members Puma, Noxa and Bim by binding to their respective promoters [62]. Finally, there is evidence that growth factors, such as granulocyte–macrophageclony-stimulating factor (GM-CSF) and insulin-like growth factor (IGF)-1, regulate activity of Bcl-2 proteins post-transcriptionally by altering the rate of protein synthesis [70, 111, 187].

Caspases

Caspase-dependent cell death is the best defined pathway in apoptosis and can be regulated by the Bcl-2 family [191]. Caspases are a group of cysteine proteases so-called due to their specific cleavage of proteins after aspartic residues. They share similarities in amino acid sequence, structure and substrate specificity and are synthesized within the cell as minimally active precursors to avoid premature activation. Under non-apoptotic conditions, each caspase is expressed as a 30- to 50-kDa inactive pro-enzyme (zymogen), which contains three domains: the NH2-terminal domain, a large subunit approximately 20 kDa in size and a small subunit approximately 10 kDa in size [22, 175, 192]. Activation of caspases results from proteolytic processing between its domains, followed by association of the small and large subunits to form a heterodimer. To initiate this activation, the caspases must have proteolytic cleavage at aspartic residues by either themselves or by other caspases. In this way, proteins that promote caspase aggregation play an important role in proximal caspase activation events and can lead on to a cascade of sequential caspase activation [22, 175, 192].

The role of caspases is to inactivate proteins that protect the cell from apoptosis. A clear example is the cleavage of I/CAD, an inhibitor of the nuclease responsible for DNA fragmentation. Other negative regulators of apoptosis cleaved by caspases are the anti-apoptotic members of the Bcl-2 family. Cleavage of these appears to not only inactivate the proteins, but also promote apoptosis in a positive feedback fashion. Direct disassembly of cell structures is another caspase function, as illustrated by the destruction of nuclear lamina, which contributes to chromatin condensation. Caspases also cleave several proteins involved in cytoskeleton regulation and inactivate proteins involved in DNA repair, replication and mRNA splicing [133, 192].

Individual caspases are numbered and can be grouped according to their function within the cell, specifically, apoptotic initiators 8, 9 and 10; executioners 2, 3, 6 and 7; or inflammatory mediators 1, 4, 5, 11 and 12 [24, 116]. Caspase-3 in particular has been labelled the quintessential executioner caspase, with its expression classed as a positive marker for apoptosis [115, 165]. Initiator caspases are those that are activated first in the apoptotic sequela, such as in response to death receptor activation and mitochondrial factor release (described in detail later in this review). While caspase-1 is essential for the production of pro-inflammatory cytokines including mature interleukin-1β, interleukin-1α and interleukin-18 [24].

Caspase-independent cell death can also be controlled by Bcl-2, with studies showing an inhibition of apoptosis in response to nitric oxide, hydrogen peroxide and menadione. This work has led authors to suggest that Bcl-2 may also work in an anti-oxidant pathway to regulate free radical generation at intracellular membranes, therefore inhibiting cell death [65, 136].

p53

Wild-type p53 is a nuclear phosphoprotein. It was first described in 1979 when it was discovered as part of studies investigating SV40 (simian virus) transformed cells. It was found to complex with the SV40 DNA tumour virus large T antigen and so was classified as a transformation-related protein [29, 106, 112]. Interestingly, p53 was first thought to be a proto-oncogene as initial studies showed that it was overexpressed in mouse and human tumour cells [30]. It

was also able to immortalise cells in culture. However, it became apparent some time later that these experiments were actually using mutant forms of the protein which was acting in a dominant negative fashion to inhibit the function of the normal protein [34, 64]. Subsequent research confirmed that wild-type p53 is a tumour suppressor gene, due to its ability to suppress transformation by oncogenes and inhibit growth of transformed cells [7]. Further to this, p53 gene knockout mice have an increased risk for development of malignancy, as do families with the Li–Fraumeni syndrome who have germ-line mutations of p53 [93].

p53 belongs to a family of proteins that contains three members: p53, p63 and p73. The p53 gene encodes a single protein containing 393 amino acid residues and is 53,000 kDa in size. It consists of four main functional domains: a transactivation domain (residues 1–42); a poly-proline-rich region (residues 61–94); a central core domain, required for sequence-specific DNA binding (residues 102–292); and the oligomerisation domain (residues 324–355). There is also the C-terminus which contains a nuclear localisation sequence and possesses both RNA and non-specific DNA-binding activities (residues 356–393) [176]. The half-life of the p53 protein is relatively short (only 20 min), and so normally, the cellular concentration remains very low. It may also exist in a latent form and may require activation to carry out functions within the cell. The functions of p53 are diverse and complex; however, the main cellular responses following activation of the protein include cell cycle regulation, DNA repair and apoptosis [177].

