BJP British Journal of Pharmacology

DOI:10.1111/j.1476-5381.2012.01900.x
www.brjpharmacol.org

REVIEW

Therapeutic implications of disorders of cell death signalling: membranes, micro-environment, and eicosanoid and docosanoid metabolism

Correspondence
HA Leaver, Cell Biology R&D, SNBTS & Department of Clinical Neurosciences, Edinburgh EH17 7QT, UK. E-mail: hanne.leaver@gmail.com

Keywords
cell death signalling; pathophysiology; therapeutics; lipids; membranes; mediators; prostaglandins

Received
22 August 2011
Revised
30 January 2012
Accepted
5 February 2012

J Davidson[1], D Rotondo[1], MT Rizzo[2] and HA Leaver[3]

[1]*SIPBS, Strathclyde University, Glasgow, UK,* [2]*Signal Transduction Laboratory, Methodist Research Institute, Indianapolis, IN, USA, and* [3]*Cell Biology R&D, SNBTS & Department of Clinical Neurosciences, Edinburgh, UK*

Disruptions of cell death signalling occur in pathological processes, such as cancer and degenerative disease. Increased knowledge of cell death signalling has opened new areas of therapeutic research, and identifying key mediators of cell death has become increasingly important. Early triggering events in cell death may provide potential therapeutic targets, whereas agents affecting later signals may be more palliative in nature. A group of primary mediators are derivatives of the highly unsaturated fatty acids (HUFAs), particularly oxygenated metabolites such as prostaglandins. HUFAs, esterified in cell membranes, act as critical signalling molecules in many pathological processes. Currently, agents affecting HUFA metabolism are widely prescribed in diseases involving disordered cell death signalling. However, partly due to rapid metabolism, their role in cell death signalling pathways is poorly characterized. Recently, HUFA-derived mediators, the resolvins/protectins and endocannabinoids, have added opportunities to target selective signals and pathways. This review will focus on the control of cell death by HUFA, eicosanoid (C20 fatty acid metabolites) and docosanoid (C22 metabolites), HUFA-derived lipid mediators, signalling elements in the micro-environment and their potential therapeutic applications. Further therapeutic approaches will involve cell and molecular biology, the multiple hit theory of disease progression and analysis of system plasticity. Advances in the cell biology of eicosanoid and docosanoid metabolism, together with structure/function analysis of HUFA-derived mediators, will be useful in developing therapeutic agents in pathologies characterized by alterations in cell death signalling.

Abbreviations

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; NSAID, nonsteroidal anti-inflammatory drug; PG, prostaglandin; AA, arachidonic acid; HUFA, highly unsaturated fatty acids with 4 or more bonds, for example, arachidonic, eicosapentaenoic and docosahexaenoic acids (4, 5 and 6 double bonds respectively); PUFA, polyunsaturated fatty acids, with 2 or more unsaturated C-C bonds; HUFA, highly unsaturated C20 fatty acid, with 3 or more unsaturated C-C bonds

Many therapeutic agents influence cell death signalling and highly unsaturated fatty acid (HUFA) metabolism (Figure 1). These agents may act at the level of metabolic events affecting apoptosis, enzyme systems and cofactors, agents affecting cell cycle progression and DNA repair, and oncogene expression. Intracellularly, agents affecting organelles and the mitochondrial intrinsic pathway, endoplasmic reticulum-associated stress pathways and lysosomal autophagy can have profound effects on cell death. There has also been development of agents affecting transcellular signalling via the extrinsic pathway, oxidative stress, growth factors and lipid mediators, ion and metabolite flux, adhesion and migration.

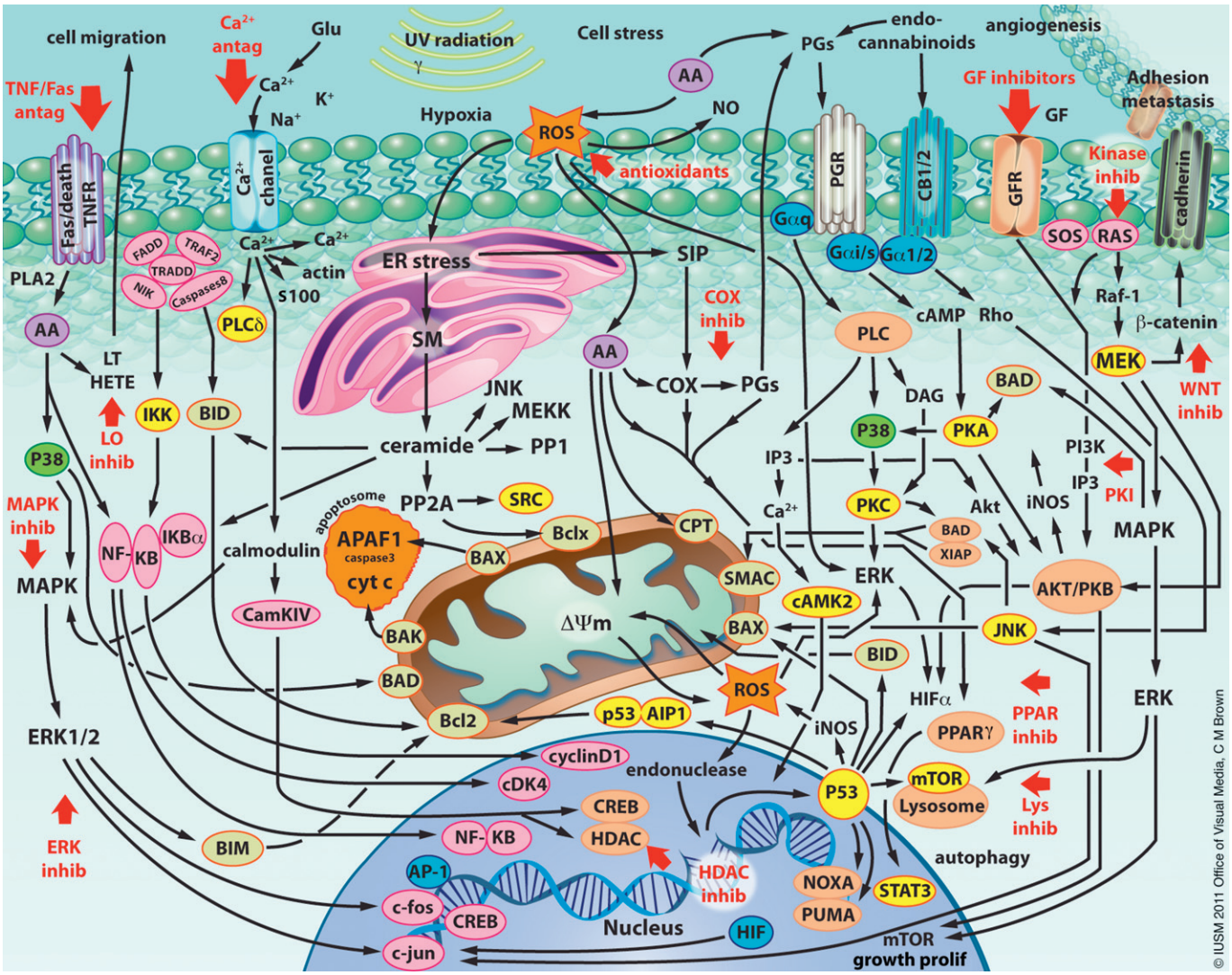

Figure 1

Cell death regulation: Signalling pathways and potential sites for pharmacological intervention. Pathways promoting cell death via extrinsic and intrinsic signalling are shown to the left, and cytoprotective pathways, to the right, although the outcome of signalling depends on signal intensity, frequency and other signals, and the micro-environment. Red arrows indicate sites of pharmaceutical intervention, for example, agonists and antagonists of prostaglandin receptors (PGRs), antagonists of Ca^{2+} transport (Ca^{2+} antag), growth factor (GF) inhibitors and lipoxygenase inhibitors (LO inhib). Both intrinsic and extrinsic pathways are activated by HUFA release and inhibited by PGs of E, F and D series via PGR signalling to PKC and cAMP. Reactive oxygen intermediates (ROS) may activate stress kinases, including extracellular regulated protein kinase (ERK). ROS may elicit DNA damage, whose repair is controlled by sensors and repair processes involving p53, acetylation/ubiquitylation and autophagy, controlled by autophagy genes and mTOR kinase. These pathways are inhibited by lysosome stabilizers (Lys inhib). Cytoplasmic phospholipase A_2 ($cPLA_2$) also activates NF-κB, and inhibits cell cycle progression via cyclin-dependent kinase 4 (CDK4) and cyclin D1. ROS may also activate the intrinsic pathway by depolarizing the mitochondrial membrane (σψm), leading to cytochrome release and apoptosis, via caspases 9 and 3. Protein phosphatase 2A (PP2A) plays a role in gene expression, cell division and signal transduction. PP2A is activated by ceramide, produced by hydrolysis of membrane sphingomyelin (SM) in response to stress-related stimuli. The cellular response to hypoxia is coordinated by hypoxia-inducible factor 1α (HIF-1α), in tumour cells this leads to up-regulation of angiogenic factors such as vascular endothelial growth factor. Disorders of HUFA metabolism have been identified in mitochondrial carnitine palmitoyl transferase (CPT), which transport HUFA into mitochondria. Sphingosine-1-phosphate (S1P) enhances survival by signalling cell adhesion in colon cancer and certain leukaemias. Transcellular signalling is shown, as PGs and their anandamide derivatives, lipoxygenase products (LT, HETE) and GFs released from activated cells, together with shorter range mediators such as Ca^{2+}, NO, AA and ROS, all of which may act as micro-environmental factors controlling cell death.

Also, recently there has been an expansion in agents affecting physiological systems, including angiogenesis, immune surveillance, and development and differentiation. These signals will be discussed, together with questions about lipid factors that lead to the decision to activate cell death or survival (Leaver and Hardingham, 2010; Wyllie, 2010). Topical issues in cell death signalling and how this signalling can be influenced by therapeutic agents will be discussed (Table 1). It will

be argued that membrane responses and membrane-associated mediators linked to HUFA play a key role in the pathophysiology of cell death.

HUFA responses to cell death signals are of vital importance in the pharmacology of some of the most complex and intractable diseases (Lukiw and Bazan, 2010). They are a primary component of cell membranes, which create cellular compartments and micro-environments, and HUFA-derived lipid mediators participate in communication between compartments. The identity of many HUFA-derived mediators is known, but the flux of mediators and micro-environmental signals controlling cell death are poorly defined at cell and systems level. Detailed analysis of the pathology of cell death signalling is being used to identify key cellular signals and agents that modulate their activity. Additionally, complex polyunsaturated fatty acid (PUFA) derivatives, for example, conjugated linoleic acids (CLAs), influence cellular metabolism, cell viability and the survival of cancer cells. These CLAs have been comprehensively reviewed (see Wahle *et al.*, 2004). In the first part of this review, developments in signalling will be outlined which are leading to potential sites of therapeutic intervention. This will be followed by specific examples of HUFA-derived mediators, whose impact on cell survival is becoming better characterized in pharmacological terms.

The pathophysiology of cell death signalling

Recent advances in cell death signalling have led to a deeper understanding of the networks and systems associated with cell pathology (Figure 1). This has been important in developing therapies in complex multifactorial diseases, such as cancer and degenerative disease (Galluzzi *et al.*, 2007). New system-based approaches to drug development, such as targeting multiple genes, and transcriptional and environmental components, are being used in diseases associated with cell death signalling (Chawla *et al.*, 2001; Kirkegaard *et al.*, 2005; Psaila and Bussel, 2008; Rader and Daugherty, 2008; Miller *et al.*, 2009; Uttara *et al.*, 2009; Wang *et al.*, 2009; Nair *et al.*, 2010; Meuillet, 2011; Prakobwong *et al.*, 2011). Advances in stem cell biology have also helped to characterize cell types important in regenerative and degenerative processes (Hardingham *et al.*, 2010; Nair *et al.*, 2010). In many cases, these approaches are in the early stages of development. However, in these systems, it is crucial to disentangle causative events and reactive changes, and to identify key events and signals, in order to develop therapeutic agents active in cell death signalling pathways.

Cell death signalling pathways

Cell death is executed by a complex and sophisticated signalling network, with multiple effectors and mediators, crosstalk, overlapping signalling pathways and diverse end points (Figure 1; Vicencio *et al.*, 2008). In this review, signalling by lipid mediators at membrane level, intracellular compartmentalization and the role of HUFA in transmitting micro-environmental signals to cell death signalling within the cell will be discussed (Table 1).

