

# The evolving role of the endocannabinoid system in gynaecological cancer

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**BACKGROUND:** The ‘endocannabinoid system’ (ECS), comprising endogenous ligands (endocannabinoids) and their regulating enzymes, together with the cannabinoid receptors, has attracted a great deal of attention because it affects not only all facets of human reproduction, from gametogenesis through to parturition and beyond, but also targets key mechanisms affecting some hallmarks of cancer. Recent evidence showing that cannabinoid receptors play a very important role in the development of malignancies outside of the reproductive organs suggests a similar role for the ECS in the establishment or continued development of gynaecological malignancy.

**METHODS:** Primary papers and review articles, and primary sources within these papers, up to December 2014, on the evolving role of the ECS in cancer, with a special focus on gynaecological cancers, were obtained by Medline and PubMed searches using the search terms: ‘cancer’, ‘cannabinoid’, ‘endocannabinoid’, ‘gynaecology’ and ‘malignancy’. Non-English manuscripts were excluded.

**RESULTS:** More than 2100 sources were obtained from which only 112 were specifically important to the topic. Analysis of those articles supports a role of the ECS in gynaecological cancers but leaves many gaps in our knowledge that need to be filled. How some of the relevant receptors are activated and cause changes in cell phenotypes that progress to malignancy remains undiscovered and an area for future research. Increasing evidence suggests that malignant transformation within the female genital tract could be accompanied by deregulation of components of the ECS, acting through rather complex cannabinoid receptor-dependent and receptor-independent mechanisms.

**CONCLUSIONS:** The paucity of studies in this area suggests that research using animal models is needed to evaluate endocannabinoid signalling in cancer networks. Future randomized clinical studies should reveal whether endocannabinoids or their derivatives prove to be useful therapeutic targets for gynaecological and other cancers.

**Key words:** endocannabinoid / cancer / endometrium / cervix / ovary

## Introduction

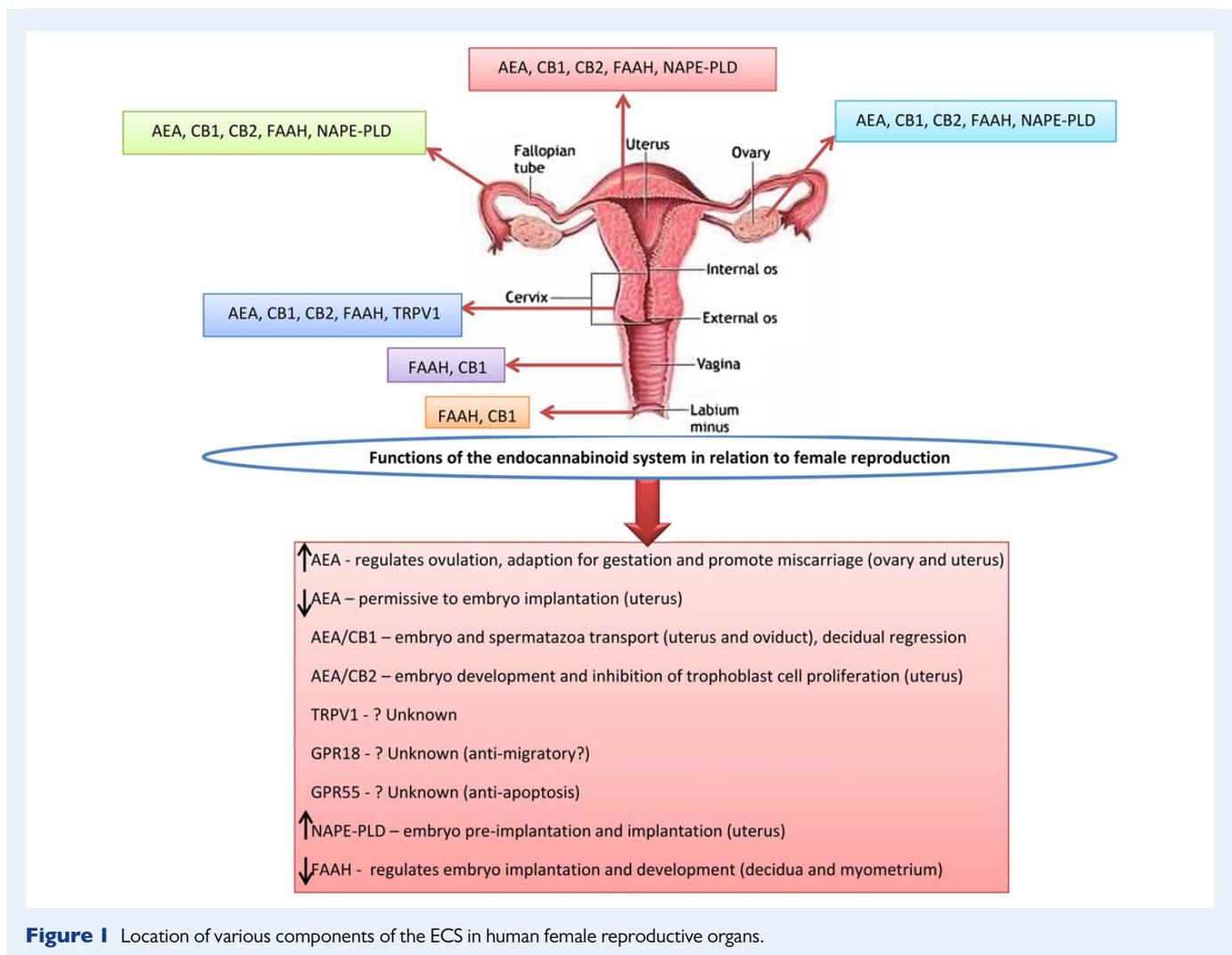
The endocannabinoid system (ECS), which is comprised of ligands, the enzymes responsible for their synthesis and degradation, and their specific G-protein-coupled receptors (GPCRs) (e.g. CB1 and CB2), has been the focus of intense research over the past decade, especially in the field of human reproduction (Fig. 1; see also Habayeb et al., 2004; Taylor et al., 2007; Maccarrone, 2009; Taylor et al., 2010; Karasu et al., 2011; Melford et al., 2014). Its prototype, endogenous ligand *N*-arachidonylethanolamide (anandamide; AEA), acts mainly on receptors to which the active component of marijuana (*Cannabis sativa*),  $\Delta$ -9-tetrahydrocannabinol ( $\Delta$ -9-THC) also binds (Pertwee et al., 2010). The fact that many of the actions attributed to endocannabinoids are involved in physiological and pathological conditions, in particular cancer (Table I: Guindon and Hohmann, 2011; Sailler et al., 2014; Thu et al., 2014), suggests that gynaecological malignancies may also be affected by perturbations in the ECS.