Levels of p53 protein are increased after various stress stimuli including hypoxia, ultraviolet radiation, hypoxia, heat shock, growth factor withdrawal, oncogene activation and cytotoxic drugs [201]. This occurs mainly due to increases in p53 stability, through phosphorylation of the protein and a decrease in association with the Mdm2 protein that normally targets it for degradation via the ubiquitin-mediated proteasome pathway. It is then able to act as a transcriptional activator of target genes to induce G_1 cell cycle arrest and/or apoptosis. Apoptosis is the dominant mechanism by which p53 inhibits tumour development and is highly conserved through evolution, with the homologues for p53 in *Drosophila* and *C. elegans* being Dmp53 and Cep-1, respectively [176, 199].

Bcl-2 family and the apoptotic pathway

Mitochondria and the Bcl-2 family in apoptosis

The mitochondria play a crucial role in the activation of apoptosis, with growing evidence that all Bcl-2 family members described so far act exclusively on the cytoplasmic face of the mitochondria (and/or the endoplasmic reticulum) [169]. Apoptotic stimuli have been shown to initiate a change in mitochondrial membrane potential prior to caspase-dependent cell death. Loss of membrane potential may be due to opening of a non-selective ion channel called the permeability transition (PT) pore or mitochondrial megachannel. Opening of this pore allows the release of proteins usually restricted to the matrix of the mitochondria that have apoptogenic potential. These proteins include apoptosis-inducing factor (AIF), which has been found to break down chromatin into large molecular weight fragments, and cytochrome *c*, which once released facilitates activation of caspases through its association with the adaptor molecule Apaf-1 [49, 185]. Also released is a second mitochondria-derived activator of caspases (Smac) which is a mammalian IAP inhibitor that binds to and neutralises their anti-apoptotic activity [60]. Further to this, the mitochondrial intermembrane space also contains certain pro-caspases, which upon release become enzymatically active [195].

The mitochondrial PT pore is thought to occur at the site of contact between the inner and outer mitochondrial membranes. Constituents of the pore include the voltage-dependent anion channel (VDAC), adenine nucleotide translocator (ANT) and cyclophilin D. Inhibitors of the membrane permeability transition are able to block apoptosis, thus suggesting that the PT pore is central to the apoptotic process [14, 117]. Furthermore, it has been found that hypoxia-induced increases in free cytosolic calcium are able to open the PT pore [25]. Bcl-2 family proteins interact with the PT pore by modulating VDAC activity [173]. Bcl-2 is anchored to the outer mitochondrial membrane, and its primary role is suggested to be regulation of cytochrome *c* release [92, 164]. Bcl-xL has fractions in both the cytosol and the mitochondrial membrane, whilst Bax is a cytosolic protein under normal conditions. After an apoptotic stimulus, Bax and Bcl-xL translocate to the mitochondrial membrane; thus, membrane association has been implicated in these proteins' regulatory functions [51, 69]. It is thought that the BH-4 domain of Bcl-2 and Bcl-xL is responsible for directly inhibiting VDAC activity, whilst Bax and Bak stimulate VDAC activity through their BH-3 domains, allowing passage of cytochrome *c* [132, 173].

Alternatively, Bcl-2 family members may act as ion channels themselves at the mitochondrial membrane [59]. Bax has been shown to form pH- and voltage-dependent ion channels in lipid bilayers which can be inhibited by Bcl-2. Thus, rather than acting on the VDAC directly, Bax may elicit its pro-apoptotic effects through an intrinsic pore-forming action [4]. Based on predicted three-dimensional structures, possibly similar to the pore-forming domain of bacterial toxins, various other proteins in the Bcl-2 family may also act at the mitochondria by forming ion-conducting channels [161].