Several evolutionarily conserved proteins protect against cell death, including Bcl-2, which regulates the intrinsic mitochondrial pathway of cell death, and p53, which is associated with genomic integrity checkpoints (Figure 1). Many

Table 1

Topical issues in cell death signalling and HUFA metabolism

1. ***Cellular organization of cell death signalling*** Relative roles of mitochondria, endoplasmic reticulum and plasma membrane in key decisions in cell death signalling (Bröker *et al.*, 2005; Alonso *et al.*, 2008; Pellicano *et al.*, 2009; Wu *et al.*, 2009b; Benadiba *et al.*, 2010; Wyllie, 2010). (pharmacological relevance includes new targets for drug development, agonists and antagonists, reversibility)
2. ***Cell-cell communication and the micro-environment*** Role in angiogenesis, oncogenesis, degenerative signalling (Adibhatla and Hatcher, 2006; Combrinck *et al.*, 2006; Rizzo and Leaver, 2010) (pharmacological relevance: how can this be modified?)
3. ***Systems communication*** Relative contribution of vascular signalling, tissue signals, cell/organ plasticity (Rush *et al.*, 2007; Miller *et al.*, 2009; Pertwee, 2009; 2010; Ahmad *et al.*, 2010; Sun *et al.*, 2010). [bioengineering of therapeutics, localization and targeting cellular membranes, vasculature]
4. ***Role of membrane HUFA*** (Bisogno *et al.*, 1999; Akiba *et al.*, 2000; Rizzo *et al.*, 2002; Bhathena, 2006; Appendino *et al.*, 2009; Brown *et al.*, 2010; 2011; Lukiw and Bazan, 2010; Picq *et al.*, 2010). (pharmacological/chemical/dietary modification)
5. ***Stress signalling*** Pro-apoptotic activities of HUFA via stress signalling pathways: pathophysiological role. Mediators and signalling pathways (Adibhatla and Hatcher, 2006; Ito *et al.*, 2006; Carrasco *et al.*, 2007; 2008; Chen and Chang, 2009; Xue *et al.*, 2009; Bidwell *et al.*, 2010). (agonists and antagonists of HUFA, stress kinases, mediators including NFκB, HIF1)
6. ***Cell survival signalling*** Cytoprotective activities. Role of PGE_2, PGD_2, 15d-PGJ_2 and their associated receptors, alternative signalling via PPAR, Bcl, endocannabinoid and resolvin pathways (Payner *et al.*, 2006; George *et al.*, 2007; Andrade da Costa *et al.*, 2009; Carlson *et al.*, 2009; Bai *et al.*, 2010) (agonists and antagonists, systems specificity)
7. ***Potential of molecular genetics and stem cell developments*** (Rader and Daugherty, 2008; Hardingham *et al.*, 2010) (pharmacological advances in diagnosis, epidemiology, bioengineering)
8. ***Multiple hits model of cell death*** Combination therapy of agents affecting different cell death signals to avoid escape via overlapping multifunctional pathways (Alonso *et al.*, 2008; Lasithiotakis *et al.*, 2008; Psaila and Bussel, 2008) (multi-system and agent approaches, especially in diseases with complex aetiology such as degenerative and oncogenic disease)

Therapeutic implications are shown in parentheses ( ).

Table 2

Organ and species specific HUFA distribution

Molecular HUFA distribution
Membrane phospholipids 2-acyl position of PtCho, PtdEt (main PLA_2 substrates), PtdSer, PtdIns (main PLC substrate), alkyl phospholipids, endocannabinoids, sphingolipids
Intracellular and secreted lipids HUFA in free fatty acids (released following PLA_2, PLC and DAG lipase activation); COX, LOX and cyt P450 metabolites, resolvins, endocannabinoid metabolites, PAF, triacylglycerols and glycerides, cholesterol esters
Subcellular HUFA distribution
Plasma membrane, endoplasmic reticulum, Golgi apparatus, mitochondria, lysosome, proteasome, secretory granules, apoptosome, peroxisome. (Distinct uptake, composition and metabolism in different membrane systems, leading to signalling compartmentation and lipid micro-environments)
Tissues and organ systems associated with HUFA metabolism
Liver (precursor chain elongation, desaturation, release of HUFA, metabolism)
Blood cells: RBC, platelets, leucocytes, plasma albumin (HUFA transport)
Vasculature endothelial cells (HUFA uptake, especially to brain)
Lung (HUFA uptake and metabolism)
Brain, CNS, retina (HUFA uptake, specific n-3 functions)
GI system (HUFA and precursor uptake, PG sensitive)
Immune system (HUFA release, LT sensitive)
Species
Mammalian (specific eicosanoid, docosanoid metabolism and activities, paracrine actions)
Fish (higher n-3/n-6 HUFA composition)
Plant (precursors of both n-6 and n-3 HUFA)

HUFA levels vary in different lipid classes, dependent on diet and pathophysiological stimuli. The cell membrane phospholipids phosphatidylcholine (PtdCho) and phospatidylethanolamine (PtdEt) generally contain the highest available cellular HUFA stores, released in response to activation of phospholipase A_2 (PLA_2). HUFA in phosphatidylinositol (PtdIns) is released by PLC and DAG/monoacylglycerol (MAG) lipase activation. Released HUFAs are rapidly metabolized by COX to form PG, lipoxygenase (LO) to form leukotriene (LT) and cytochrome P450 (cyt P450) enzymes, or are re-esterified. Resolvins and endocannabinoids and platelet activating factor (PAF) may use HUFA pathways of metabolism, but also have distinct synthetase pathways and receptors. HUFA are present in all subcellular membranes, although phospholipid and HUFA distribution is membrane specific. Tissue and species HUFA levels are also specific, and depend on supply and demand, and specific functions, for example, neurotransmission or inflammatory processes require selective uptake and release, and both processes are often compromised in degenerative disease.

key genes associated with cell death exert other vital functions associated with survival. Indeed, it has been postulated that no specific 'cell death' genes exist, only genetic and epigenetic elements that control cell survival under certain circumstances. Thus, mediators, metabolites, signalling systems and organelles such as mitochondria are involved in the pathophysiology of cell death as well as other physiological functions. This has implications in therapeutics, where partial agonist and antagonists may be important in order to preserve physiological functions, while targeting pathological changes with overlapping pathways and mediators.

The characteristics of cell death are diverse: necrosis, apoptosis and autophagy may be different and distinct modes of cell death, although many pathophysiological processes exhibit characteristics of multiple modes of cell death (Kroemer *et al.*, 2009). Thus, the catastrophic stress and necrosis of vascular stroke differ from slower degenerative changes in vascular disease. Yet, both processes use overlapping pathways and mediators, for example, endothelial cells responding to death signals such as hypoxia and stress signals via the intrinsic pathway (Figure 1, Rizzo and Leaver, 2010). A further cell death pathway involving lysosomes has been identified. Recent studies on lysosomal membrane metabolism have implicated lysosomes in autophagy, and have led to development of agents that affect lysosomal stability (Alonso *et al.*, 2008; Galluzzi *et al.*, 2008; Geng *et al.*, 2010). A fruitful field of drug development has focused on early signalling elements, such as agents acting on protein kinases (Bain *et al.*, 2007).

Triggers of cell death may include physical or chemical insult, and hormonal and other cell- and system-derived signals, activating various cellular mediators. The transduction pathways of cell death are diverse (Vicencio *et al.*, 2008; Harr and Distelhorst, 2010) involving membrane systems, including the plasma membrane, intracellular membranes and organelles, and membrane-derived lipid mediators with nuclear and transcriptional actions (Table 1, Colquhoun, 2010; Leaver *et al.*, 2010; Cui *et al.*, 2011; Rizzo, 2011). A characteristic of eukaryotic plasma and intracellular membranes is their high PUFA content (Table 2). PUFAs may be released from membranes in response to pathophysiological stimuli, and either exert a direct action, or be metabolized by

lipoxygenase or COX to mediators with pathophysiological activities (Figure 2). These mediators have a short half-life and physical range, being limited to intracellular compartments in the case of free radicals, and highly reactive lipid peroxides, or having transcellular and local systemic activity in the case of PGE_2. Lipid mediator synthesis may be influenced by micro-environmental factors (Nair *et al.*, 2010), and pharmacological agents such as aspirin may result in the synthesis of novel anti-inflammatory mediators (Lukiw and Bazan, 2010; Serhan and Haeggstrom, 2010).

PUFA release under pathological conditions

The HUFA cascade

Mediators and key regulatory points of the cell death cascade are shown in Figure 1. Pathways of arachidonic acid (AA) release and metabolism are shown, although n-3 HUFA (Figure 2) may play a role in certain tissues and species (Table 2). HUFA release is initiated by phospholipase activation. Phospholipases A_2, C and D are activated in response to cell surface ligand binding, intracellular calcium mobilization and activation of cell stress signals (Figure 1). The type and amount of released lipid mediators depend on the stimulus, cell type, nutritional and metabolic state, and membrane composition (Table 2). The release of fatty acids can also be regarded as physiological when the actions of lipases are constitutive (require no external stimulus) or occur in response to hormones, for example, vascular cell release of AA in response to vasopressin (Liu and Taylor, 2006), which is a calcium-dependent response. There is not always a requirement for increased intracellular calcium to activate phospholipases, indeed in monocytes both processes can occur in parallel when both calcium-dependent and calcium-independent release of AA may elicit increased eicosanoid formation (Hoffman *et al.*, 1988). HUFA signalling influences early events in two interacting pathways of cell death, intrinsic and extrinsic pathways (Figure 1). The intrinsic pathway, activated by stress signals, involves mitochondrial factors and Bcl family members, while extrinsic signalling is initiated by cell surface receptors of the TNF family and extrinsic signals. PUFA/ HUFA release may occur at the plasma membrane, or at intracellular membranes, such as endoplasmic reticulum and mitochondrial membranes. AA and other PUFA may exert direct effects on stress signalling factors and genes (Rizzo *et al.*, 1999; 2002). AA regulates gene expression directly via p38 MAPK, ERK and JNK, increasing transcription of AP-1-containing genes. These events are inhibited by tyrosine kinase inhibitors. These signalling systems present potential therapeutic targets, and the opportunity for specifically targeting pathological pathways, while protecting physiologically important signals, such as basal COX activity essential for gastric integrity, endothelial and vascular protection, or brain specific signalling via n-3 HUFA-associated pathways.

Pathology of PUFA release

PUFA released in response to stress or TNFR signalling may be oxidized by lipoperoxidation (Figure 2) to reactive oxygen species (ROS), which rapidly depolarize mitochondria, leading to cytochrome c release, apoptosis inducing factor release and cell death (Figure 1). ROS may be generated intracellularly or extracellularly in response to ionizing radiation, stress signals, hypoxia/reperfusion, mitochondrial uncoupling, free radical generation, or from NO or HUFA peroxidation, to activate stress kinases, including p38 MAPK or JNK (Ito *et al.*, 2006; Chen and Chang, 2009; Uttara *et al.*, 2009). ROS may also exert genotoxic activity, activating endonuclease and ceramide cell stress signalling (Miller *et al.*, 2009). These pathways may be exaggerated, for instance, in tumours over-expressing Akt, a key apoptotic signal sensitive to ROS (Kirkegaard *et al.*, 2005; Meuillet, 2011). Also, pathological changes in the ceramide stress pathway, affecting sensitivity to chemotherapy and radiotherapy, have been detected (Orgertman and Hannun, 2004). HUFA-derived ROS may also be formed directly within membrane phospholipids, but these appear to have similar pro-apoptotic activities via stress signalling pathways (Deigner and Hermetter, 2008).

Pathological control over PUFA release and metabolism may be exerted at the level of phospholipase activation, for example, $sPLA_2$ and $cPLA_2$ stimulate tumour cell migration and proliferation (Rizzo *et al.*, 2002; Denizot *et al.*, 2009). Hypoxia during stroke or vascular injury may elicit cell death via ROS-dependent activation of apoptosis (Adibhatla and Hatcher, 2006). PUFA and associated ROS activity are limited by rapid re-esterification pathways, which are also important in membrane remodelling (Farooqui and Horrocks, 2006).

Selective intracellular uptake of PUFA is critical, and disorders of PUFA uptake have been identified, for example, mitochondrial carnitine palmitoyl transferase, involved in transport of HUFA into mitochondria, which is inhibited by PGE_2 (Colquhoun, 2010). Additionally, as shown in Figure 1, PUFA and their metabolites can act as transcellular mediators in both activation of and protection from cell death signals. This notion emphasizes a critical role of lipid mediators in influencing the micro-environment, and creating conditions for generation of apoptotic or anti-apoptotic signals (Greenhough *et al.*, 2009). Thus, the decision of cells to survive or undergo death is influenced by PUFA and their metabolites in the micro-environment.

Anti-apoptotic survival pathways involving HUFA are relevant in pathologies characterized by increased angiogenesis, where HUFA-derived eicosanoids, such as PGE_2, may play a critical role in affecting endothelial cell angiogenic responses, and release of angiogenic growth factors from tumour cells (Baryawno *et al.*, 2008; Bai *et al.*, 2010; Rizzo, 2011).