## Gynaecological cancer

Cancers of the female reproductive system, which make up approximately one out of six cancers in women (Sankaranarayanan and Ferlay,

2006), are a diverse group of malignancies with different epidemiological and pathological features, clinical presentations and treatment modalities. Gynaecological cancers remain an important cause of morbidity and mortality in the United Kingdom (UK) with the 2014 Cancer Research UK statistics website showing that uterine and ovarian cancers were in the top 10 most commonly diagnosed female malignancies (Cancer Research UK, 2014). Approximately 8500 and 7100 new cases of uterine and ovarian cancers, respectively, were diagnosed in 2011 (Cancer Research UK, 2014). In 2012, there were 2000 deaths from uterine and 4300 deaths from ovarian cancers (Cancer Research UK, 2014). After uterine and ovarian cancers, cervical cancer is the third most common gynaecological cancer with 3100 new cases in 2011 and 920 women deaths from cervical cancer in 2012 in the UK (Cancer Research UK, 2014). While significant progress has been made in reducing the incidence of some cancers, the same cannot be said of other genital tract cancers. This may partly be due to the lack of a thorough understanding of their pathogenesis.

Cancers, including those of gynaecological origin, are marked by deregulation of critical cellular mechanisms including cell division, differentiation and death. Various compounds or factors thought to be involved in these processes may thus be critical in the development of cancer.



**Figure 1** Location of various components of the ECS in human female reproductive organs.

**Table 1** Main signalling pathways affected by the ECS in carcinogenesis.

Cancer type	Ligand	Classification	Effect and putative pathway (reference)
Breast	Anandamide	Endocannabinoid	Inhibition of the proliferation in MDA-MB31 cells by modulating Wnt/ $\beta$ -catenin signalling pathway <a href="#">Laezza et al. (2012)</a> , <a href="#">Laezza et al. (2013)</a> Inhibition of cancer cell proliferation by acting via the CB1 receptor to activate the cAMP/PKA/MAPK <a href="#">Portella et al. (2003)</a> Blockade of cell cycle (S phase arrest) progression by activation of Chk1 or cyclin-dependent kinase inhibitor p27/kip1 <a href="#">Portella et al. (2003)</a> and up-regulation of p21 <sup>waf</sup> and reduction in Cdk2 formation and activity <a href="#">Laezza et al. (2006)</a> Inhibition of mitogen-induced stimulation of G <sub>0</sub> /G1-S phase in MCF7, T47D and EFM-19 (human breast cancer cell lines) <a href="#">De Petrocellis et al. (1998)</a> Inhibition of migration and invasion through CB1 and CB2 via blocking the FAK-Src-RhoA pathway <a href="#">Grimaldi et al. (2006)</a>
	THC	Phytocannabinoid (derived from plants)	Blockade of cell cycle (G2-M phase arrest) via Cdc2 regulation <a href="#">Caffarel et al. (2006)</a> Inhibition of cell proliferation via activation of transcription factor JunD <a href="#">Caffarel et al. (2008)</a> Reduction in cancer progression via Akt inhibition <a href="#">Caffarel et al. (2010)</a> Anti-angiogenic and anti-invasive effects by modulating MMP-2 and MMP-9 <a href="#">Ursini-Siegel et al. (2007)</a> , <a href="#">Zhang et al. (2007)</a>
	2-AG	Endocannabinoid	Down-regulation of prolactin receptors and nerve growth factor tyrosine receptor kinase (TRK) receptors <a href="#">Melck et al. (2000)</a>
	WIN55,212-2	Aminoalkylindole (Synthetic)	Anti-proliferation effect via of the COX-2/PGE2 <a href="#">Hoellen et al. (2011)</a> , <a href="#">Qamri et al. (2009)</a>
	R-(+)-MET	Eicosanoid (synthetic)	Decreased tyrosine phosphorylation of both FAK and Src affects migration and adhesions <a href="#">Grimaldi et al. (2006)</a>
	JWH-133	Cannabinoid (synthetic)	Anti-proliferation effect via modulation of the COX-2/PGE2 <a href="#">Qamri et al. (2009)</a> and the Akt <a href="#">Caffarel et al. (2010)</a> Inhibition of migration and invasion of MDA-MB231 <a href="#">Farsandaj et al. (2012)</a>
	Met-F-AEA	Endocannabinoid (synthetic derivative)	Inhibition of the FAK and RhoA-ROCK involved in cell migration <a href="#">Laezza et al. (2008)</a>
	JWH-015	Aminoalkylindoles (synthetic)	Anti-proliferation effect via the inhibition of cytokine/chemokine <a href="#">Nasser et al. (2011)</a> Inhibition of metastasis by modulating CXCL12/CXCR4 <a href="#">Nasser et al. (2011)</a> , <a href="#">Zlotnik et al. (2011)</a>
	CBD	Phytocannabinoid CBD	Involvement in metastasis—modulation of a basic helix-loop-helix transcription factor inhibitor (ERK) and reactive oxygen species (ROS) pathways leading to down-regulation of Id-1 expression <a href="#">McAllister et al. (2007)</a> , <a href="#">McAllister et al. (2011)</a> , <a href="#">Wallace (2012)</a> Invasion via the vanilloid receptors <a href="#">Farsandaj et al. (2012)</a> Inhibition of Akt and mTOR Inhibition of association between beclin 1 and Bcl-2 <a href="#">Shrivastava et al. (2011)</a> Inhibition of breast cancer-resistance protein via stimulation of the xenobiotic permeability through human placental barrier <a href="#">Feinshtein et al. (2013)</a>
	CBDA	Phytocannabinoid cannabidiolic acid	Inhibition of migration of MDA-MB-231 cells via cAMP-dependent protein kinase A and RhoA <a href="#">Takeda et al. (2012)</a>
Prostate	Anandamide	Endocannabinoid	Down-regulation of EGFR levels via CB1 receptor results in inhibition of proliferation <a href="#">Mimeault et al. (2003)</a> Cell death by apoptosis/necrosis through CB1/CB2 <a href="#">Mimeault et al. (2003)</a>
	THC	Phytocannabinoid (derived from plants)	Cannabinoid receptor-independent/-dependent activation in prostate cancer (PC) cells activates the PI3K/Akt/Raf-1/ERK 1/2 (G1 cell cycle arrest) and nerve growth factor stimulation <a href="#">Nithipatikom et al. (2004)</a> , <a href="#">Ruiz et al. (1999)</a> Apoptosis via non-CB receptors in PC3 cells <a href="#">Ruiz et al. (1999)</a>
	2-AG	Endocannabinoid	Suppression of nerve growth factor TRK receptors and prolactin receptors <a href="#">Melck et al. (2000)</a> Inhibitor of androgen-independent prostate cancer cell invasion <a href="#">Nithipatikom et al. (2004)</a>
	HU 120	Cannabinoid (synthetic)	Anti-tumour affect involving Akt pathways <a href="#">Sarfaraz et al. (2006)</a>