Distribution of Bcl-2 family proteins after an apoptotic stimulus in relation to the mitochondria appears to influence greatly the effect on the cell. Khaled and colleagues found that after withdrawal of IL-7 and IL-3 from a

dependent cell line, a transient rise in intracellular pH occurred. This alkalisation resulted in exposure of the hydrophobic transmembrane domain of Bax and translocation to the mitochondria [89]. Hsu and colleagues looked at normal mouse thymocytes treated with dexamethasone to induce apoptosis. This treatment did not cause an overall increase in the level of Bcl-2, Bcl-xL or Bax protein but did induce the translocation of Bcl-xL and Bax to the outer mitochondrial membrane. Inhibition of apoptosis by the drug cycloheximide blocked the shift of these proteins to the membranes. Furthermore, removing the C-terminus from the proteins halted membrane insertion but did not alter their death-promoting or death-inhibiting action [69]. Although little data are published, proteins that may be involved in binding the Bcl-2 family to the mitochondria or putative membrane receptors include the TOM-proteins (TOM-20 and 22) and mitochondrial FK506-binding protein 38 [169].

ER and the Bcl-2 family in apoptosis

Bcl-2 family proteins are also localised to the endoplasmic reticulum membrane, although little is known about their action there. Several ER membrane proteins though have been shown to interact with the Bcl-2 proteins to enhance their anti-apoptotic ability, for example Bax inhibitor-1 and Bcl-2-/Bcl-xL-associated Bap-31. Similarly, Cnx1 interacts with Bak to increase its pro-apoptotic potential. In contrast, members of the ER-anchored reticulon family NSP-C and RTN-Xs bind to Bcl-xL and Bcl-2 and contribute to apoptosis [60]. It is known that the ER plays an active role in apoptosis by activating stress response pathways JNK, NF-κB and caspase-12, and now, growing evidence suggests that there is considerable cross talk between the ER and mitochondria. It is also thought that Bcl-2 may exert its protective effect in the compartments by controlling calcium homeostasis [41]. Furthermore, it is thought that expression of Bcl-2 decreases the free Ca^{++} concentration within the ER lumen by increasing the Ca^{++} permeability of the ER membrane and hence works in an ion channel function possibly similar to the mitochondria [36].

Death receptors and the Bcl-2 family

The Bcl-2 family can also regulate apoptosis at the cell surface level through the death receptor pathway. The death receptor Fas (which belongs to the tumour necrosis factor superfamily) and its ligand have been shown to activate caspases rapidly after an apoptotic stimulus. Fas utilises an adaptor protein Fadd to recruit caspase-8 to initiate a cascade of sequential caspase activation that leads to cell death [6, 130]. There is also significant cross talk with the mitochondrial pathway of cell death, particularly through the Bcl-2 family member Bid. Bid is activated by caspase-8, which in turn initiates the conformational transformation of Bax and its subsequent insertion into the mitochondrial membrane [114]. The Bax/Bcl-2 ratio within a cell has also been recognised as a predictor of the susceptibility of cells to Fas-mediated apoptosis [160], and in many cells, overexpression of Bcl-xL or Bcl-2 can inhibit the Fas signaling pathway [167]. High levels of Fas and FasL have been found in the intestine [144].

Bcl-2 and apoptosis in the intestine

Research conducted by Krajewski and colleagues has detailed the expression of different Bcl-2 family members throughout normal and neoplastic tissue [101–105]. Using immunohistochemistry, they have observed differences in expression profiles between the small and large intestines and also within the tissues regions for the Bcl-2 family members. Most importantly, Bcl-2 is strongly expressed within the crypts of the colon, whereas there is very little to no expression within the crypts of the small intestine. Conversely, there is intense staining of Bax within the crypts of the small intestine and weak expression within the colonic crypts. This pattern of expression for pro- and anti-apoptotic family members may be a contributing factor in the increased sensitivity of small intestinal stem cells to apoptosis compared to those of the large intestine [120, 156].