Therapeutic aspects of cell death signalling

Topical issues in therapeutics

The inappropriate regulation of cell death has been implicated in many pathological processes, ranging from cancer to vascular disease (Galluzzi *et al.*, 2007). There is demand for drugs that selectively induce cell death (e.g. in neoplastic cells) or agents that antagonize or attenuate it (in degenerative disease or ischaemia). Increasing numbers of therapeutic agents act on cell death signalling pathways (Figure 1 and

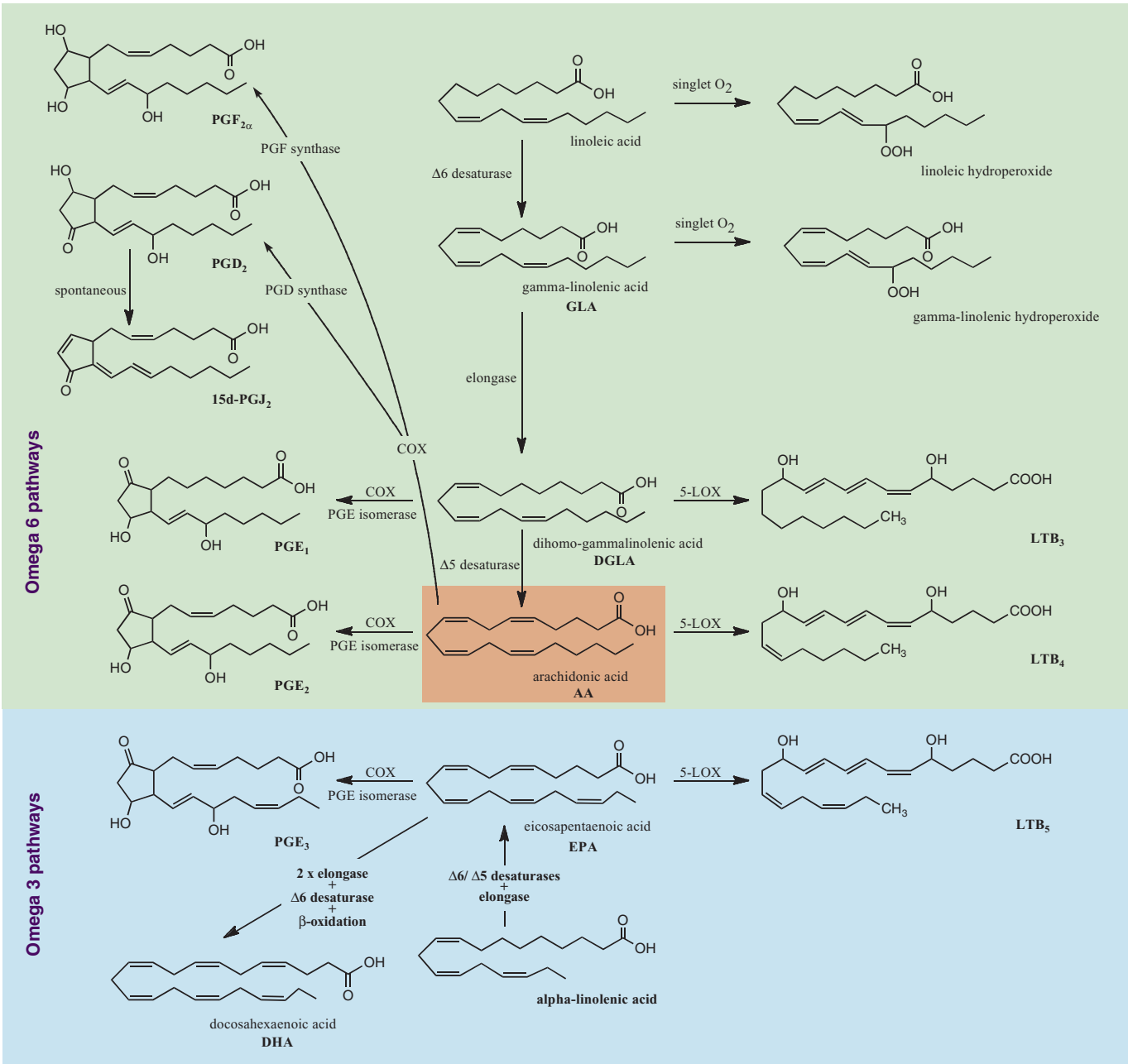

Figure 2

Pathways of PUFA release and metabolism. PUFA released from cell membranes in response to pathophysiological stimuli either exerts a direct action localized within the cell, or may be metabolized by lipoperoxidation or COX to mediators with a wider range of activity and capacity to act on cells, organs, tissues, and exert pathophysiological activities (endothelial/cancer cell migration, proliferation, adhesion). Selectivity lies at the level of uptake, transport, esterification/lipase cycles and phospholipase. There may also be specificity at the level of n-3 and n-6 series metabolism. PGs are synthesized from essential fatty acids derived from diet, predominantly esterified to complex lipids. Three primary PG precursor PUFAs are dihomo-gamma-linolenic acid (DGLA, cis-5,8,11-eicosatrienoic acid; 20:3 *n*-6), AA (cis-5, 8, 11, 14 eicosatetraenoic acid; 20:4 *n*-6) and EPA (cis-5,8,11,14,17-eicosapentaaenoic acid; 20:4 *n*-6). PUFAs, mainly esterified in the *sn*-2-position of membrane phospholipids, are released via phospholipase A_2 (PLA_2, Akiba *et al.*, 2000) or in a two-step process involving phospholipase C and diglyceride/monoglyceride lipase. Free DGLA, AA and EPA within mammalian cells are metabolized via COX to series 1, 2 and 3 PGs with 1, 2 and 3 double bonds respectively. The most abundant PUFA is AA. AA and EPA are converted to unstable endoperoxide intermediate by COX, and to 2- and 3-series PGs, PGE, PGD and $PGF_{2\alpha}$ by specific PG synthases.

Table 3

HUFA metabolism in pathological conditions, indicating pharmacological targets

Pathology	Pharmacological target	Reference
Age-related degenerative processes	ROS/SOD	Ito *et al.*, 2006
Aging and stress (retinal ischaemia)	PGE_2 agonists, EP2R	Osborne *et al.*, 2009
	Resolvin, PAF	Lukiw and Bazan, 2010
Microglia (neuroprotection)	PGE_2 agonists, EP1R	Carlson *et al.*, 2009
Alzheimer's disease	PGE_2 agonists, EP2R	Combrinck *et al.*, 2006
Neuroprotection	PGD_2 agonists, DP1R	Liang *et al.*, 2005b
Neovascularization in oncology	COX inhibitors	Rizzo, 2011
Endothelial proliferation	Lipoperoxide, p38, MAPK	Cui *et al.*, 2011
Invasion, proliferation, survival	COX inhib (aspirin, NSAID)	Rothwell *et al.*, 2011
(Various cancers)		Elwood *et al.*, 2009
		Cuzick *et al.*, 2009
Osteolytic tumour growth	EP2R	Takahashi *et al.*, 2008
Glioma, leukemia growth	Lysosomal membrane, autophagy	Alonso *et al.*, 2008
		Vicencio *et al.*, 2008
Glioma cell growth	mPGE synthase-1, pKAII	Payner *et al.*, 2006
Anaplastic astrocytoma	PGD_2 synthase	Payne *et al.*, 2008
Medulloblastoma growth	EP2	Baryawno *et al.*, 2008
Colorectal cancer	COX-2 metabolites	Chell *et al.*, 2006
Colon cancer	Fas ligand EP1R	O'Callaghan *et al.*, 2008
Esophageal adenocarcinoma	PGE_2R antagonists	Piazuelo *et al.*, 2006
Oral squamous cell carcinoma growth	PGE_2, EP3R	Hoshikawa *et al.*, 2009
CML	Mitochondrial disruption	Pellicano *et al.*, 2009
	Src tyrosine kinase	Nair *et al.*, 2010
Endometrial cancer (epithelial cell invasion)	Epithelial $PGF_{2\alpha}$ ADAMTS1	Keightley *et al.*, 2010
Stroke (neuroprotection)	PPAR-γ	Pereira *et al.*, 2006
Cerebral infarction	DP1 receptor	Thura *et al.*, 2009
Brain ischaemia-reperfusion injury	PGD(2) DP1R	Saleem *et al.*, 2007
	PGE_2, EP2R	McCullough *et al.*, 2004
Neurons oxidative stress (β-amyloid exposure)	EP2 and EP4 agonists	Echeverria *et al.*, 2005
Pulmonary apoptosis (haemorrhagic shock, sepsis)	PPAR-γ, mito $\Delta\psi m$	Chima *et al.*, 2010
Gastric epithelial *Helicobacter pylori*-induced apoptosis	NF-kB, Bcl-2	Chu *et al.*, 2003

Superoxide dismutase (SOD) inhibitors increased lifespan in rodent models, and inhibitors of reactive oxygen (ROS) generation attenuate degenerative processes involving glutaminergic neurotransmission. Receptors for PGE_2 (EP) and PGD_2 (DP) are cytoprotective in neurodegenerative processes and vascular disease. In endometrial adenocarcinoma cells, $PGF_{2\alpha}$ via the FP receptor regulates disintegrin and metalloproteinase with thrombospondin repeat 1 (ADAMTS1). Microsomal PGE (mPGE) synthase-1 regulates human glioma cell growth via PGE_2-dependent activation of type II protein kinase A (pKAII). Ciglitazone, a novel inhibitor of PPAR, attenuated the intrinsic pathway of apoptosis and mitochondrial membrane potential ($\Delta\psi m$).

Table 3). However, limitations in clinical trials using inhibitors of terminal cell death effectors, the caspases, indicate the importance of selecting early triggering events and mediators, before the cascade leading to cell death becomes irreversible.

Targeting early signals and pathological processes has been the basis of inhibitors of, for example, dual SRC/BCR-Abl kinase inhibition of tumour-initiating cells (Bain *et al.*, 2007; Nair *et al.*, 2010). Also, targeting early events involving mitochondrial disruption is effective in killing chronic myeloid leukemia (CML) progenitor cells (Pellicano *et al.*, 2009). Other pharmacological agents include those affecting ion flux associated with HUFA release (Harr and Distelhorst, 2010). The role of antioxidants in limiting excessive ROS in inflammatory, hypermetabolic and degenerative disease is also the subject of current research (Uttara *et al.*, 2009). The PPARs are another group of HUFA receptors with up-regulated cell death signalling activity in hypoxia and various pathologies (Table 4).

Angiogenesis is a current area of therapeutic development, targeting vascular endothelial growth receptors and endothelial cell signalling. Endothelial cell growth and

Table 4

Pro- and anti-apoptotic activities of PPAR agonists and antagonists, indicating proposed mediators, and selective actions in tumour, normal tissue and tissue/cells affected by degenerative disorders

PPAR species	Signalling elements	Pathological target*	Reference
α KO	NF-κβ	+ Tumour radiation-induced stress	Zhao *et al.*, 2007
β	H2O2	– Endothelial cell	Jiang *et al.*, 2009
β	FA oxidation	– Pancreatic β cell	Wan *et al.*, 2010
βδ	Oedema	– Spinal cord injury	Paterniti *et al.*, 2010
δ	COX	+ Colon cancer	Wu *et al.*, 2009a
δ	TGF β	– Vascular smooth muscle	Kim *et al.*, 2009
γ	Bcl-2	– Oxid stress cardiomyocyte	Ren *et al.*, 2009
γ	Akt	– Cardiomyocyte	Kilter *et al.*, 2009
γ	Ψm, Bcl-2	– Uvb, mitochondria	Wu *et al.*, 2009b
γ	NO	+ Retinoid assoc tumour	Shankaranarayanan *et al.*, 2009
γ	Cannabinoid	+ Hep92 apoptosis	Giuliano *et al.*, 2009
γ	P63 p73	+ Ovarian cancer	Kim *et al.*, 2011
γ	15LO	+ Non-small cell lung cancer	Mao *et al.*, 2010
γ	Chromatin	– Growth HL-60 cells	Liu *et al.*, 2009b
γ	NF-κβ, STAT-	– Growth biliary cancer	Prakobwong *et al.*, 2011
γ	XIAP, CZG	+ Promyelocytic leukaemia	Liu *et al.*, 2009a
γ		– Liver in haemorrhagic shock	Zingarelli *et al.*, 2010
γ	COX-2	– Proliferation, pancreatic cancer	Sun *et al.*, 2009
γ	C/EBP, p53	+ Hepatic stellate cells	Wang *et al.*, 2009
γ	NF-κβ, GSK-3β	– Growth colon cancer	Ban *et al.*, 2010

* indicates protection against apoptosis, + pro-apoptotic activity, T tumour cells, mito mitochondria.
KO, gene knockout study; FA, fatty acid; LO, lipoxygenase.

migration play a key role in angiogenesis (Tables 3 and 5) and are controlled by autocrine and paracrine growth factors and lipid mediators which influence endothelial cell survival (Rizzo and Leaver, 2010). Survival mechanisms may be important in endothelial cell function, where advances in adhesion biology have helped define processes associated with angiogenesis and repair in damaged tissue (Rader and Daugherty, 2008). On the other hand, pro-apoptotic endothelial targeting has recently been the focus of anti-angiogenic therapy in invasive tumours (Lord-Dufour *et al.*, 2009; Rizzo and Leaver, 2010). The role of vasoactive paracrine HUFA-derived signals, such as eicosanoids and docosanoids, is an important area of therapeutic investigation. This will be discussed further, see following sections on the role of prostaglandins (PGs) in control of cell death signalling, and advances in cyclooxygenase pharmacology: receptors and signals that confer protection by preventing cell death (the drug/receptors nomenclature use conforms to the British Journal of Pharmacology's guide to receptors and channels, Alexander *et al.*, 2011).