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Table I Continued

Cancer type	Ligand	Classification	Effect and putative pathway (reference)
	WIN55,212-2	Aminoalkylindole (synthetic)	Stimulation of ERK1/2 resulting in cell cycle arrest in Go/G1 phase Inhibition of NF-κB, cyclin D1 and E Induction of apoptosis via CBI/CB2 Decreased expression of AR and PSA (LNCaP cells) <a href="#">Sarfaraz et al. (2005)</a>
	R-(+)-MET	Eicosanoid (synthetic)	Inhibition of mitogen-induced proliferation via CBI/CB2 <a href="#">Mimeault et al. (2003)</a> Enhancement of androgen receptor expression <a href="#">Sanchez et al. (2003)</a> Inhibition of tumour (PC3 cells) growth <a href="#">Nithipatikom et al. (2012)</a> Induction of interleukin secretion (PC3 cells) <a href="#">Olea-Herrero et al. (2009)</a> Mitogenic effect on LNCaP cells at very low doses via activation of phosphoinositide 3-kinase/PKB pathway by CBI/CB2 <a href="#">Sanchez et al. (2003)</a>
	CAY 10401	Cannabinoid (synthetic)	Decreased cell migration through inhibition of FAAH in PC3 cells <a href="#">Endsley et al. (2008)</a>
	JWH-015	Aminoalkylindoles	Induction of cell death following synthesis of ceramide JNK (C-Jun N-terminal kinase) activation Akt inhibition <a href="#">Olea-Herrero et al. (2009)</a>
	CBD	Phytocannabinoid	Sustained activation of ERK1/2 and inhibition of Akt resulting in induction of phosphatases in LNCaP cells <a href="#">Sreevalsan et al. (2011)</a>
Lung	Anandamide	Endocannabinoid	Anti-tumorigenic effects (cell cycle arrest and apoptosis)—FAAH inhibition augments AEA mediated effects by down-regulating the EGF/EGFR pathway in non-small cell lung cancer <a href="#">Ravi et al. (2014)</a> Inhibition of tumour progression and Akt phosphorylation <a href="#">Preet et al. (2011)</a>
	THC	Phytocannabinoid (derived from plants)	Down-regulation of EGF/inhibition of ERK1/2, JNK1/2 and Akt pathways results in inhibition of angiogenesis and tumour invasiveness <a href="#">Preet et al. (2008)</a> Increased cell proliferation (NCI-H292) via EGFR <a href="#">Hart et al. (2004)</a> Up-regulation of the TIMP-1 expression <a href="#">Massi et al. (2013)</a> Inhibition of growth of Lewis lung adenocarcinoma via the following pathways inhibition of ERK1/2, c-Jun-NH2-kinase1/2 and Akt <a href="#">Guzman, (2003), Preet et al. (2008)</a>
	CBD	Phytocannabinoid	Induction of apoptosis via up-regulation of PPAR-γ and COX-2 <a href="#">Ramer et al. (2013)</a> Inhibition of invasion via up-regulation of TIMP-1 and down-regulation of plasminogen activator inhibitor 1 (PAI-1) <a href="#">Massi et al. (2013), Ramer and Hinz (2008)</a> Stimulation of TIMP-1 through the up-regulation of intracellular adhesion molecule 1 (ICAM-1) <a href="#">Ramer et al. (2012)</a>
	JWH-133	Cannabinoid (synthetic)	Anti-proliferative effect through DNA fragmentation in non-small lung cancer cells (A549 cell line) <a href="#">Vidinsky et al. (2012)</a> Anti-angiogenic effect through inhibition of MMP-2 pathways <a href="#">Vidinsky et al. (2012)</a>
	JWH-015	Aminoalkylindoles	Inhibition of chemotaxis, chemo-invasion, tumour growth and lung metastasis through inhibition of Akt, matrix MMP-9 <a href="#">Preet et al. (2011)</a>
	WIN55,212-2	Aminoalkylindole (synthetic)	Inhibition of Akt and MMP-9 results in inhibition tumour growth and progression <a href="#">Preet et al. (2011)</a>
Glioma	THC	Phytocannabinoid (derived from plants)	Inhibition of invasion through down-regulation of MMP-2 <a href="#">Blazquez et al. (2008b)</a> Stimulation of cell death or autophagy through activation of ER stress <a href="#">Salazar et al. (2009)</a> Anti-tumour action-sustained ceramide accumulation and extracellular signal-regulated kinase activation <a href="#">Galve-Roperh et al. (2000)</a> Apoptosis by down-regulation of PI3K/Akt pathways and stimulation BAD protein <a href="#">Ellert-Miklaszewska et al. (2005)</a> Anti-tumour action by down-regulation of TIMP-1 <a href="#">Blazquez et al. (2008a)</a>
	2-AG	Endocannabinoid	Apoptosis—TRPV2-dependent Ca <sup>2+</sup> influx <a href="#">Nabissi et al. (2013)</a>
	WIN55,212-2	Aminoalkylindole (synthetic)	Cell death and anti-inflammatory caused via accumulation of ceramide <a href="#">Galve-Roperh et al. (2000)</a> Inhibition of PGN-activated cell growth and NF-κB pathways <a href="#">Echigo et al. (2012)</a>
	CBD	Phytocannabinoid	Inhibition of angiogenesis through MMP-2 and ID-1 <a href="#">Freimuth et al. (2010), Soroceanu et al. (2013)</a>