The Bcl-x gene can produce two protein products, anti-apoptotic Bcl-xL and pro-apoptotic Bcl-xS. It is believed that Bcl-xS is not expressed within the intestine, but the long isoform Bcl-xL has widespread expression within the small and large intestine. This anti-apoptotic family member has shown expression within the crypts and villi of the small intestine and predominant staining in the apical region of the colonic crypts [102]. The pro-apoptotic family member Bak is also found to be strongly expressed within the intestine and is functionally similar to Bax [19]. Indeed, tissues which express little to no Bax, such as the absorptive epithelial cells of the jejunum and ileum, often contain prominent Bak immunostaining. It has also been suggested that Bak may be the principal promoter of apoptosis in the intestinal epithelium. This is due to its increased expression in regions of apoptosis and the lack of consistent changes in expression after an insult in the other family members [126].

Bim is a BH-3-domain-only member of the Bcl-2 family that is also expressed throughout the intestine. Its pattern of expression is somewhat reciprocal to that of Bax, with staining seen in the base of the colonic crypts and also throughout the villi [138]. Another BH3-only protein, Bad, has significantly more restricted expression than other pro-apoptotic Bcl-2 family proteins with very weak or absent expression in the intestine [91]. By Western blot analysis, the anti-apoptotic Bcl-2 family protein, Bcl-w, was found

to be expressed strongly in the colon and moderately in the small intestine [139]. Lastly, Mcl-1, an anti-apoptotic family member which is closely related to Bcl-2, has been examined. In the small intestine, it has strong expression throughout the epithelium, although more intense in the cells located at the upper portions of the villi. In the colon, this gradient of moderate staining in the crypt base to intense staining at the luminal surface is roughly opposite to that of Bcl-2 [103] (Table 1; Fig. 3).

The role of Bcl-2 in damage-induced apoptosis in the intestine

Studies using gene knockout mice have highlighted the importance of Bcl-2 family protein expression in the intestinal crypt response to radiation and chemotherapy. Bcl-2-null mice have a greatly larger apoptotic response in the colonic crypts after radiation compared with wild-type litter-mates [156], whilst mice with no Bcl-w gene have much higher apoptosis levels in the crypts of the small intestine after treatment compared to control mice with an intact Bcl-w gene [157]. In contrast, the intestinal crypts of Bax-null mice do not have a decreased apoptotic response compared to control mice [156]. This may be due to other pro-apoptotic members of the Bcl-2 family substituting for Bax and taking over its role as the presumed quintessential apoptotic protein. Evidence to support this has come from experiments using Bax-null, Bak-null or both models which show that damage-induced apoptosis is only ameliorated when both genes are knocked-out [204]. Thus, it is apparent that apoptosis can be controlled by a number of different Bcl-2 family members, and the balance of expression may determine the response to chemotherapy in the crypts.

The small and large intestines have different susceptibility to spontaneous and damage-induced apoptosis. Under normal conditions, approximately one apoptotic

Table 1 Summary of immunohistochemistry results found by varying authors in the gastrointestinal tract

Author	Bcl-2 family member	Function	Protein expression: small intestine	Protein expression: large intestine
Krajewski	Bcl-2	Anti-apoptotic	Villi, low/moderate; crypts, absent	Apical cells, low; basal cells, high
	Bax	Pro-apoptotic	Villi, moderate; crypts, high	Apical, high; basal, low
	Bcl-xL	Anti-apoptotic	Villi, moderate; crypts, moderate	Apical, moderate; basal, low
	Bak	Pro-apoptotic	Villi, high; crypts, high	Apical, high; basal, low
	Mcl-1	Anti-apoptotic	Villi, moderate; crypts, very low	Apical, moderate; basal, absent
Hirose	Bcl-2	Anti-apoptotic	Not investigated	Immunopositivity detected in lower half of crypts
	Bcl-xL	Anti-apoptotic	Not investigated	Immunopositivity detected in entire crypt length
	Bax	Pro-apoptotic	Not investigated	Weak immunopositivity detected in apex of crypt
Krajewska	Bid	Pro-apoptotic	Staining present absorptive epithelium	Not investigated
Wilson	Bax	Pro-apoptotic	Immunoreactivity strongest crypt base	Immunoreactivity seen upper portion of crypts
	Bad	Pro-apoptotic	Low expression villus tip and apical crypt cells	Immunoreactivity moderate in table region and absent from crypt base
O'Reilly	Bim	Pro-apoptotic	High expression villi and crypt cell	Moderate expression in crypt cells
	Bcl-w	Anti-apoptotic	Moderate expression detected	Strong expression detected
Hockenbery	Bcl-2	Anti-apoptotic	Expression detected lower half of crypts	Expression detected lower half of crypts
Lu	Bcl-2	Anti-apoptotic	Bcl-2-positive cells not detected	Positive cells present in crypt base only