Additionally, the principle of combined therapy is currently used in selecting targets to evade alternative signalling, for example, in many oncology trials, combinations of agents acting at different targets, for example. Growth factor antagonists, acting via intrinsic and extrinsic apoptotic pathways, are often combined with agents that affect DNA damage repair, or cell cycle checkpoints. Membrane, mediator and micro-environmental signalling at multiple locations is also relevant to stem cell strategies, where more than one cell type may be involved in pathogenesis.

Targeting n-3 HUFA metabolism

The n-3 essential fatty acids (Figure 2 and Table 2) are currently a focus of interest, because of the ability of n-3 HUFA-based drugs, dietary approaches and nutrachemicals to modify membrane HUFA content. This has arisen because of perceived beneficial cardiovascular effects, but brain targets may also be important. Recent advances in genetics, proteomics and lipidomics have given insights into the substrate specificity of HUFA release (Denizot *et al.*, 2009). Additional approaches have included using naturally occurring n-3 HUFA, development of specific n-3 HUFA-derived agonists and antagonists (Picq *et al.*, 2010), and agonists with neuroprotective properties (Lukiw and Bazan, 2010). Dietary and epidemiological studies have concentrated primarily on effects of dietary HUFA precursors, but have been complemented by pharmacological studies characterizing metabolically active mediators (Cui *et al.*, 2011). Both approaches are important in analysing the actions of rapidly released and metabolized mediators, and cell biology has bridged the gap by analysing metabolism at cellular and system levels, for example, direct effects at the level of lipogenic and peroxiso-

Table 5

Pro- and anti-apoptotic activities of prostaglandin receptor (PGR), and PGR agonists and antagonists, indicating proposed mediators, and selective actions in tumour, normal tissue and tissue/cells affected by degenerative disorders

PGR	Protective	Analogue	Pathology target	Species	Reference	
EP1	–	SC-51089	Haemorrhage	Neurones	Rabbit	Vinukonda *et al.*, 2010
EP1	+	17-phenyl trinor PGE_2	Liver carcinoma	Liver cells	Human	Bai *et al.*, 2010
EP1	–	ONO 8711/ SC 51089	Excitotoxicity	Microglia	Mouse	Carlson *et al.*, 2009
EP1	+	ONO-8713	Medulloblastoma (mb)	Mb cells	Human	Baryawno *et al.*, 2008
EP1	+	EP1 expression	Colon cancer	Colorectal cells	Human	O'Callaghan *et al.*, 2008
EP1	+	17-phenyl trinor PGE_2	Parkinson's	Neurones	Rat	Carrasco *et al.*, 2007
EP2	+	Soluble EP2 receptor	Bone cancer	Osteoblasts	Human	Takahashi *et al.*, 2008
EP2	+	Butaprost	Medulloblastoma (mb)	Mb cells	Human	Baryawno *et al.*, 2008
EP2	+	Butaprost	Parkinson's	Neurones	Rat	Carrasco *et al.*, 2008
EP3	+	ONO-AE3-240	Oral carcinoma	Squamous cells	Human	Hoshikawa *et al.*, 2009
EP3	+	ONO-AE3-240	Medulloblastoma (mb)	Mb cells	Human	Baryawno *et al.*, 2008
EP4	+	EP4A	T-cell leukaemia	T-cells	Human	George *et al.*, 2007
EP4	+	AH23848B	Adenocarcinoma (ad)	Ad cells	Human	Piazuelo *et al.*, 2006
DP1	+	BW245C/ BWA868C	Cerebral ischaemia	Neurones	Rat	Thura *et al.*, 2009
DP1	+	BWA868C	Colorectal cancer	Crypt cells	Rat	Zamuner *et al.*, 2005
DP1/2	–	PGD_2	Glioblastoma	A172 cells	Human	Payne *et al.*, 2008
DP2	+	CAY10471	T-cell apoptosis	TH2 cells	Human	Xue *et al.*, 2009
FP	+	$PGF_{2\alpha}$	Endometrial cancer	Epithelial cells	Human	Keightley *et al.*, 2010
FP	+	Fluprostenol	Skin cancer	JB6 epidermal	Mouse	Weber *et al.*, 2002

mal gene expression (Clarke *et al.*, 1997; see also 'Developing strategies: Hydroperoxy fatty acid signalling and endocannabinoid' sections of this review).

The mechanisms of n-3 HUFA action at cellular level are complex and incompletely understood. Part of their signalling involves substrate specificity for COX and PG synthase, but metabolites of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the resolvins and (neuro) protectins, may also play a part, as they have anti-inflammatory and immunoregulatory actions (Lukiw and Bazan, 2010; Serhan and Haeggstrom, 2010). Compounds derived from EPA (Figure 2) are designated E resolvins, while those formed from DHA are denoted D resolvins or protectins (neuroprotectins). The identification of protectins, which are formed in the presence of aspirin, and are associated with COX acetylation and active site modification, has increased the understanding of drug interactions with biological systems, and biomodulation of metabolism. There is evidence of increased protectin synthesis in pathological processes, for example, neuroprotectin D1 is released in response to ischaemia-reperfusion, oxidative stress or physiological stimulation by neurotrophins. Certain activities of resolvin/protectins are associated with resolution of inflammation, while others appear independent of classical inflammatory cells and pathways (Serhan *et al.*, 2009; Serhan and Haeggstrom, 2010). Like the n-6 PUFA, n-3 HUFA precursors and their lipoxygenase metabolites often have opposing, primarily pro-apoptotic and cell death stimulating activities, while their major COX metabolites are predominantly anti-apoptotic (see topical issues in eicosanoids section in this review). However, other targets for n-3 HUFA have recently been identified (see 'Developing strategies: PPAR and Endocannabinoid' sections of this review. The n-3 derived endocannabinoids represent a new area of development, which may be especially relevant to tissues and cells in which n-3 HUFA supply is significant).

The role of lipidomics

The cell biology of HUFA signalling has been advanced by improved analytical techniques. Subcellular HUFA release may be analysed using microdissection and mass spectroscopy. Together with other imaging techniques, this provides information on mediator localization and release, spatiotemporal aspects of, for example, mitochondrial signalling and the intrinsic pathway of cell death, and lysosomal activation (e.g. chloroquine, in addition to its recently postulated lysosomal stabilization properties, is a also a potent inhibitor of phospholipase A_2).

Prostaglandins and the control of cell death signalling

Lipid metabolites of AA and DHA, the eicosanoids and docosanoids, have been productive targets of pharmacological research. Selective agonists and antagonists with efficacy in cardiovascular disease and anti-inflammatory actions have been developed, and other actions affecting cell death signal-

ling have been identified. The role of eicosanoids in cell death signalling will be discussed in this review. Additionally, lipoperoxidation, PPAR and cannabinoid signalling will be covered, as evidence of their therapeutic potential has emerged.

Prostaglandin signalling may be intracellular or transcellular. Thus, in pathological processes, altered PG metabolism may selectively target the micro-environment, for example, cell and tissue selective HUFA metabolism to $PGF_{2\alpha}$ in endometrial carcinoma, where $PGF_{2\alpha}$ is involved in endothelial cell invasion (Keightley *et al.*, 2010), or loss of prostaglandin D (PGD) synthase in the transition of a low-grade astrocytoma to anaplastic astrocytoma (Payne *et al.*, 2008).

Certain common PGs, present in high concentrations in mammalian tissues and cells (Table 2), have cytoprotective activity, for example, PGE_2 and PGD_2 attenuate neuronal cell death in response to neurotoxic stimuli (Sawyer *et al.*, 2002; Lee *et al.*, 2004; Liang *et al.*, 2005a,b). 15d-PGJ_2 may also be neuroprotective (Lin *et al.*, 2006), and PGE_2 prevented death of neurones in response to TNF-α (Lee *et al.*, 2004). There is current interest in roles of these PGs in angiogenesis and neovascularization (Tables 3 and 5).

Therapeutic aspects of prostaglandin metabolism

Aspirin is the most consumed pharmaceutical agent worldwide and aspects of its activity are still emerging (Warner and Mitchell, 2004). Recently, low-dose aspirin has shown efficacy in cancer trials (Elwood *et al.*, 2009; Rothwell *et al.*, 2011). In an epigenetic analysis of 25 000 patients, analysing death rates and prophylactic treatment with 75 $mg \cdot d^{-1}$ aspirin, reduced incidence of cancer in gastrointestinal and solid tumours was detected, although the trials were originally set up to study mainly cardiovascular, rather than oncological outcomes (Cuzick *et al.*, 2009). This supports studies suggesting that eicosanoids enhance the ability of cancer cells to resist cell death (Table 5). There is evidence that increased tumour cell proliferation and migration may be associated with prostaglandin E (PGE) synthesis (Rush *et al.*, 2007; Baryawno *et al.*, 2008; Rizzo and Leaver, 2010) and this has implications for angiogenesis (Rizzo, 2011, Table 3). Recent structure/activity analysis of proliferative activity of PGE_2 implicated specific regions of PGE_2, including C5, cyclopentane ring, 9-ketone, C13-14 double bond and 15-hydroxy group (Payner *et al.*, 2006). The signalling pathways affecting key survival decisions affected by nonsteroidal anti-inflammatory drug (NSAID) remain unclear, although the Bcl-2 pathway appears important. Signalling elements have been identified, showing that NSAIDs promoted apoptosis in human HT-1080 fibrosarcoma cell lines by up-regulating p53, p21 and Bax expression, and down-regulating Bcl-2 (Mahdi *et al.*, 2006). Some of these changes have been also been observed in glioma cells treated with PUFA (Benadiba *et al.*, 2010; Colquhoun, 2010). It is therefore possible that COX inhibition diverted PUFA into cytotoxic metabolites in fibrosarcoma cells and that this is an effective cytotoxic pathway in transformed cells.

Another topical issue in eicosanoid pharmacology is the relative importance of COX subtypes and the actions of specific COX antagonists. Recent advances in genetic analysis of COX subtypes have led to development of agents targeted against COX-1 and -2 isoforms, which also have activity in cell death signalling. An aim of NSAID development was inhibition of inducible COX-2 at sites of inflammation, avoiding side effects (e.g. gastric damage) due to inhibition of constitutive COX-1. Although COX-2 selectivity was associated with reduced gastrointestinal damage, COX-2 antagonists also revealed roles for constitutive COX-2 within tissues such as brain, kidney, pancreas, intestine and blood vessels. This has given a better understanding of COX-1 and COX-2 activity in functions as disparate as pain perception and cancer progression (Greenhough *et al.*, 2009). However, clinical use of COX-2 selective compounds has also indicated potential cardiovascular side effects such as myocardial infarction, stroke and elevated blood pressure (Warner and Mitchell, 2004). Also, tumour cells frequently over-express the inducible COX-2 isoform (Rizzo, 2011) and the anti-neoplastic activity of celecoxib was initially assumed to result from selective inhibition of COX-2 and PG synthesis. However, recently celecoxib was also found to inhibit apoptosis in a COX-2-independent manner, which may involve cell death signals and the intrinsic pathway of cell death. Rudner *et al.* (2010) reported that celecoxib induced apoptosis in Jurkat cells via Mcl-1/Noxa, and this effect was inhibited by over-expression of anti-apoptotic Bcl-xL.

Pathology of prostaglandin activity

Prostanoids have been associated with a variety of pathological responses and may act as a primary cellular defence mechanism (Tables 4 and 5). This may be partly due to activation of inflammatory pathways (Portanova *et al.*, 1996), although non-inflammatory actions involving cell death signalling have been observed. During inflammation, PGs may be directly cytoprotective and also act as negative feedback regulators, suppressing cytokine production via JAK/STAT signalling (Tamiya *et al.*, 2011). Gastric mucosa is one of the best characterized tissues with respect to the cytoprotective properties of PGs (Sibilia *et al.*, 2009). However, PGs also suppress cell necrosis in many other tissues in response to chemical and immune-induced cell death, for example, in liver, PGE_2 analogues suppressed cell death in response to galactosamine or complement (Mizoguchi *et al.*, 1987; Kurebayashi and Honda, 1991). More recently, neuroprotective activity of PGs was identified in conditions similar to those following stroke, that is ischaemia reperfusion-induced cell death (Govoni *et al.*, 2001; McCullough *et al.*, 2004), and in systemic inflammatory responses, elevation of PGE_2 in CSF was detected (Davidson *et al.*, 2001). These cytoprotective actions appeared to be mediated, at least in part, via EP2 receptor (EP2R) and intracellular cAMP (McCullough *et al.*, 2004).

Recent advances in cyclooxygenase pharmacology: receptors and signal systems that confer protection by preventing cell death

Pathological PUFA release may exert pro-apoptotic activity via various stress signalling pathways (Figure 1). However, HUFA metabolism via COX is predominantly anti-apoptotic, effectively down-regulating the initial cell stress response.