Continued

**Table I** *Continued*

Cancer type	Ligand	Classification	Effect and putative pathway (reference)
	CBD and THC	Phytocannabinoids	Inhibition of angiogenesis and tumour growth <a href="#">Hernan Perez de la Ossa et al. (2013)</a> Inhibition of cell proliferation, modulation of cell cycle, apoptosis, induction of ROS and modulation of caspases activities and the extracellular signal-regulated kinase <a href="#">Marcu et al. (2010)</a>
	HU-210 and JWH-133	Cannabinoid (synthetic)	Involvement in neural differentiation of glioma stem-like cells (GSC) and blockade of GSC-mediated gliomagenesis <a href="#">Aguado et al. (2007)</a>
	THC and temozolomide (TMZ)	Phytocannabinoid (derived from plants) and cannabinoid (synthetic)	Anti-tumoural action via autophagy <a href="#">Torres et al. (2011)</a>
	KM-233	Cannabinoid ligand	Modulation in the phosphorylation of MEK, ERK1/2, Akt, BAD, STAT3 and p70SgK in human GBM cells (U87MG) <a href="#">Gurley et al. (2012)</a>
Pancreas	CBD	Phytocannabinoid	Inhibition of metabolism and stimulation of AMPK-dependent pathways result in autophagy <a href="#">Dando et al. (2013)</a>
	CBD and gemcitabine		Autophagy via the ROS-mediated pathways <a href="#">Donadelli et al. (2011)</a>
Oral	CBD	Phytocannabinoid	Inhibition of cellular respiration of human oral cancer cells <a href="#">Whyte et al. (2010)</a>
Embryonal	CBD	Phytocannabinoid	Effect on viability in P19 embryonal carcinoma cells <a href="#">Gustafsson et al. (2013)</a>
	HU210, WIN55,212-2, AEA and metAEA	All groups	Induction of cytotoxicity (concentration dependent) in P19 cells <a href="#">Gustafsson et al. (2013)</a> , <a href="#">Takakura (2012)</a>

One such group of compounds are the endocannabinoids, which have been reported to play vital roles in the regulation of cell proliferation, differentiation and survival. The ECS is accordingly emerging as a new, integral, cell homeostatic regulatory machine involved in protecting against dysregulatory processes, which are essential to cancer pathogenesis. In this review, the evidence for the involvement of the ECS in cancers, and in particular gynaecological cancers, will be presented.

## The endocannabinoid system

### Overview

The main active ingredient of cannabis,  $\Delta$ -9-THC, produces its pathophysiological effects through activation of CB1 ([Zimmer et al., 1999](#)) and CB2 receptors ([Buckley et al., 2000](#)). The phytocannabinoids consists of a group of fat-soluble molecules of which  $\Delta$ -9-THC, cannabidiol (CBD) and cannabinol (CBN) are the most prevalent ([Russo, 2011](#)).  $\Delta$ -9-THC, at submicromolar concentration, binds to CB1 and CB2 with similar affinities. In the brain, it behaves mainly as a CB1 receptor agonist, whilst acting as a tissue-specific CB1/CB2 receptor antagonist. It has been suggested that it acts by preventing the normal action of the prevailing endogenous cannabinoid, i.e. endocannabinoid ([Paronis et al., 2012](#)). Endocannabinoids, in addition to acting via these receptors, also exert their effects through the activation of putative cannabinoid receptors, such as the transient potential vanilloid receptor 1 (TRPV1) and the orphan GPCRs GPR55, GPR119 ([Pertwee, 2009](#)) and GPR18 ([Qin et al., 2011](#)). Endocannabinoids, which are endogenous lipids, mimic the effects of  $\Delta$ -9-THC on these receptors and are involved in various processes essential to the establishment and maintenance of human health ([Pertwee, 2009](#); [Bosier et al., 2010](#); [Pertwee et al., 2010](#); [Fonseca et al., 2013](#)). The ECS is widely distributed throughout the body and is found in the brain, the gastrointestinal tract, connective

tissues, the reproductive system and the immune system ([Maccarrone et al., 2002](#); [Marczylo et al., 2010](#)).

### Cannabinoid and non-cannabinoid receptors

The cannabinoid receptors (CB1, CB2) belong to the Gi/o family of seven trans-membrane GPCRs. CB1, the central receptor, was first described as predominantly expressed in the central nervous system, while CB2 was first isolated from splenic cells. Pharmacological studies have indicated the existence of other cannabinoid receptor subtypes ([Mackie and Stella, 2006](#)), and recently two orphan GPCRs, GPR55 and GPR119, have been characterized as cannabinoid receptors ([Godlewski et al., 2009](#); [Okuno and Yokomizo, 2011](#)). GPR55 has been identified in the brain and peripheral tissues, such as the gut, spleen, adrenals and the reproductive tract, and two endocannabinoid ligands, 2-arachidonoylglycerol (2-AG) and *N*-palmitoylethanolamide (PEA), seem to have the greatest affinity for this receptor. In a recent study, it was reported that 2-arachidonoyl lysophosphatidyl-inositol may also be a ligand for GPR55 ([Okuno and Yokomizo, 2011](#)). GPR55 is found in skin, and it drives skin carcinogenesis, with its expression significantly increased in human squamous cell cancers ([Perez-Gomez et al., 2013](#)). GPR119, on which *N*-oleoylethanolamide (OEA) has the greatest affinity, has a more limited tissue distribution, being predominantly expressed in the pancreas and intestinal tissues ([Godlewski et al., 2009](#)), whilst the transient receptor potential vanilloid subtype 1 (TRPV1), a ligand-gated,  $\text{Ca}^{2+}$  permeable ion channel (thought to be involved in the ion-mediated action of cannabinoids), has an almost ubiquitous distribution ([Huang et al., 2002](#)). *N*-Arachidonylethanolamide, i.e. anandamide (AEA), and *N*-arachidonoyldopamine (NADA) bind to this receptor and have therefore been considered part of a group of ligands known as endovanilloids that act on the endovanilloid receptor, TRPV1 ([Huang et al., 2002](#)). Lately, the peroxisome proliferator-