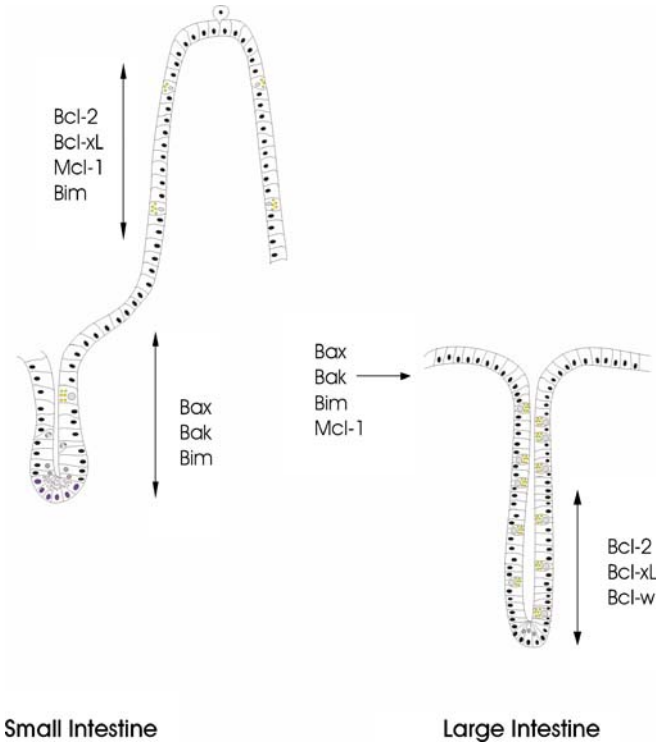

Fig. 3 Diagram showing the distribution patterns of a selection of Bcl-2 family members in the small and large intestine, as investigated by immunohistochemistry by the authors, summarised in Table 1

cell per villus can be seen in sections cut at 3 μm in the small intestine. Furthermore, one cell in every five crypts (or less than 1% of crypt cells) will be undergoing apoptosis at any one time, although this can be as high as 10% within the stem cell region. In the large intestine, it is very rare to see an apoptotic cell in the stem cell region, with only occasional apoptotic cells seen scattered throughout the crypt and table region. Overall, spontaneous apoptosis occurs tenfold less in the large intestine compared to the small intestine, and this may contribute to the differential cancer incidence between the two tissues [63, 153].

In studies examining the effect of radiation on the intestine, it was found that the small intestine is much more susceptible to damage-induced apoptosis than the colon. This increased sensitivity has been attributed partly to the lack of expression of anti-apoptotic Bcl-2 within the crypts of the small intestine [76, 119, 120]. The intestine responds with a dose-dependent increase in apoptosis, which peaks approximately 3 to 6 h after treatment. Increasing numbers of cells in the small intestine die up to a dose of approximately 1 Gy, where ~6 cells per crypt are killed. Radiation primarily attacks at the site of the stem cell region of the crypts of the small intestine. Within the large intestine, an increasing number of cells die up to 6–8 Gy of radiation, but when specific doses are studied, apoptosis occurs at a lower level compared to the small intestine. The site of apoptotic cells is not specifically located at the stem cell region within the colon but instead scattered throughout the crypt [153].

Further studies have also examined the difference in sensitivity to apoptotic stimuli between the small and large intestine. After treatment with a number of pharmacological compounds that inhibit signal transduction molecules, it was found that in all cases, there was a greater increase in apoptosis seen in the small intestine than that in the large intestine. It was concluded that segment-specific expression of Bcl-2 family members was at least in part responsible for cell survival within the colon after disruptions to signaling pathways [38, 39].