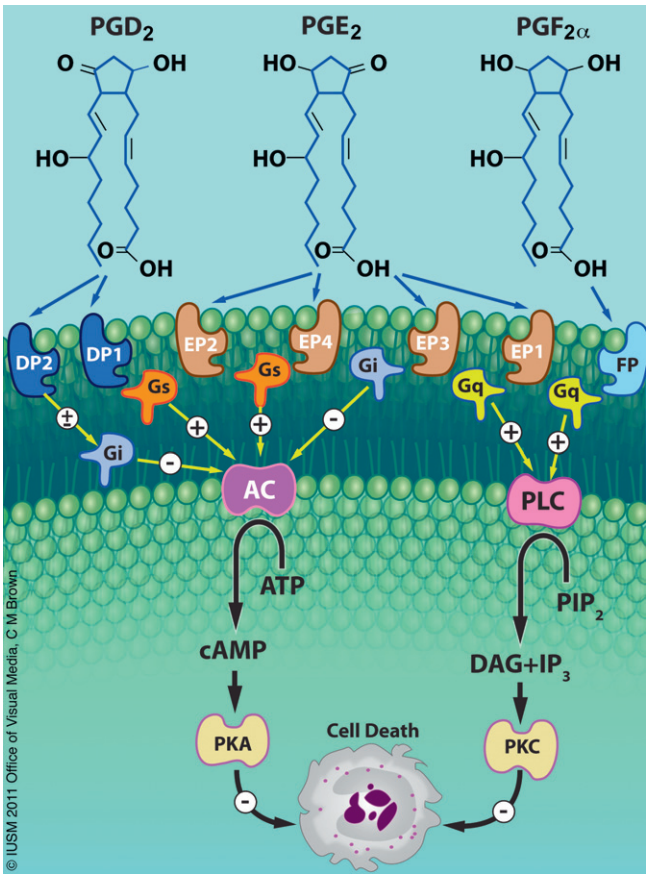

Figure 3

Prostaglandin structure and signalling via prostaglandin receptors. PGE_2, $PGF_{2\alpha}$ and PGD_2 bind to individual specific membrane-bound cellular receptors, EP1-4, FP and DP1-2, each with specific agonists and antagonists (Table 5). Prostaglandin receptors (PGRs) are coupled via G proteins Gi, Gs and Gq to two principal pathways, linked to either inner plasma membrane-bound adenylate cyclase (AC) or to PLC enzymes. PLC hydrolyses phosphatidylinsitol 2 phosphate (PIP2) to diacylglycerol (DAG) and inositol 3 phosphate (IP3), while AC cyclizes adenosine triphosphate (ATP) to cAMP. Both pathways are predominantly anti-apoptotic in most cell types. The PGRs and their signalling pathways represent potential molecular sites of selective pharmacological intervention. PGR agonist and antagonist studies also indicate activities independent of these receptors, possibly via additional receptors that have not been fully characterized (e.g. endocannabinoid metabolite receptors), or receptor-independent signalling.

These cytoprotective actions may be partly mediated via cAMP or PLC (Figure 3), although evidence is emerging of actions involving other lipid receptors such as PPAR and endocannabinoid receptors, and cell death signalling pathways involving NF-κB and Bcl. EP2 or DP1 receptors are linked to Gs/adenylate cyclase, and activate cAMP-dependent pathways, such as PKA (Figure 3). The activities of therapeutic agents affecting multiple signalling pathways require careful analysis and systems have been developed for analysing G protein-coupled receptors which initiate downstream signalling (Urban *et al.*, 2007).

Cytoprotective activities of PGE receptors

Many studies have attempted to identify PG receptors involved in preventing cell death, using selective agonists and antagonists (Table 5). These studies have yielded ambiguous interpretations, partly because of overlapping activities with other PG receptors, and also because additional, atypical EP receptors and alternative signalling pathways may exist (Table 5, Bidwell *et al.*, 2010). There are at least four subtypes of PGE_2R, EP1, EP2, EP3 and EP4, linked to different signal systems, with a complex distribution, even within the same cell types (Figure 3, Tables 4 and 5, Andrade da Costa *et al.*, 2009). McCullough *et al.* (2004) used pharmacological and genetic approaches to identify the role of the EP2R. Following focal ischaemia, there was greater infarct volume, with no effect on cerebral blood flow, in EP2R knockout animals. EP2R involvement was supported by neuroprotective actions of the EP2R agonist butaprost (Andrade da Costa *et al.*, 2009; Osborne *et al.*, 2009). Similar cytoprotective effects of PGE_2 were observed in neurodegenerative disease: in the extrinsic pathway involving TNF (Figure 1), Lee *et al.* (2004) showed cytotoxicity to cultured neurones which was ablated by PGE_2. Also, in a cell model of Alzheimer's disease, butaprost (but not EP1/EP3 agonist sulprostone) prevented neurotoxicity in a cAMP-dependent manner following exposure to beta-amyloid protein (Echeverria *et al.*, 2005). Furthermore, in Alzheimer's disease, there was increased PGE_2 in CSF of patients who survived longer (Combrinck *et al.*, 2006) indicating a protective role for PGE_2. This has implications for the design of EP2R selective agonists with neuroprotective activity in neurodegenerative disease and stroke. However, as EP2R is involved in many other functions (Sugimoto and Narumiya, 2007), it may be too general a target.

Cytoprotective activities of PGD and 15-deoxy-PGJ

Recently, PGD_2 has attracted attention as a cytoprotective molecule with fewer potential side effects than PGE_2 (Table 3). PGD_2 is abundant in brain (Hertting and Seregi, 1989), and its receptors may be an appropriate CNS target. Indeed, PGD_2 protected cultured neurones from glutamate-induced toxicity, an action dependent on cAMP (Liang *et al.*, 2005b). Two PGD_2 receptors, DP1 and DP2, have been identified, and the DP1 agonist BW245C mimicked the cytoprotective effects of PGD_2. Similarly, in reperfusion-ischaemia, DP1 receptor knockout animals showed larger necrotic lesions following cerebral artery occlusion, without changes in cerebral blood flow (Saleem *et al.*, 2007; Ahmad *et al.*, 2010). These studies demonstrated protective actions of PGD_2 via DP1 receptors. Thus, DP1R may present another target for therapeutic suppression of neuronal cell death.

A complication in understanding PGD_2 action arises from metabolism of PGD_2 to 15-deoxy-PGJ_2 (15d-PGJ_2), which also has cytoprotective activity (Lin *et al.*, 2006; Ou *et al.*, 2006). 15d-PGJ_2 reduced infarct volume following cerebral ischaemia in mice, coincident with up-regulation of transcription factor PPAR-γ and enhanced nuclear binding of PPAR-γ (Table 4). This suggested that PPARγ mediated some of the cytoprotective actions of 15d-PGJ_2. However, 15d-PGJ_2 may also act

independently of PPAR-γ via cell death signalling pathways. Pereira *et al*. (2006) showed PPAR-γ activation reduced necrosis following cerebral artery occlusion independently of 15d-PGJ_2. Also, 15d-PGJ_2 associated neuroprotection through PPAR-γ-independent mechanisms was reported (Saleem *et al*., 2007; Thura *et al*., 2009), and PPAR-γ-independent actions of 15d-PGJ_2 are supported by (i) evidence of 15d-PGJ_2 activity in PPAR-γ knockout cells (Chawla *et al*., 2001); and (ii) concentrations of 15d-PGJ_2 required to exert an action several orders of magnitude lower than those activating PPAR-γ in the same tissues (Bell-Parikh *et al*., 2003; Ide *et al*., 2003). An additional site of action of 15d-PGJ_2 in cell death signalling is nuclear factor NF-κB signalling (Figure 1). 15d-PGJ_2 reacts with nucleophiles such as free sulfhydryls of glutathione and cysteine residues in cellular proteins, and inhibited activation of NF-κB via inhibition of phosphorylation and degradation of IκBα (Ackerman *et al*., 2005). Indeed, it has also been shown that 15d-PGJ_2 can covalently bind to the cysteine residues of PPAR-γ (Soares *et al*., 2005).

A gastrointestinal effect of 15d-PGJ_2 has been identified, also involving NF-κB and Bcl-2 signalling. *Helicobacter pylori* infection, associated with peptic ulcer, gastric atrophy and gastric adenocarcinoma, appears linked to *H. pylori*-induced apoptosis in gastric epithelial cells. Exposure of gastric epithelial cells to *H. pylori* activated transcription factor NF-kB, which promoted increased pro-apoptotic gene expression (Chu *et al*., 2003). Recently, Cha *et al*. (2009) demonstrated that 15d-PGJ_2 inhibited apoptosis in *H. pylori*-infected gastric epithelial cells by inhibiting NF-κB activation, resulting in down-regulation of apoptotic Bax, and up-regulation of anti-apoptotic Bcl-2 gene expression.

Topical issues in eicosanoid pharmacology

Although aspirin and NSAIDs are widely prescribed, their molecular and cellular sites of action are incompletely understood. Recent studies have implicated novel mediators such as the resolvins, PGD_2 and direct actions of HUFA on cell death signalling pathways. The beneficial actions of NSAIDs have been linked to their ability to inhibit COX, and COX-2 selective inhibitor SC58236 exhibited neuroprotective activity in cerebral ischaemia, with marked reduction in lesions (Govoni *et al*., 2001). This study also showed that ischaemia was accompanied by increased PGD_2, and that COX-2 inhibitor reduced lesions and PGD_2 levels. This is an example of paradoxes reported in the actions of COX inhibitors, that is COX inhibitors being cytoprotective, while the products they inhibit (PGs) may also be cytoprotective! An explanation may lie in COX inhibitor cell death signalling independently of PGE_2 or PGD_2, for example, Vartiainen *et al*. (2001) demonstrated that NS398 (COX-2 selective) and piroxicam (non-selective COX inhibitor) protected neurones following ischaemia-reperfusion-induced necrosis, without up-regulating COX-1 or COX-2, and with little PGE_2 being produced. However, other cytoprotective signalling systems, such as ERK, were activated by COX inhibitors, and it is possible that COX inhibition allowed precursor HUFAs to accumulate. AA has apoptotic activity in many cell types, including leukaemic and vascular cells (Rizzo *et al*., 1999; 2002; Kalyankrishna *et al*., 2002; Rizzo and Leaver, 2010). Such PUFA release and signalling would be transient, as millimolar concentrations of fatty acids are unlikely to accumulate for extended periods, due to rapid re-esterification. The activity and extent of such transient localized signals need further investigation.

Developing strategies: agonist and antagonist design based on substrate specificity and host metabolism: neuroprotectin D1, hydroperoxy fatty acid signalling, endocannabinoids

Analysis of cell death signalling by membrane and lipid mediators has identified potential sites of drug development, ranging from COX metabolism to agonists and antagonists of lysosomal and ceramide signalling pathways. Strategies already discussed include (i) membrane modification via diet, neutrachemicals, specific uptake pathways, often involving n-3/n-6 PUFA modification (Bhathena, 2006; Farooqui and Horrocks, 2006); (ii) the specificity and selectivity of phospholipase A_2, studies extended by recent identification of molecular subtypes and systems which control of their activity (Akiba *et al*., 2000; Denizot *et al*., 2009; Sun *et al*., 2010); (iii) the generation of ROS, including those derived from lipid peroxides, superoxide, nitric oxide (NO being particularly relevant to vascular disease and pathology of endothelial cells), Bcl-2 family proteins acting at the level of mitochondrial permeability, antioxidant functions and Nicotinamide adenine dinucleotide phosphate oxidase (Colquhoun, 2010); (iv) sphingolipid and ceramide pathways (Orgertman and Hannun, 2004; Harr and Distelhorst, 2010); (v) eicosanoids and docosanoids and their receptors (Tables 4 and 5); and (vi) lipoxygenase and platelet activating factor (Esquenazi and Bazan, 2010; Serhan and Haeggstrom, 2010). Additionally, two recently developed areas for therapeutic intervention include the following lipid mediators.

Hydroperoxy-fatty acid signalling

The PPAR nuclear receptors are transcription factors that regulate gene transcription in response to lipid ligands and are involved in cell death signalling (Table 4, Hardwick and Chiang, 2009). The PPAR includes receptors for a wide range of lipids, including steroid and thyroid hormones, vitamin D, retinoic acid, HUFA, HUFA metabolites, and fibrate and thiazolidinedione (TZD) hypolipidemic and antidiabetic agents. PPAR exerts pro- and anti-apoptotic activities in different cells and pathologies. PPAR-γ, the most studied member of the PPAR family, is involved in adipocyte development and is the molecular target for TZD antidiabetic agents. Although PPAR-γ ligands have been useful in treatment of metabolic syndrome, their use is limited by side effects, including oedema, increased plasma volume, adiposity and adverse cardiovascular effects (Ajjan and Grant, 2008). Further analysis of PPAR-γ effects on the kidney and vasculature may help overcome these limitations. PPARs are of pharmacological interest, as they appear to have selective action on transformed cells and cells affected by degenerative disorders (Hardwick and Chiang, 2009). The fatty acid specificity of

PPAR is wide in comparison to cyclooxygenase and lipoxygenase, and PPAR-γ has also been reported to respond to cannabinoids (Giuliano *et al.*, 2009).