activated receptors (PPARs) have also been added to the list of potential endocannabinoid receptors. PPARs have been shown to be stimulated by endocannabinoids under both physiological and pathological conditions and of interest is the fact that they have a higher affinity for OEA and PEA than other endocannabinoids (Pistis and Melis, 2010). Furthermore, recent studies have suggested that another orphan G-protein receptor, GPR18, plays a vital role in tumourigenesis and metastasis of human cancer. Although GPR18 also binds endocannabinoids, it is constitutively active and inhibits apoptosis in metastatic melanoma, suggesting that activated GPR18 plays a vital role in tumour cell survival (Qin et al., 2011). Whether this receptor is present in gynaecological cancers remains unknown. *N*-Arachidonoyl glycine (NAGLy), an endogenous metabolite of AEA, has in fact been reported to be a GPR18 ligand (Kohno et al., 2006), perhaps through pre-activation by AEA, which is a precursor of NAGLy and produced by either oxidation by cytochrome c (McCue et al., 2008) or by alcohol dehydrogenase (Bradshaw et al., 2006).

### Synthesis and degradation of endocannabinoids

The synthesis and degradation pathways are known for the two most widely studied endocannabinoids, AEA (anandamide) (Devane et al., 1992) and 2-AG (Mechoulam et al., 1995). Endocannabinoids are generally thought to be synthesized on demand from phospholipid precursors residing in the cell membrane, through the catalytic activities of a variety of intracellular enzymes, the activation of which normally occurs in response to a rise in intracellular calcium levels (Matias and Di Marzo, 2006).

AEA was the first endogenous ligand identified and remains the most frequently investigated endocannabinoid (Devane et al., 1992). It is produced rapidly by the hydrolysis of *N*-arachidonoyl phosphatidylethanolamine (NAPE) by a specific phospholipase D (NAPE-PLD) (Matias and Di Marzo, 2006). 2-AG, on the other hand, is synthesized through the activation of phospholipase C and the subsequent production of diacylglycerol, which is then converted to 2-AG by diacylglycerol lipase (DAGL) (Matias and Di Marzo, 2006). Upon release, AEA and 2-AG activate molecular targets and are then subsequently inactivated following cellular re-uptake (McFarland and Barker, 2004). Diverse transport systems have been proposed for AEA, including endocytosis, passive and active diffusions and a specific carrier protein (McFarland and Barker, 2004). Once inside the cell, AEA is degraded into arachidonic acid (AA) and ethanolamine by the enzyme fatty acid amide hydrolase (FAAH), while 2-AG is degraded mainly, but not exclusively, by monoacyl-glycerol lipase (MAGL) into AA and glycerol (Matias and Di Marzo, 2006).

## Methods

Primary papers and review articles, up to December 2014, on the evolving role of the ECS in cancer as a whole, with a special focus on gynaecological cancers, were obtained by Medline and PubMed searches using the search terms: 'cancer', 'cannabinoid', 'endocannabinoid', 'gynaecology' and 'malignancy'. Non-English manuscripts were excluded. Furthermore, reference lists of the retrieved articles were examined for further evaluative sources.

## Results

### Endocannabinoids and cancer

The ECS has been implicated in a wide range of physiological and pathological conditions, such as obesity or metabolic syndrome (Merroun et al., 2013), intractable cancer-related pain (Johnson et al., 2010; Gu

et al., 2011), reduced inflammation (Burstein et al., 2011), such as that observed in inflammatory bowel disease (De Petrocellis et al., 2012; Borrelli et al., 2013; Liu et al., 2013), immunomodulation (Disis, 2010), and has neurological effects in attenuated catalepsy (Gomes et al., 2013) and Alzheimer's disease (Hill et al., 2012; Aso et al., 2013; Deiana, 2013; Shinjyo and Di Marzo, 2013) as well as other neurophysiological effects (Parolaro et al., 2002; Walker and Huang, 2002). It has been shown to have potential therapeutic effects in the alleviation of symptoms, such as chemotherapy-induced neuropathic pain and bone cancer pain, via increased levels of 2-AG (Khasabova et al., 2011; Lozano-Ondoua et al., 2013), appetite loss, and nausea and vomiting. For example, 2AG stimulation and MAGL inhibition attenuate nausea and vomiting in rodent models (Sticht et al., 2012; Todaro, 2012) and in cancer patients (Hall et al., 2005). In addition, the ECS plays a vital role in the regulation of metabolism in several peripheral tissues (Ruminska and Dobrzyn, 2012). Furthermore, these lipids are increasingly being shown to play a role in cancer stem cells (CSC) (Takakura, 2012), to have a close relationship with lipid mediators in cancer (Santos and Schulze, 2012), and to be involved in the regulation of key processes in the development of cancer (Pisanti et al., 2009; Hanahan and Weinberg, 2011; Velasco et al., 2012; Pisanti et al., 2013; Van Dross et al., 2013), particularly sex steroid hormone-dependent cancers (Ayakannu et al., 2013b; Meccariello et al., 2014). These exciting initial observations make a strong case for more research to substantiate these anti-neoplastic effects in humans (Malfitano et al., 2011; Fowler, 2012; Velasco et al., 2012; Massi et al., 2013). The main pathways that have been demonstrated for the involvement of the ECS in carcinogenesis are summarized in Table 1.