Effect of chemotherapy agents on p53 and Bcl-2 expression

Chemotherapy and Bcl-2

Breast cancer cells treated with various cytotoxic agents to induce apoptosis upregulate Bax and downregulate Bcl-2 in a time-dependent and dose-dependent manner [42, 109]. Similar experiments using colon cancer cell lines treated with 5-FU found that Bax and Bak expression was significantly increased without consistent change to other family members [134]. An increase in Bax expression was observed in the intestinal crypts of mice, concurrent with sites of apoptosis following 5-FU treatment [75]. Many chemotherapy agents induce apoptosis in a p53-dependent manner which is known to critically involve the Bcl-2 family [63, 107, 119]. Hence, it is plausible that similar results could be seen in studies using normal intestinal tissue. A report by Kitada et al. has shown that gamma-radiation increases Bax expression in mouse small intestine [91]. Recently, it has also been shown that combined chemoradiotherapy (MTX + whole body irradiation) causes an increase in Bax, Bak and Bad RNA expression in the mouse intestine [9].

Chemotherapy and p53

The effect of chemotherapy treatment on p53 expression and subsequent apoptosis in the intestine has been thoroughly investigated compared to the Bcl-2 family. Activation of p53 occurs in response to DNA damage and results in either the arrest of the cell cycle at one of its checkpoints or the induction of apoptosis [10]. The activation of p53 is mainly at the post-translational level, where the protein is stabilised through phosphorylation by a number of cell protein kinases. Possibly the most important activator of p53 is the protein kinase ATM, which recognises double-stranded DNA breaks [8]. This stabilisation allows it to accumulate in the nucleus, where it

binds to and modulates transcription of genes including Bax and Bcl-2 (described above) and the cyclin-dependent kinase inhibitor $p21^{waf1/cip1}$. The protein products of these genes regulate apoptosis and cell cycle progression, respectively. The action on these genes in part explains the cells' response to stress [96]. Enhanced transcription of the death receptor gene Fas by p53 also occurs after chemotherapy treatment, which enhances the sensitivity of the cell to apoptosis [61, 127, 128].

Transcriptional upregulation of effector genes is not the only way in which p53 can induce cell death. Recently, it has been shown that cytoplasmic p53 can directly interact with members of the Bcl-2 family at the mitochondrial membrane to induce cytochrome *c* release [31]. Although the exact mechanism remains to be defined, it has been postulated that accumulation of p53 in the cytoplasm can function analogously to BH-3-only proteins (such as Bid), causing oligomerisation and membrane insertion of the pro-apoptotic proteins Bax and Bak [20]. Alternatively, p53 could disrupt Bax/Bak heterodimers with anti-apoptotic family members and effectively become the dominant complex. This would release Bax and Bak to associate with the mitochondrial membrane. Evidence to support this is the fact that mitochondrial localised p53 has been shown to disrupt the Bak/Mcl-1 complex preceding caspase activation [108]. As such, it appears p53 has the ability to modulate apoptosis independent of new protein synthesis.

It is suggested that the amount of p53 protein present in the tissue determines its response to genotoxic stress [96, 107, 205], and studies investigating the correlation between p53 status and radiosensitivity in the small and large intestine have supported this statement. Wilson and colleagues found that ionizing radiation induced p53 expression in the crypts of the small intestine and that sites of p53 immunopositivity were coincident with distribution of apoptotic cells [207]. Further to this, experiments using p53 null mice given whole body irradiation showed no difference in apoptosis levels compared to non-treated animals. Hence, the intestinal epithelial cells became radio-resistant with loss of p53 [119]. Finally, a landmark study by Pritchard and colleagues showed that p53 is responsible for crypt damage after treatment with 5-FU. The experiment used $p53^{-/-}$ mice which after cytotoxic treatment had significantly reduced apoptosis and inhibition of cell cycle progression compared to $p53^{+/+}$ mice. In these mice, crypt integrity was maintained, proving that the p53-dependent response to cell stress is responsible for gastrointestinal damage during chemotherapy [155]. Thus, both radiation and chemotherapy induce a p53-dependent apoptosis of crypt cells.