Endocannabinoids and their receptors

A novel group of HUFAs containing compounds with therapeutic potential are the naturally occurring cannabinoids, the endocannabinoids, including anandamide (arachidonyl ethanolamide), 2-arachidonoyl glycerol (Pertwee, 2006), O-arachidonyl ethanolamine, 2-arachidonyl glyceryl ether and N-arachidonyl dopamine (Ashton *et al.*, 2008). The reason for the arachidonyl component is unclear, but may be related to the biological activity of this moiety. In addition to the n-6 series of endocannabinoids, n-3 series, specifically docosanoid ethanolamide has also been identified. Bisogno *et al.* (1999) demonstrated the presence of docosahexaenoylethanolamide and 2-docosahexaenoylglycerol in the retina which accumulates DHA. Two receptors associated with endocannabinoid signalling, cannabinoid receptors 1 (CB1) and 2 (CB2), have been identified. Additionally, there is evidence that endocannabinoid metabolites may be effective ligands of PGE receptors (Ross *et al.*, 2002) and of endocannabinoid metabolism via cyclooxygenase and lipoxygenase pathways (Figure 2), and action on vanilloid and capsaicin receptors (Craib *et al.*, 2001; Appendino *et al.*, 2009). CB1 and CB2 are active in cell death signalling pathways. CB1 and CB2 are transmembrane GPCRs which inhibit adenylyl cyclase and activate MAP kinase (see Figure 1). CB1 receptors are present in highest concentration in brain, but are also found in gastrointestinal tract, liver and adipose tissue. CB1 receptors inhibit presynaptic N- and P/Q-type calcium channels and activate inwardly rectifying potassium channels (Pertwee, 2006; 2009; 2010). CB1 receptors are highly expressed in hypothalamic areas involved in food intake. Also, in peripheral tissues, antagonism of CB1 receptors increases insulin sensitivity and oxidation of fatty acids in muscles and liver. CB2 receptors are predominantly located in immune and haematopoietic systems. The discovery of the endogenous cannabinoids led to development of CB1 receptor antagonists in 1994. However, early CB1 antagonists, developed for treatment of obesity, had serious psychiatric side effects, and CB1 antagonists that target peripheral CB1 receptors by restricting their ability to cross the blood brain barrier are currently under development. Possibly of even greater potential are cannabinoid receptor agonists that target the brain, for instance, pain receptor antagonists currently used in chemotherapy-induced nausea and vomiting, relief of neuropathic pain in multiple sclerosis, and agents affecting CB2 receptors in the immune and haematopoietic systems may also be useful (Pertwee, 2009; Bolognini *et al.*, 2010). Recently, it has been shown that n-3 PUFA ethanolamides such as DHA-ethanolamide and EPA-ethanolamide can be antiproliferative towards prostate cancer cells and that part of these actions is mediated via cannabinoid receptors (Brown *et al.*, 2010). It has also been definitively shown that cancer cells possess the capacity to produce DHA-ethanolamide and EPA-ethanolamide (Brown *et al.*, 2011). In developing these agents, better understanding of endocannabinoid pathways, signalling systems and micro-environmental signals modulating their activity is essential, for example, neuroprotective, anti-apoptotic actions of the phytocannabinoid cannabidiol (Ryan *et al.*, 2009).

Future directions in cell death signalling: membranes, mediators and micro-environments

Strategies in drug design should be informed by signalling pathways at the cellular level. These approaches are being used to investigate the complex biology of cell death. However, genetic and proteomic approaches have diverted attention from the role of membranes in compartmentalization and signalling via membrane metabolism and lipid mediators, especially those associated with HUFA (Figures 1 and 2).

The HUFA is essential for cell function. These epigenetic elements are crucial at cellular level, initiating and integrating key events in cell signalling at the plasma membrane, intracellular organelles, responding to stress signals, and controlling transcription and regulatory factors. HUFA-associated membrane responses and mediator actions are involved in complex pathological processes, and key signalling events associated with disorders of cell death (Table 3). These events are integrated at the level of signal modulation, involving the micro-environment and systems biology (Table 1). Agents affecting HUFA metabolism include the NSAIDs, a pharmacognosy that extends over a century, but which is still yielding insights into the treatment of complex multifactorial diseases (Table 5).

The identity and activity of key mediators is a crucial issue, and novel intermediates associated with prostanoid, cannabinoid, resolvin and endoperoxide pathways are providing new therapeutic opportunities. Topical issues in cell death signalling include how and why membrane metabolism signalling occurs, its role in intracellular and transcellular communication, and interactions with micro-environmental and epigenetic factors involved in pathogenic changes (Table 1). New developments have focused on key initiating events in cell death signalling, interactions at molecular, cellular and system levels, using bioengineering and cell biology.

Acknowledgements

We are very grateful to Professors Nicolas and Haydee Bazan for their help in organizing a British Pharmacological Society Symposium on Cell Death Signalling, to the Royal Society of Edinburgh, Amarin, Antoxis Ltd., Enzo Life Sciences Ltd. and Promega for support of the meeting, and to Chris Brown of Indiana University School of Medicine, Department of Visual Media.

Conflicts of interest

None.

References

Ackerman WE, Zhang XL, Rovin BH, Kniss DA (2005). Modulation of cytokine-induced cyclooxygenase 2 expression by PPARG ligands through NFkappaB signal disruption in human WISH and amnion cells. Biol Reprod 73: 527–535.

Adibhatla RM, Hatcher JF (2006). Phospholipase A_2, reactive oxygen species, and lipid peroxidation in cerebral ischemia. Free Radic Biol Med 40: 376–387.

Ahmad AS, Ahmad M, Maruyama T, Narumiya S, Dore S (2010). Prostaglandin D2 DP1 receptor is beneficial in ischemic stroke and in acute exicitotoxicity in young and old mice. Age (Dordr) 32: 271–282.

Ajjan RA, Grant PJ (2008). The cardiovascular safety of rosiglitazone. Expert Opin Drug Saf 7: 367–376.

Akiba S, Hatazawa R, Ono K, Hayama M, Matsui H, Sato T (2000). Transforming growth factor-alpha stimulates prostaglandin generation through cytosolic phospholipase A(2) under the control of p11 in rat gastric epithelial cells. Br J Pharmacol 131: 1004–1010.

Alexander SPH, Mathie A, Peters JA (2011). Guide to Receptors and Channels (GRAC), 5th Edition. Br J Pharmacol 164 (Suppl. 1): S1–S324.

Alonso MM, Jiang H, Yokoyama T, Xu J, Bekele NB, Lang FF *et al.* (2008). Delta-24-RGD in combination with RAD001 induces enhanced anti-glioma effect via autophagic cell death. Mol Ther 16: 487–493.

Andrade da Costa BL, Kang KD, Rittenhouse KD, Osborne NN (2009). The localization of PGE_2 receptor subtypes in rat retinal cultures and the neuroprotective effect of the EP2 agonist butaprost. Neurochem Int 55: 199–207.

Appendino G, Ligresti A, Minassi A, Cascio MG, Allar M, Taglialatela-Scafati OV *et al.* (2009). Conformationally constrained fatty acid ethanolamides as cannabinoid and vanilloid receptor probes. J Med Chem 52: 3001–3009.

Ashton JS, Wrigth JL, McPartland JM, Tyndall JDA (2008). Cannabinoid CB1 and CB2 receptor LIGAND specificity and the development of CB2-selective agonists. Curr Med Chem 15: 1428–1443.

Bai XM, Jiang H, Ding JX, Peng T, Ma J, Wang YH *et al.* (2010). Prostaglandin E2 upregulates survivin expression via the EP1 receptor in hepatocellular carcinoma cells. Life Sci 86: 214–223.

Bain J, Plater L, Elliott M, Shpiro N, Hastie CJ, Mclauchlan H *et al.* (2007). The selectivity of protein kinase inhibitors: a further update. Biochem J 408: 297–315.

Ban JO, Kwak DH, Oh JH, Park EJ, Cho MC, Song HS *et al.* (2010). Suppression of NF-kappaB and GSK-3beta is involved in colon cancer cell growth inhibition by the PPAR agonist troglitazone. Chem Biol Interact 188: 75–85.

Baryawno N, Sveinbjornsson B, Eksborg S, Orrego A, Segerstrom L, Oqvist CO *et al.* (2008). Tumor-growth-promoting cyclooxygenase-2 prostaglandin E2 pathway provides medulloblastoma therapeutic targets. Neuro-Oncol 10: 661–674.

Bell-Parikh LC, Ide T, Lawson JA, McNamara P, Reilly M, FitzGerald GA (2003). Biosynthesis of 15-deoxy-delta12,14-PGJ_2 and the ligation of PPARγ. J Clin Invest 112: 945–955.

Benadiba M, Dos Santos RR, Silvade O, Colquhoun A (2010). Inhibition of C6 rat glioma proliferation by [Ru2Cl(Ibp)4] depends on changes in p21, p27, Bax/Bcl2 ratio and mitochondrial membrane potential. J Inorg Biochem 104: 928–935.

Bhathena SJ (2006). Relationship between fatty acids and the endocrine and neuroendocrine system. Nutr Neurosci 9: 1–10.

Bidwell P, Joh K, Leaver HA, Rizzo MT (2010). Prostaglandin E2 activates cAMP response element-binding protein in glioma cells via a signaling pathway involving PKA-dependent inhibition of ERK. Prostaglandins Other Lipid Mediat 91: 18–29.

Bisogno T, Delton-Vandenbroucke I, Milone A, Lagarde M, Di Marzo V (1999). Biosynthesis and inactivation of N-arachidonoylethanolamine (anandamide) and N-docosahexaenoylethanolamine in bovine retina. Arch Biochem Biophys 370: 300–307.

Bolognini D, Costa D, Maione S, Comelli F, Marini P, Di Marzo V *et al.* (2010). The plant cannabinoid Δ9-tetrahydrocannabivarin can decrease signs of inflammation and inflammatory pain in mice. Br J Pharmacol 160: 677–687.

Bröker LE, Kruyt FAE, Giaccone G (2005). Cell death independent of caspases: a review. Clin Cancer Res 11: 3155–3162.

Brown I, Cascio MG, Wahle KW, Smoum R, Mechoulam R, Ross RA *et al.* (2010). Cannabinoid receptor-dependent and -independent anti-proliferative effects of omega-3 ethanolamides in androgen receptor-positive and -negative prostate cancer cell lines. Carcinogenesis 31: 1584–1591.

Brown I, Wahle KW, Cascio MG, Smoum-Jaouni R, Mechoulam R, Pertwee RG *et al.* (2011). Omega-3 N-acylethanolamines are endogenously synthesised from omega-3 fatty acids in different human prostate and breast cancer cell lines. Prostaglandins Leukot Essent Fatty Acids 85: 305–310.

Carlson NG, Rojas MA, Black JD, Redd JW, Hille J, Hill KE *et al.* (2009). Microglial inhibition of neuroprotection by antagonists of the EP1 prostaglandin E2 receptor. J Neuroinflammation 6: 5.

Carrasco E, Casper D, Werner P (2007). PGE(2) receptor EP1 renders dopaminergic neurons selectively vulnerable to low-level oxidative stress and direct PGE(2) neurotoxicity. J Neurosci Res 85: 3109–3117.

Carrasco E, Werner P, Casper D (2008). Prostaglandin receptor EP2 protects dopaminergic neurons against 6-OHDA-mediated low oxidative stress. Neurosci Lett 441: 44–49.

Cha B, Kim KH, Kim H (2009). 15-Deoxy-delta 12, 14,-prostaglandin J2 suppresses nuclear factor-kappaB-mediated apoptosis of Helicobacter pylori-infected gastric epithelial cells. Ann N Y Acad Sci 1171: 457–463.

Chawla A, Barak Y, Nagy L, Liao D, Tontonoz P, Evans RM (2001). PPAR-γ dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation. Nat Med 7: 48–52.

Chell S, Kaidi A, Williams AC, Paraskeva C (2006). Mediators of PGE_2 synthesis and signalling downstream of COX-2 represent potential targets for the prevention/treatment of colorectal cancer. Biochim Biophys Acta 1766: 104–119.

Chen KC, Chang LS (2009). Arachidonic acid-induced apoptosis of human neuroblastoma SK-N-SH cells is mediated through mitochondrial alteration elicited by ROS and Ca2+-evoked activation of p38α MAPK and JNK1. Toxicology 262: 199–206.

Chima RS, Hake PW, Piraino G, Mangeshkar P, O'Connor M, Zingarelli B (2010). Ciglitazone, a novel inhibitor of lung apoptosis following hemorrhagic shock. Int J Clin Exp Med 3: 1–9.

Chu SH, Lim JW, Kim KH, Kim H (2003). NF-kappaB and Bcl-2 in Helicobacter pylori-induced apoptosis in gastric epithelial cells. Ann N Y Acad Sci 1010: 568–572.

Clarke SD, Turini M, Jump D (1997). Polyunsaturated fatty acids regulate lipogenic and peroxisomal gene expression by independent mechanisms. Prostaglandins Leukot Essent Fatty Acids 57: 65–69.

Colquhoun A (2010). Lipids, mitochondria and cell death: implications in neuro-oncology. Mol Neurobiol 42: 76–88.