### Cell cycle regulation and endocannabinoids

AEA has been shown to arrest human MDA-MB-231 breast cancer cells in the S phase of the cell cycle primarily because of a loss in Cdk2 activity, up-regulation of p21 waf and a reduced formation of the active complex cyclin E/Cdk2 kinase (Laezza et al., 2006). In addition, AEA arrests cells in the S phase through Chk1 activation and Cdc25A proteolysis, which prevents Cdk2 activation by dephosphorylation on critical inhibitory residues (Thr14/Tyr15) (Laezza et al., 2006). In a CB2 receptor-dependent mechanism,  $\Delta$ -9-THC inhibits breast cancer cell proliferation by blocking the cell cycle at the G2/M phase through the down-regulation of Cdc2; however, CB2-selective antagonists significantly but not totally prevent such effects, suggesting that a CB2-receptor-independent mechanism is also present (Caffarel et al., 2006). Prostate cancer (LNCaP) cells, when treated with the CBI agonist WIN55,212-2, arrest cell division at the G0/G1 phase of the cycle, with this arrest being sustained by the activation of ERK1/2, induction of p27/kip1 and inhibition of cyclin D (Sarfaraz et al., 2006). The arrest at G0/G1 can result in stimulation of apoptosis via a change in the Bax/Bcl-2 ratio and the activation of caspases (Sarfaraz et al., 2006). Importantly, WIN55,212-2 treatment of LNCaP cells results in a dose-dependent decrease in the expression of cyclins D1, D2 and E, as well as cdk2, cdk4 and cdk6, phosphorylated retinoblastoma protein and its molecular partner, the transcriptional factor E2F (Sarfaraz et al., 2006). WIN55,212-2 also causes a dose-dependent decrease in the expression of the transcription factors DP-1 and DP-2, leading to a decrease in the formation of the heterodimeric complex with E2F, which is essential for its activity (Sarfaraz et al., 2006). In glioblastoma multiforme cells,  $\Delta$ -9-THC elicits a G0/G1 cell cycle blockade via suppression of E2F1 and cyclin A and also through the up-regulation of the cell cycle inhibitor p16 (INK4A) (Galanti et al., 2008).

### *Inhibition of cancer cell proliferation by endocannabinoids*

The proliferation of various cancer cells can be inhibited by many different mechanisms, such as a decrease in the expression and/or activity of nerve growth factor, prolactin or vascular endothelial growth factor (VEGF) tyrosine kinase receptors (De Petrocellis *et al.*, 1998; Melck *et al.*, 2000; Portella *et al.*, 2003), a decrease in epidermal growth factor receptor (EGFR) expression and/or attenuation of EGFR tyrosine kinase activity (Mimeault *et al.*, 2003), cell cycle blockade with induction of the cyclin-dependent kinase inhibitor p27/Kip1 (Portella *et al.*, 2003) or the inhibition of adenylate cyclase activity and the cAMP/protein kinase A pathway (Melck *et al.*, 1999). Breast cancer cell proliferation is inhibited by AEA via down-regulation of the prolactin receptor, BRCA 1 gene product and the high-affinity neurotrophin receptor trk A (Melck *et al.*, 1999; Melck *et al.*, 2000; Caffarel *et al.*, 2012). The anti-proliferative effect of AEA is proportional to the hormone dependency of the cell lines, and the mechanism relies on the inhibition of the phosphokinase A (PKA) pathway (Melck *et al.*, 2000). In addition, synthetic cannabinoids such as JWH-018, JWH-073, JWH-122 and JWH-210 containing a naphthoylindole ring show anti-estrogenic properties in the MCF-7 breast cancer cell (Koller *et al.*, 2013). Furthermore, WIN55,212-2 and JWH-133 also cause inhibition of breast cancer cell proliferation (MDA-MB-231) by causing cell cycle arrest at G1 to S phase and induction of apoptosis (Qamri *et al.*, 2009). Interestingly, a novel synthetic hexahydrocannabinol analogue (LYR-7 and LYR-8) acts as an anti-proliferative and anti-angiogenesis agent and has also been suggested to cause a reduction in tumour growth by targeting the VEGF-mediated angiogenesis signalling pathways in both tamoxifen-sensitive and tamoxifen-resistant MCF-7 breast cancer cell lines (Thapa *et al.*, 2011).

A number of intraepithelial or invasive prostatic cancers exhibit evidence of increased expression of EGFR, EGF and transforming growth factor  $\alpha$  (TGF  $\alpha$ ) (Mimeault *et al.*, 2003). Micromolar concentrations of AEA inhibit the EGF-induced proliferation of PC3, DU145 and LNCaP prostate cancer cells through G1 arrest and down-regulation of EGFR expression. These effects are mediated via CBI receptors (Mimeault *et al.*, 2003). In addition, omega-3 ethanolamides show evidence of anti-proliferative effects through cannabinoid receptor-dependent pathways in androgen receptor-positive prostate cancer cell lines (Brown *et al.*, 2010; Brown *et al.*, 2013). Furthermore, treatment of LNCaP cells with the CBI agonist WIN55,212-2 decreases cell proliferation, androgen receptor expression, VEGF protein expression and prostate-specific antigen (PSA) secretion (Sarfaraz *et al.*, 2005). Moreover, the putative cannabinoid receptor GPR55 is also expressed in prostate cancer cell lines, such as PC3 and DU-145 cells, and this putative cannabinoid receptor has been suggested to define an autocrine loop in prostate cancer cell proliferation that is unique to malignant prostate cells (Pineiro *et al.*, 2011). As more research is undertaken, the role of this orphan receptor in preventative oncogenesis is becoming more important (Andradas *et al.*, 2011). For example, activation of GPR55 receptors in cholangiocarcinoma by AEA results in an anti-proliferative effect (Huang *et al.*, 2011). Furthermore, the demonstration of an anti-proliferative effect in BV-2 microglial and HEK 293 cells via the GPR18 receptor, when activated by NAGly, which results in p44/42 MAPK pathway activation (McHugh *et al.*, 2010), suggests that multiple endocannabinoid signalling pathways converge to attenuate malignant cell growth (Bradshaw *et al.*, 2009).