Prevention of mucositis, p53 and pifithrin-α

Considerable effort has gone into developing ways to prevent intestinal mucositis, with studies investigating a wide range of possible therapeutical strategies. Growth factors including KGF (Palifermin), IGF and TGF-β have each been shown in different settings to reduce damage in intestinal tissue to some degree after chemotherapy treatment [11, 43, 44, 46, 68]. Worth noting is the recent approval of the use of recombinant human KGF (Palifermin, Amgen) in the prevention of oral mucositis during haematological malignancy. Recently, a study using TGF-β2 has had modest success in preventing small intestinal side-effects of MTX treatment in rats possibly through modulation of stem cell cycle time [196]. Oral administration of anti-doxorubicin (doxorubicin is an anti-metabolite chemotherapy agent) monoclonal antibodies to mice has also had some success in reducing gastrointestinal toxicity. This treatment inhibited crypt cell apoptosis, weight loss and mortality after mice were given 12 mg/kg of doxorubicin [125]. A synthetic bacterial lipopeptide, JBT-3002, has shown to inhibit irinotecan-induced damage in the intestine by protecting the epithelium and lamina propria [174]. In another study, an intestinal trophic peptide that is secreted by enteroendocrine cells in response to injury called glucagon-like peptide (GLP-2) was administered to mice before treatment with the chemotherapy drugs irinotecan hydrochloride and 5-FU. Mice that received GLP-2 showed significantly improved survival rates and attenuation of epithelial damage compared with buffer-treated animals [13]. In contrast to this, an experiment using lactoferrin to inhibit GLP-2-induced proliferation in the intestine was effective in reducing MTX-induced damage. The authors suggested that temporarily arresting epithelial cell cycling in the intestine protected it from the deleterious side-effects of the anti-cancer therapy [197]. A somewhat natural approach to limiting the gastrointestinal side-effects of MTX treatment in the rat was investigated by Horie and colleagues, who used extracts of aged garlic. By measuring small intestinal permeability of the compound fluorescein-isothiocyanate-labelled dextran, they showed that rats fed with the aged garlic extract diet maintained a similar level of permeability to untreated rats, indicating they had ameliorated MTX-induced damage [66]. Rats fed soybean extract also appeared to be somewhat protected from the gastrointestinal side-effects of MTX treatment, with improved incidence of diarrhoea, anorexia and crypt cell death compared to animals fed a normal rat chow diet [37]. While milk growth factors enriched from cheese whey reduce bacterial translocation and increase jejunal crypt

area in rats following MTX treatment [67]. Other factors trialed as anti-mucotoxics include vitamin A, bombesin, intestinal trefoil factor, zinc and glutamine [9, 21, 129, 140, 193]. Many other studies not mentioned have attempted to find a way to prevent mucositis, but currently, there is no effective anti-mucositis treatment.

p53 and pifithrin-α

p53 is one of the major determinants of the side-effects of chemotherapy [155]. Thus, it seems likely that inhibiting the p53-dependent apoptotic response may effectively reduce damage in the intestine. Suppression of p53 as a method for overcoming the side-effects of antitumour therapy is a relatively new idea. Previously, efforts have concentrated on re-establishing p53 function in tumours rather than further suppressing p53, which was seen as too dangerous. However, more than 50% of human cancers do not have functional p53, and so it may prove a useful therapy. Of course, p53 suppression with molecules during chemotherapy is only suited to those patients already shown to lack active p53 in their cancer [95–97].

Pifithrin-alpha (PFTα) is an anti-parasitic compound effective in protecting cultured neurones from death induced by DNA-damaging topoisomerase 1 and 2 inhibitors. It has a potential role in the prevention of neurodegenerative diseases such as Alzheimer's, Parkinson's and cerebrovascular stroke [212]. PFTα has also been shown to block activation of p53 in response to doxorubicin, ultraviolet and gamma radiation, etoposide, taxol, cytosine arabinoside, ischaemia and hyperthermia [211]. However, it is unable to prevent cell death due to trophic factor withdrawal, which is a p53-independent form of cell death, proving its specificity to p53-mediated apoptosis [212]. Importantly, PFTα has been shown to be effective in saving mice from lethal doses of radiation, without promoting tumour formation [94]. No tumours were found in mice 1 year after treatment; therefore, it does not appear to increase the risk of cancer development. Thus, temporary suppression of p53 is somewhat different from complete p53 deficiency in terms of cancer predisposition. The most interesting point from that study was that PFTα administered directly before high doses of gamma irradiation significantly abrogated apoptosis seen in crypts and villi of mice compared to untreated controls [94]. In experiments with p53-null mice, PFTα did not affect apoptosis incidence, further proving its specificity to p53-dependent apoptosis. Other possible applications for PFTα could include hyperthermia stress, ischaemic diseases and brain degeneration [97]. This small, stable and lipophilic molecule has since been investigated for its potential to decrease neuronal death in a model of stroke and neurodegenerative disorders and also for acceleration of wound healing, with both having encouraging results [26, 200]. And more recently, PFTα has been used in numerous studies testing its ability to protect against cisplatin treatment, heat shock, glucocorticord signaling and endotoxin-induced cell death [98, 168, 211].