Combrinck M, Williams J, De Berardinis MA, Warden D, Puopolo M, Smith AD *et al.* (2006). Levels of CSF prostaglandin E2, cognitive decline, and survival in Alzheimer's disease. J Neurol Neurosurg Psychiatry 77: 85–88.

Craib SJ, Ellington HC, Pertwee RG, Ross RA (2001). A possible role of lipoxygenase in the activation of vanilloid receptors by anandamide in the guinea pig bronchus. Br J Pharmacol 134: 30–37.

Cui PH, Petrovic N, Murray M (2011). The W-3 epoxide of eicosapentaenoic acid inhibits endothelial cell proliferation by p38 MAPK activation and cyclin D1/CRK4 down-regulation. Br J Pharmacol 162: 1143–1155.

Cuzick J, Otto F, Baron JA, Brown PH, Burn J, Greenwald P *et al.* (2009). Aspirin and non-steroidal anti-inflammatory drugs for cancer prevention: an international consensus statement. Lancet Oncol 10: 501–507.

Davidson J, Abul HT, Milton AS, Rotondo D (2001). Cytokines and cytokine inducers stimulate prostaglandin E2 entry into the brain. Pflugers Arch 442: 526–533.

Deigner HP, Hermetter A (2008). Oxidized phospholipids: emerging lipid mediators in pathophysiology. Curr Opin Lipidol 19: 289–294.

Denizot Y, De Armas R, Durand K, Robert S, Moreau JJ, Caire F *et al.* (2009). Analysis of several PLA_2 mRNA in human meningiomas. Mediators Inflamm 2009: 689430.

Echeverria V, Clerman A, Dore S (2005). Stimulation of PGE receptors EP2 and EP4 protects cultured neurons against oxidative stress and cell death following beta-amyloid exposure. Eur J Neurosci 22: 2199–2206.

Elwood PC, Gallagher AM, Duthie GG, Mur LA, Morgan G (2009). Aspirin, salicylates, and cancer. Lancet 373: 1301–1309.

Esquenazi S, Bazan H (2010). Role of platelet-activating factor in cell death signaling in the cornea. Mol Neurobiol 42: 32–38.

Farooqui AA, Horrocks LA (2006). Phospholipase A_2-generated lipid mediators in the brain: the good, the bad, and the ugly. Neuroscientist 12: 245–260.

Galluzzi L, Maiuri MC, Vitale I, Zischka H, Castedo M, Zitvogel L *et al.* (2007). Cell death modalities: classification and pathophysiological implications. Cell Death Differ 14: 1237–1243.

Galluzzi L, Morselli E, Vicencio JM, Kepp O, Joza N, Tajeddine N *et al.* (2008). Life, death and burial: multifaceted impact of autophagy. Biochem Soc Trans 36: 786–790.

Geng Y, Kohli L, Klocke BJ, Roth KA (2010). Chloroquine-induced autophagic vacuole accumulation and cell death in glioma cells is p53 independent. Neuro-Oncol 12: 473–481.

George RJ, Sturmoski MA, Anant S, Houchen CW (2007). EP4 mediates PGE_2 dependent cell survival through the PI3 kinase/AKT pathway. Prostaglandins Other Lipid Mediat 83: 112–120.

Giuliano M, Pellerito O, Portanova P, Calvaruso G, Santulli A, De Blasio A *et al.* (2009). Apoptosis induced in HepG2 cells by the synthetic cannabinoid WIN: involvement of the transcription factor PPARγ. Biochimie 91: 457–465.

Govoni S, Masoero E, Favalli L, Rozza A, Scelsi R, Viappiani S *et al.* (2001). The cyclooxygenase-2 inhibitor SC58236 is neuroprotective in an in vivo model of focal ischemia in the rat. Neurosci Lett 303: 91–94.

Greenhough A, Smartt HJM, Moore AE, Roberts HR, Williams AC, Paraskeva C *et al.* (2009). The COX-2/PGE_2 pathway: key roles in the hallmarks of cancer and adaptation to the tumour microenvironment. Carcinogenesis 30: 377–386.

Hardingham GE, Patani R, Baxter P, Wyllie DJ, Chandran S (2010). Human embryonic stem cell-derived neurones as a tool for studying neuroprotection and neurodegeneration. Mol Neurobiol 42: 97–102.

Hardwick JP, Chiang JY (2009). PPARs, RXRs, and drug-metabolizing enzymes. PPAR Res 2009: 589626.

Harr MW, Distelhorst CW (2010). Apoptosis and autophagy: decoding calcium signals that mediate life or death. Cold Spring Harb Perspect Biol 2: a005579.

Hertting G, Seregi A (1989). Formation and function of eicosanoids in the central nervous system. Ann N Y Acad Sci 559: 84–99.

Hoffman T, Lizzio EF, Suissa J, Rotrosen D, Sullivan JA, Mandell GL *et al.* (1988). Dual stimulation of phospholipase activity in human monocytes. Role of calcium-dependent and calcium-independent pathways in arachidonic acid release and eicosanoid formation. J Immunol 140: 3912–3918.

Hoshikawa H, Goto R, Mori T, Mitani T, Mori N (2009). Expression of prostaglandin E2 receptors in oral squamous cell carcinomas and growth inhibitory effects of an EP3 selective antagonist, ONO-AE3-240. Int J Oncol 34: 847–852.

Ide T, Egan K, Bell-Parikh LC, FitzGerald GA (2003). Activation of nuclear receptors by prostaglandins. Thromb Res 110: 311–315.

Ito K, Hirao A, Arai F, Takubo K, Matsuoka S, Miyamoto K *et al.* (2006). Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells. Nat Med 12: 446–451. (Corrigendum Jan 2010).

Jiang B, Liang P, Zhang B, Song J, Huang X, Xiao X (2009). Role of PPAR-β in hydrogen peroxide-induced apoptosis in human umbilical vein endothelial cells. Atherosclerosis 204: 353–358.

Kalyankrishna S, Parmentier JH, Malik KU (2002). Arachidonic acid-derived oxidation products initiate apoptosis in vascular smooth muscle cells. Prostaglandins Other Lipid Mediat 70: 13–29.

Keightley MC, Sales KJ, Jabbour HN (2010). PGF2alpha-F-prostanoid receptor signalling via ADAMTS1 modulates epithelial cell invasion and endothelial cell function in endometrial cancer. BMC Cancer 10: 488.

Kilter H, Werner M, Roggia C, Reil JC, Schafers HJ, Kintscher U *et al.* (2009). The PPAR-γ agonist rosiglitazone facilitates Akt rephosphorylation and inhibits apoptosis in cardiomyocytes during hypoxia/reoxygenation. Diabetes Obes Metab 11: 1060–1067.

Kim HJ, Kim MY, Jin H, Kang SS, Lee JH, Chang KC *et al.* (2009). Peroxisome proliferator-activated receptor {delta} regulates extracellular matrix and apoptosis of vascular smooth muscle cells through the activation of transforming growth factor-{beta}1/Smad3. Circ Res 105: 16–24.

Kim S, Lee JJ, Heo DS (2011). PPARγ ligands induce growth inhibition and apoptosis through p63 and p73 in human ovarian cancer cells. Biochem Biophys Res Commun 406: 389–395.

Kirkegaard T, Witton CJ, McGlynn LM, Tovey SM, Dunne B, Lyon A *et al.* (2005). AKT activation predicts outcome in breast cancer patients treated with tamoxifen. J Pathol 207: 139–146.

Kroemer G, Galluzzi L, Vandenabeele P, Abrams J, Alnemri ES, Baehrecke EH *et al*. (2009). Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009. Cell Death Differ 16: 3–11.

Kurebayashi Y, Honda Y (1991). Protection by 16,16-dimethyl prostaglandin E2 and dibutyryl cyclic AMP against complement-mediated hepatic necrosis in rats. Hepatology 14: 545–550.

Lasithiotakis KG, Sinnberg TW, Schittek B, Flaherty KT, Kulms D, Maczey E *et al*. (2008). Combined inhibition of MAPK and mTOR signaling inhibits growth, induces cell death, and abrogates invasive growth of melanoma cells. J Invest Dermatol 128: 2013–2023.

Leaver HA, Hardingham GE (2010). To die or not to die? Current questions in cell death signaling. Mol Neurobiol 42: 1–3.

Leaver HA, Rizzo MT, Whittle IR (2010). Glioma cell death: cell–cell interactions and signalling networks. Mol Neurobiol 42: 89–96.

Lee EO, Shin YJ, Chong YH (2004). Mechanisms involved in prostaglandin E2-mediated neuroprotection against TNF-alpha: possible involvement of multiple signal transduction and beta-catenin/T-cell factor. J Neuroimmunol 155: 21–31.

Liang X, Wang Q, Hand T, Wu L, Breyer RM, Montine TJ *et al*. (2005a). Deletion of the prostaglandin E2 EP2 receptor reduces oxidative damage and amyloid burden in a model of Alzheimer's disease. J Neurosci 25: 10180–10187.

Liang X, Wu L, Hand T, Andreasson K (2005b). Prostaglandin D2 mediates neuronal protection via the DP1 receptor. J Neurochem 92: 477–486.

Lin TN, Cheung WM, Wu JS, Chen JJ, Lin H, Liou JY *et al*. (2006). 15d-prostaglandin J2 protects brain from ischemia-reperfusion injury. Arterioscler Thromb Vasc Biol 26: 481–487.

Liu JJ, Guo YW, Fang ZG, Si XN, Wu XY, Liu PQ *et al*. (2009a). Activation of peroxisome proliferator-activated receptor-gamma induces apoptosis on acute promyelocytic leukemia cells via downregulation of XIAP. Int J Mol Med 24: 623–632.

Liu JJ, Xu Y, Zhang Y, Xiao RZ, Lin DJ (2009b). Peroxisome proliferator-activated receptor γ (PPAR-γ) agonist rosiglitazone (RGZ) inhibits HL-60 cell growth by induction of apoptosis. Lab Med 40: 297–302.

Liu Y, Taylor CW (2006). Stimulation of arachidonic acid release by vasopressin in A7r5 vascular smooth muscle cells mediated by Ca2+-stimulated phospholipase A_2. FEBS Lett 580: 4114–4120.

Lord-Dufour S, Copland IB, Levros LC, Post M, Das A, Khosla C *et al*. (2009). Evidence for transcriptional regulation of the glucose-6-phosphate transporter by HIF-1α: targeting G6PT with mumbaistatin analogs in hypoxic mesenchymal stromal cells. Stem Cells 27: 489–497.

Lukiw WJ, Bazan NG (2010). Inflammatory, apoptotic, and survival gene signaling in Alzheimer's disease. A review on the bioactivity of neuroprotectin D1 and apoptosis. Mol Neurobiol 42: 10–16.

Mahdi JG, Alkarrawi MA, Mahdi AJ, Bowen ID, Humam D (2006). Calcium salicylate-mediated apoptosis in human HT-1080 fibrosarcoma cells. Cell Prolif 39: 249–260.

Mao JT, Nie WX, Tsu IH, Jin YS, Rao JY, Lu QY *et al*. (2010). White tea extract induces apoptosis in non-small cell lung cancer cells: the role of peroxisome proliferator-activated receptor-{gamma} and 15-lipoxygenases. Cancer Prev Res (Phila) 3: 1132–1140.

McCullough L, Wu L, Haughey N, Liang X, Hand T, Wang Q *et al*. (2004). Neuroprotective function of the PGE_2 EP2 receptor in cerebral ischemia. J Neurosci 24: 257–268.

Meuillet J (2011). Novel inhibitors of AKT: assessment of a different approach targeting the pleckstrin homology domain. Curr Med Chem 18: 2727–2742.

Miller TW, Isenberg JS, Roberts DD (2009). Molecular regulation of tumor angiogenesis and perfusion via redox signaling. Chem Rev 109: 3099–3124.

Mizoguchi Y, Tsutsui H, Miyajima K, Sakagami Y, Seki S, Kobayashi K *et al*. (1987). The protective effects of prostaglandin E1 in an experimental massive hepatic cell necrosis model. Hepatology 7: 1184–1188.

Nair RR, Tolentino J, Hazlehurst LA (2010). The bone marrow microenvironment as a sanctuary for minimal residual disease in CML. Biochem Pharmacol 80: 602–612.

O'Callaghan G, Kelly J, Shanahan F, Houston A (2008). Prostaglandin E2 stimulates Fas ligand expression via the EP1 receptor in colon cancer cells. Br J Cancer 99: 502–512.

Orgertman B, Hannun YA (2004). Biologically active sphingolipids in cancer pathogenesis and treatment. Nat Rev Cancer 4: 604–616.

Osborne NN, Li GY, Ji D, Andrade da Costa BL, Fawcett RJ, Kang KD *et al*. (2009). Expression of prostaglandin PGE_2 receptors under conditions of aging and stress and the protective effect of the EP2 agonist butaprost on retinal ischemia. Invest Ophthalmol Vis Sci 50: 3238–3248.