### *Induction of apoptosis by endocannabinoids*

BAD, a pro-apoptotic member of the Bcl-2 family of signalling molecules, is hypothesized to play a role in endocannabinoid-dependent apoptosis (Ellert-Miklaszewska *et al.*, 2005). Increasingly, the endocannabinoids together with ceramide have been shown to play a vital role in cancer cell signalling (Morad and Cabot, 2013). The pro-apoptotic effects may also depend on CBI-independent stimulation of sphingomyelin breakdown (Sanchez *et al.*, 1998). In leukaemia and lymphoma cell lines, depolarization of mitochondria via cytochrome c release is a common event in endocannabinoid-induced apoptosis (Jia *et al.*, 2006). These effects may be caused by CB agonists such as WIN55,212-2, which induces cytoplasmic vacuolation in apoptosis-resistant mantle cell lymphoma (MCL) cells (Wasik *et al.*, 2011a) and  $\Delta$ -9-THC, which induces CB-dependent apoptosis via ceramide accumulation and caspase stimulation through the p38MAPK signalling pathway, down-regulation of the RAF1/MAPK pathway and translocation of BAD to mitochondria (Jia *et al.*, 2006). Moreover, CBI and CB2 receptors are also expressed at a high concentration in MCL and B cell non-Hodgkin lymphoma, when compared with non-malignant states, whilst  $\Delta$ -9-THC causes increased apoptosis in EL4 and MCL cells when cultured *in vitro* (Flygare *et al.*, 2005; Wasik *et al.*, 2011b). CB agonists are mitochondrial inhibitors, since they decrease the oxygen consumption and mitochondrial membrane potential while increasing mitochondrial hydrogen peroxide production, thus inducing apoptosis (Athanasίου *et al.*, 2007). The anti-cancer actions of the endocannabinoids on glioma cells have been reported to be exerted either through the CBI or the CB2 receptor (Lorente *et al.*, 2011a, b). THC induces apoptosis of C6 glioma cells via a network involving the CBI receptor, sustained generation of the pro-apoptotic lipid ceramide and prolonged activation of the Raf1/MEK/ERK cascade (Galve-Roperh *et al.*, 2000). THC treatment has been documented to induce apoptosis through CBI inhibition of the RAS-MAPK/ERK and PI3K-Akt survival signalling cascade and stimulation of the BCL-2 family member BAD-mediated apoptosis pathway in colorectal cancer (Greenhough *et al.*, 2007). In addition, MAGL knock-down inhibits progression of tumour cell growth in colorectal cancer (Ye *et al.*, 2011). Furthermore,  $\Delta$ -9-THC induces apoptosis in oral squamous cell carcinoma (Lopes *et al.*, 2012). CB receptor agonist-induced colon cancer cell death is via TNF $\alpha$ -stimulated ceramide synthesis; therefore, TNF $\alpha$  may act as a link between endocannabinoid receptor activation and ceramide production (Cianchi *et al.*, 2008). It has been shown that prolonged AEA incubation (5–6 days) induces massive apoptosis in DU145 and PC3 prostate cancer cells, and this is also mediated through CBI/2 via cellular ceramide accumulation, but noted to be absent in LNCaP cells (Mimeault *et al.*, 2003). In addition, fluorinated methanandamide (MET-F-AEA), a metabolically stable analogue of anandamide, inhibits human thyroid cell line growth through apoptosis (Cozzolino *et al.*, 2010).

### *The endocannabinoid system and cancer cell invasion*

Cancer cell invasion is an important step in tumour growth and metastasis that defines the aggressiveness, degree of malignancy and disease outcome. Several studies have evaluated the effects of endocannabinoids on cancer cell invasion and the signalling pathways involved in the inhibition of invasion (Hermanson and Marnett, 2011), such as that of the TIMP-1 endogenous tissue inhibitor of metalloproteinase (TIMP) (Ramer and Hinz, 2008), inhibition of FAK/Src signalling (Grimaldi

et al., 2006) and inhibition of matrix metalloproteinase 2 (MMP-2) (Blazquez et al., 2008b). In relation to the matrix metalloproteinase (MMP) system, endocannabinoids have a direct effect that plays a key role in their anti-invasive action. MMP-2 and MMP-9 promote cancer invasion by proteolytic degradation of major basement membrane components, such as laminin, collagen and nidogens (Curran and Murray, 2000). An important action of MMPs is the degradation of the extracellular matrix (ECM) components, which are important in cancer angiogenesis, invasion and metastasis (Stamenkovic, 2000). MMP proteolytic activity is inhibited by the TIMPs. In lung cancer cells, inhibition of invasion by AEA and  $\Delta$ -9-THC depends on the induction of TIMP-1 expression (Ramer and Hinz, 2008). In addition, cannabinoid treatment of glioma cell lines and primary tumour cells from glioblastoma multiforme inhibits the expression of TIMP-1 (Blazquez et al., 2008a). Furthermore,  $\Delta$ -9-THC inhibits MMP-2 expression and cell invasion in glioma cells (Blazquez et al., 2008b). Down-regulation of MMP-2 plays a vital role in  $\Delta$ -9-THC-mediated inhibition of cancer cell invasion (Blazquez et al., 2008b). In addition, monoacyl-glycerols (MAGs), such as 2-AG, are metabolized to free fatty acids (FFAs) and glycerol by MAGL, which are elevated in aggressive prostate cancer, whilst anti-survival MAGs are down-regulated in aggressive prostate cancer when compared with that of non-aggressive tumours and thus MAGL exerts dual control over endocannabinoids and fatty acid pathways in prostate cancer (Nomura et al., 2011). In contrast, FAAH expression in prostate cancer is associated with disease severity and outcome and CBI receptor expression, and this is regulated by IL-4 (Thors et al., 2010). Whether this also occurs in gynaecological cancers remains unknown. Studies in androgen-independent prostate cancer cell lines (PC3 and DU145) show that endogenous 2-AG and CBI agonists reduce invasion through the CBI-dependent inhibition of adenylate cyclase and the subsequent decreased PKA activity (Nithipatikom et al., 2004).