It has been shown that PFTα works by inhibiting p53 DNA-binding activity and activation of Bax and p21, without affecting overall production of p53 protein in cells

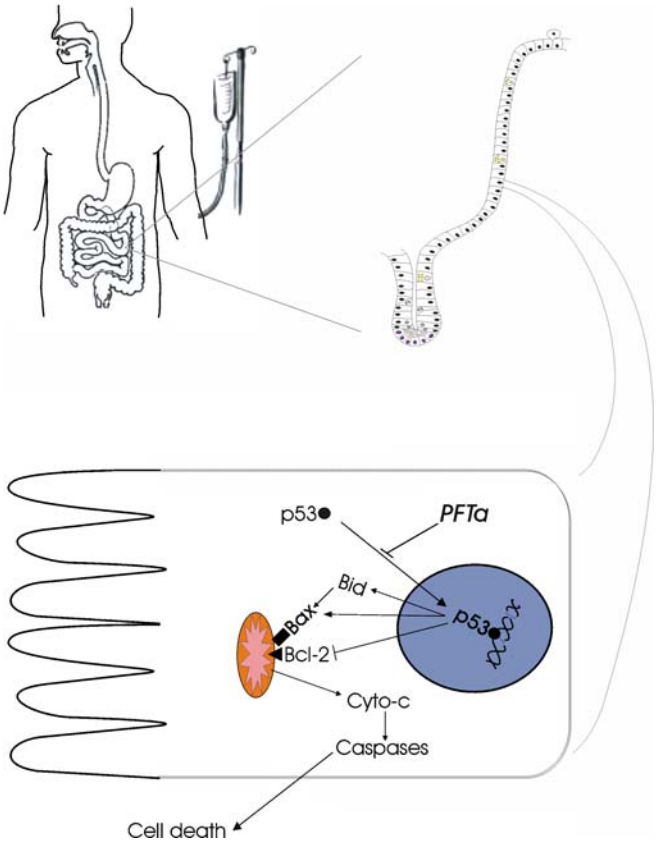

Fig. 4 Diagram of the mitochondrial-regulated apoptosis pathway. Chemotherapy causes genotoxic damage which is recognised by p53. Translocation of p53 into the nucleus allows transcriptional regulation of multiple Bcl-2 family members. Insertion of Bax into the mitochondrial membrane induces release of cytochrome *c*, which in turn activates caspases. PFTα blocks movement of p53 into the nucleus to prevent initiation of cell death pathway

undergoing apoptosis. It has yet to be shown how PFTα affects expression of other members of the Bcl-2 family in response to damage. PFT also stabilises mitochondrial function and suppresses caspase activation [94], making it a promising candidate as an anti-mucositis agent. Pertaining to this, an experiment using both a single and a fractioned cumulative radiation dose which were relevant to clinical anti-cancer treatment doses protected mice and increased survival [99]. An outline of the apoptotic pathway and how PTFα modulates this is shown in Fig. 4.

Summary

Intestinal mucositis occurs as a consequence of cytotoxic treatment through complex interactions within the tissue, most notably crypt cell death and cytostasis. The molecular control of these actions, although known to involve p53, the Bcl-2 family and caspases, is yet to be fully elucidated within the gastrointestinal epithelium. This review has attempted to provide an overview of the current research in the area and identify areas in which further elucidation is required.

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