Ou Z, Zhao X, Labiche LA, Strong R, Grotta JC, Herrmann O (2006). Neuronal expression of peroxisome proliferator-activated receptor-gamma (PPARγ) and 15d-prostaglandin J2-mediated protection of brain after experimental cerebral ischemia in rat. Brain Res 1096: 196–203.

Paterniti I, Esposito E, Mazzon E, Galuppo M, Di Paola R, Bramanti P *et al*. (2010). Evidence for the role of peroxisome proliferator-activated receptor-beta/delta in the development of spinal cord injury. J Pharmacol Exp Ther 333: 465–477.

Payne CA, Maleki S, Messina M, O'Sullivan MG, Stone G, Hall NR *et al*. (2008). Loss of prostaglandin D2 synthase: a key molecular event in the transition of a low-grade astrocytoma to an anaplastic astrocytoma. Mol Cancer Ther 7: 3420–3428.

Payner T, Leaver HA, Knapp B, Whittle IR, Trifan OC, Miller S *et al*. (2006). Microsomal prostaglandin E synthase-1 regulates human glioma cell growth via prostaglandin E(2)-dependent activation of type II protein kinase A. Mol Cancer Ther 5: 1817–1826.

Pellicano F, Copland M, Jorgensen HG, Mountford J, Leber B, Holyoake TL (2009). BMS-214662 induces mitochondrial apoptosis in chronic myeloid leukemia (CML) stem/progenitor cells, including CD34+38- cells, through activation of protein kinase C beta. Blood 114: 4186–4196.

Pereira MP, Hurtado O, Cardenas A, Bosca L, Castillo J, Davalos A *et al*. (2006). Rosiglitazone and 15-deoxy-delta12,14-prostaglandin J2 cause potent neuroprotection after experimental stroke through noncompletely overlapping mechanisms. J Cereb Blood Flow Metab 26: 218–229.

Pertwee RG (2006). Cannabinoid pharmacology: the first 66 years. Br J Pharmacol 147: S163–S171.

Pertwee RG (2009). Emerging strategies for exploiting cannabinoid receptor agonists as medicines. Br J Pharmacol 156: 397–411.

Pertwee RG (2010). Receptors and channels targeted by synthetic cannabinoid receptor agonists and antagonists. Curr Med Chem 17: 1360–1381.

Piazuelo E, Jimenez P, Strunk M, Santander S, Garcia A, Esteva F *et al.* (2006). Effects of selective PGE_2 receptor antagonists in esophageal adenocarcinoma cells derived from Barrett's esophagus. Prostaglandins Other Lipid Mediat 81: 150–161.

Picq M, Chen P, Perez M, Lagarde M (2010). DHA metabolism: targetting the brain and lipoxygenation. Mol Neurobiol 42: 48–51.

Portanova JP, Zhang Y, Anderson GD, Hauser SD, Masferrer JL, Seibert K *et al.* (1996). Selective neutralization of prostaglandin E2 blocks inflammation, hyperalgesia, and interleukin 6 production in vivo. J Exp Med 184: 883–891.

Prakobwong S, Gupta SC, Kim JH, Sung B, Pinlaor P, Hiraku Y *et al.* (2011). Curcumin suppresses proliferation and induces apoptosis in human biliary cancer cells through modulation of multiple cell signaling pathways. Carcinogenesis 32: 1372–1380.

Psaila B, Bussel JB (2008). Refractory immune thrombocytopenic purpura: current strategies for investigation and management. Br J Haematol 143: 16–26.

Rader DJ, Daugherty A (2008). Translating molecular discoveries into new therapies for atherosclerosis. Nature 451: 904–913.

Ren Y, Sun C, Sun Y, Tan H, Wu Y, Cui B *et al.* (2009). PPARγ protects cardiomyocytes against oxidative stress and apoptosis via Bcl-2 upregulation. Vascul Pharmacol 51: 169–174.

Rizzo MT (2011). Cyclooxygenase-2 in oncogenesis. Clin Chim Acta 412: 671–687.

Rizzo MT, Leaver HA (2010). Brain endothelial cell death: modes, signaling pathways, and relevance to neural development, homeostasis, and disease. Mol Neurobiol 42: 52–63.

Rizzo MT, Regazzi E, Garau D, Akard L, Dugan M, Boswell HS *et al.* (1999). Induction of apoptosis by arachidonic acid in chronic myeloid leukemia cells. Cancer Res 59: 5047–5053.

Rizzo MT, Pudlo N, Farrell L, Leaver A (2002). Specificity of arachidonic acid-induced inhibition of growth and activation of c-jun kinases and p38 mitogen-activated protein kinase in hematopoietic cells. Prostaglandins Leukot Essent Fatty Acids 66: 31–40.

Ross RA, Craib SJ, Stevenson LA, Pertwee RG, Henderson A, Toole J *et al.* (2002). Pharmacological characterization of the anandamide cyclooxygenase metabolite: prostaglandin E2 ethanolamide. J Pharmacol Exp Ther 301: 900–907.

Rothwell PM, Fowkes FG, Belch JF, Ogawa H, Warlow CP, Meade TW (2011). Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. Lancet 377: 31–41.

Rudner J, Elsaesser SJ, Muller AC, Belka C, Jendrossek V (2010). Differential effects of anti-apoptotic Bcl-2 family members Mcl-1, Bcl-2, and Bcl-xL on celecoxib-induced apoptosis. Biochem Pharmacol 79: 10–20.

Rush S, Khan G, Bamisaiye A, Bidwell P, Leaver HA, Rizzo MT (2007). c-jun amino-terminal kinase and mitogen activated protein kinase 1/2 mediate hepatocyte growth factor-induced migration of brain endothelial cells. Exp Cell Res 313: 121–132.

Ryan D, Drysdale AJ, Lafourcade C, Pertwee RG, Platt B (2009). Cannabidiol targets mitochondria to regulate intracellular Ca2+ levels. J Neurosci 29: 2053–2063.

Saleem S, Zhuang H, de Brum-Fernandes AJ, Maruyama T, Narumiya S, Dore S (2007). PGD(2) DP1 receptor protects brain from ischemia-reperfusion injury. Eur J Neurosci 26: 73–78.

Sawyer N, Cauchon E, Chateauneuf A, Cruz RP, Nicholson DW, Metters KM *et al.* (2002). Molecular pharmacology of the human prostaglandin D2 receptor, CRTH2. Br J Pharmacol 137: 1163–1172.

Serhan CN, Haeggstrom JZ (2010). Lipid mediators in acute inflammation and resolution: eicosanoids, PAF, resolvins, and PRotectins. In: Serhan CN, Ward PA, Gilroy PW (eds). Fundamentals of Inflammation. Cambridge University Press: Cambridge, pp. 153–174.

Serhan CN, Yang R, Martinod K, Kasuga K, Pillai PS, Porter TF *et al.* (2009). Maresins: novel macrophage mediators with potent anti-inflammatory and proresolving actions. J Exp Med 206: 15–23.

Shankaranarayanan P, Rossin A, Khanwalkar H, Alvarez S, Alvarez R, Jacobson A *et al.* (2009). Growth factor-antagonized rexinoid apoptosis involves permissive PPARγ/RXR heterodimers to activate the intrinsic death pathway by NO. Cancer Cell 16: 220–231.

Sibilia V, Pagani F, Rindi G, Lattuada N, Rapetti D, De Luca V *et al.* (2009). Central ghrelin gastroprotection involves nitric oxide/prostaglandin cross-talk. Br J Pharmacol 154: 688–697.

Soares AF, Nosjean O, Cozzone D, D'Orazio D, Becchi M, Guichardant M *et al.* (2005). Covalent binding of 15-deoxy-delta12,14-prostaglandin J2 to PPARgamma. Biochem Biophys Res Commun 337: 521–525.

Sugimoto Y, Narumiya S (2007). Prostaglandin E receptors. J Biol Chem 282: 11613–11617.

Sun GY, Shelat PB, Jensen MB, He Y, Sun AY, Simonyi A (2010). Phospholipases A_2 and inflammatory responses in the central nervous system. Neuromolecular Med 12: 133–148.

Sun WH, Chen GS, Ou XL, Yang Y, Luo C, Zhang Y *et al.* (2009). Inhibition of COX-2 and activation of peroxisome proliferator-activated receptor gamma synergistically inhibits proliferation and induces apoptosis of human pancreatic carcinoma cells. Cancer Lett 275: 247–255.

Takahashi T, Uehara H, Bando Y, Izumi K (2008). Soluble EP2 neutralizes prostaglandin E2-induced cell signaling and inhibits osteolytic tumor growth. Mol Cancer Ther 7: 2807–2816.

Tamiya T, Kashiwagi I, Takahashi R, Yasukawa H, Yoshimura A (2011). Regulation of T-Cell inflammation by SOCS1 and SOCS3 suppressors of cytokine signaling (SOCS) proteins and JAK/STAT pathways. Arterioscler Thromb Vasc Biol 31: 980–985.

Thura M, Hokamura K, Yamamoto S, Maeda M, Furuta K, Suzuki M *et al.* (2009). GIF-0173 protects against cerebral infarction through DP1 receptor activation. Exp Neurol 219: 481–491.

Urban JD, Clarke WP, von Zastrow M, Nichols DE, Kobilka B, Weinstein H *et al.* (2007). Functional selectivity and classical concepts of quantitative pharmacology. J Pharmacol Exp Ther 320: 1–13.

Uttara B, Singh AV, Zamboni AV, Mahajan RT (2009). Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol 7: 65–74.

Vartiainen N, Huang CY, Salminen A, Goldsteins G, Chan PH, Koistinaho J (2001). Piroxicam and NS-398 rescue neurones from hypoxia/reoxygenation damage by a mechanism independent of cyclo-oxygenase inhibition. J Neurochem 76: 480–489.

Vicencio JM, Galluzzi L, Tajeddine N, Ortiz C, Criollo A, Tasdemir E *et al*. (2008). Senescence, apoptosis or autophagy? When a damaged cell must decide its path – a mini-review. Gerontology 54: 92–99.

Vinukonda G, Csiszar A, Hu F, Dummula K, Pandey NK, Zia MT *et al*. (2010). Neuroprotection in a rabbit model of intraventricular haemorrhage by cyclooxygenase-2, prostanoid receptor-1 or tumour necrosis factor-alpha inhibition. Brain 133: 2264–2280.

Wahle KW, Heys SD, Rotondo D (2004). Conjugated linoleic acids: are they beneficial or detrimental to health? Prog Lipid Res 43: 553–587.

Wan J, Jiang L, Lu Q, Ke L, Li X, Tong N (2010). Activation of PPARδ up-regulates fatty acid oxidation and energy uncoupling genes of mitochondria and reduces palmitate-induced apoptosis in pancreatic beta-cells. Biochem Biophys Res Commun 391: 1567–1572.

Wang X, Huang G, Mei S, Qian J, Ji J, Zhang J (2009). Over-expression of C/EBP-alpha induces apoptosis in cultured rat hepatic stellate cells depending on p53 and peroxisome proliferator-activated receptor-gamma. Biochem Biophys Res Commun 380: 286–291.

Warner TD, Mitchell JA (2004). Cyclooxygenases: new forms, new inhibitors, and lessons from the clinic. FASEB J 18: 790–804.

Weber TJ, Markillie LM, Chrisler WB, Vielhauer GA, Regan JW (2002). Modulation of JB6 mouse epidermal cell transformation response by the prostaglandin F2alpha receptor. Mol Carcinog 35: 163–172.

Wu JS, Cheung WM, Tsai YS, Chen YT, Fong WH, Tsai HD *et al*. (2009a). Ligand-activated peroxisome proliferator-activated receptor-gamma protects against ischemic cerebral infarction and neuronal apoptosis by 14-3-3 epsilon upregulation. Circulation 119: 1124–1134.

Wu JS, Lin TN, Wu KK (2009b). Rosiglitazone and PPARγ overexpression protect mitochondrial membrane potential and prevent apoptosis by upregulating anti-apoptotic Bcl-2 family proteins. J Cell Physiol 220: 58–71.

Wyllie AH (2010). Where O death is thy sting? A brief review of apoptosis biology. Mol Neurobiol 42: 4–9.

Xue L, Barrow A, Pettipher R (2009). Novel function of CRTH2 in preventing apoptosis of human Th2 cells through activation of the phosphatidylinositol 3-kinase pathway. J Immunol 182: 7580–7586.

Zamuner SR, Bak AW, Devchand PR, Wallace JL (2005). Predisposition to colorectal cancer in rats with resolved colitis: role of cyclooxygenase-2-derived prostaglandin D2. Am J Pathol 167: 1293–1300.

Zhao W, Iskandar S, Kooshki M, Sharpe JG, Payne V, Robbins ME (2007). Knocking out peroxisome proliferator-activated receptor (PPAR) α inhibits radiation-induced apoptosis in the mouse kidney through activation of NF-kappaB and increased expression of IAPs. Radiat Res 167: 581–591.

Zingarelli B, Chima R, O'Connor M, Piraino G, Denenberg A, Hake PW (2010). Liver apoptosis is age dependent and is reduced by activation of peroxisome proliferator-activated receptor-gamma in hemorrhagic shock. Am J Physiol Gastrointest Liver Physiol 298: G133–G141.