#### *Inhibition of cancer angiogenesis by endocannabinoids*

Since angiogenesis has been shown to be closely linked with cancer development, and implicated in metastasis, the development of a cancer-specific anti-angiogenesis therapy has been proposed to be possibly more effective than current regimens (Cao et al., 2013). In this respect, endocannabinoids might be potential new therapeutics, because they are thought to inhibit tumour growth by reducing the vascular concentration in the tumour, decreasing the production of pro-angiogenic factors and/or by directly modulating endothelial cells (Freimuth et al., 2010). The pro-angiogenic growth factor VEGF and its receptor VEGFR-1 are major cancer cell-released chemo-attractants in angiogenesis, and several studies have reported that endocannabinoids can modulate the expression of VEGF and VEGFR-1 (Saia et al., 2007). Indeed, endocannabinoids inhibit angiogenesis in thyroid cancer by decreasing the levels of VEGF and VEGFR-1 (Portella et al., 2003). In addition, intra-tumoural administration of  $\Delta$ -9-THC to glioblastoma patients decreases both VEGF and VEGFR-2 expression (Blazquez et al., 2004). Endocannabinoid treatment of glioma inhibits the expression of VEGF, angiopoietin-2, MMP-2 and hypoxia-inducible factor 1- $\alpha$  (HIF-1 $\alpha$ ), which is the major factor responsible for VEGF expression (Blazquez et al., 2004). The anti-angiogenic activity of AEA has also been reported in capillary-like tube formation and morphogenesis assays, and it acts via inhibition of basic fibroblast growth factor (bFGF)-induced chemotaxis and MMP-2 degrading activity (Pisanti et al., 2007). HU-331, a derivative of CBD, is an anti-carcinogenic drug that acts as an anti-angiogenic factor

via a different mechanism, whereby it directly induces apoptosis of vascular endothelial cells without modulating the expression of pro- and anti-angiogenic factors and their receptors (Kogan et al., 2006). In addition, cannabinoids that activate CBI and/or CB2 receptors, including  $\Delta$ -9-THC, JWH-133, HU-210 and WIN55,212-2, have been reported to inhibit vascular endothelial cell migration and survival as part of their direct anti-angiogenic mechanism of action (Blazquez et al., 2003). In contrast, the genetic and pharmacological interventions that inactivate CBI receptors may result in inhibition of angiogenesis (Pisanti et al., 2011). The metabolically stable AEA-derivative met-fluoro-AEA suppresses angiogenesis in an *in vivo* chick chorioallantoic membrane assay, inhibits capillary-like tube formation *in vitro* and reduces the sprout number and length of endothelial cell spheroids (Pisanti et al., 2007).

#### *Effects of the endocannabinoid system on cancer cell adhesion*

Matrix proteins, such as cell adhesion molecules of the immunoglobulin superfamily (IgSF CAMs), integrins, selectins and cadherins, are integral to the adhesive property of cells to the ECM. Changes to the adhesive properties of cancer cells and their interactions with the surrounding microstructures play a vital role in cancer growth, invasion, migration and metastases. Studies of endocannabinoids have reported that they have various effects on the adhesion of cancer cells to the ECM. AEA inhibits the adhesion of the highly invasive MDA-MB-231 and TSA-E1 metastatic breast cancer cell lines on type IV collagen, the major component of the basement membrane (Grimaldi et al., 2006) and of SW480 colon cancer cells via the stimulation of endocannabinoid receptors (CBI) (Joseph et al., 2004). AEA has no effect on adhesion of these cancer cells to fibronectin and laminin (Grimaldi et al., 2006) but decreases the affinity of integrins to collagen via the suppression of the pro-oncogenic tyrosine kinase c-Src and by the phosphorylation of the focal adhesion kinase (FAK) (Grimaldi et al., 2006).

#### *Effects of the endocannabinoid system on cancer cell migration*

The ECS has been shown to be involved in the direct regulation of cancer cell migration. In the scratch wound healing assay, cancer cell migration is reduced by 2-AG and WIN55,212-2 in a CBI receptor-dependent manner (Grimaldi et al., 2006). Migration of the highly invasive metastatic breast cancer cell lines MDA-MB-231 and TSA-E1 (when grown on type IV collagen) is inhibited by AEA (Grimaldi et al., 2006). In SW480 colon carcinoma cells, AEA inhibits cell migration via activation of CBI receptors (Joseph et al., 2004). Inhibition of migration of lung cancer cells has been reported for both AEA and  $\Delta$ -9-THC (Ramer and Hinz, 2008), while migration of human glioma cells is inhibited by CBD via a receptor-independent method (Vaccani et al., 2005). The epidermal growth factor (EGF) and its EGFR (Preet et al., 2008), neurotransmitters (Joseph et al., 2004), mast cells (Rudolph et al., 2008) and other paracrine or endocrine chemo-attractants are among other factors that play a vital role in cancer cell migration.

#### *Cannabinoid receptor-independent modes of action in carcinogenesis*

In addition to the effects mediated via the endocannabinoid receptors, endocannabinoids, in particular AEA and CBD, are known to have CB-receptor-independent effects (Begg et al., 2005). AEA, for example, induces both neuroblastoma and lymphoma cell death via vanilloid receptor-mediated mechanisms (Maccarrone et al., 2000), whilst methanandamide (MA) inhibits cancer cell invasion through TIMP-1 degradation, which is mediated via TRPV1 activation (Ramer and Hinz,

**Table II** The ECS in the female reproductive tract.

Organ	ECS	Description	Reference
Ovary	CBI	Immunostaining shows expression of CBI in the medulla and cortex, in granulosa cells of primordial, primary, secondary and tertiary follicles, in thecal cells of secondary and tertiary follicles and in corpus luteum and corpus albicans	El-Talatini <i>et al.</i> (2009)
	CB2	Immunostaining shows expression of CB2 in the medulla and cortex, in granulosa cells of primordial, primary, secondary and tertiary follicles, in thecal cells of secondary and tertiary follicles and in corpus luteum and corpus albicans	El-Talatini <i>et al.</i> (2009)
	NAPE-PLD	Expressed in granulosa and thecal cells of the secondary and tertiary follicles, corpus luteum and corpus albicans	El-Talatini <i>et al.</i> (2009)
	FAAH	Expressed in granulosa and thecal cells of the secondary and tertiary follicles, corpus luteum and corpus albicans	El-Talatini <i>et al.</i> (2009)
	AEA	Suggested to be produced from granulosa of growing follicles but not from oocytes May be involved in follicle and oocyte maturation	Schuel <i>et al.</i> (2002), El-Talatini <i>et al.</i> (2009)
	$\Delta$ -9-THC	Inhibitory effect on both folliculogenesis and ovulation	Adashi <i>et al.</i> (1983)
Oviduct	AEA	Higher concentrations in the plasma of women with ectopic pregnancy	Gebeh <i>et al.</i> (2012)
	CBI	Noted in mouse oviductal muscularis at the isthmus region and associated with $\alpha$ 1 and $\beta$ 2 adrenergic receptor actions Reduced expression in ectopic pregnancy	Wang <i>et al.</i> (2004), Horne <i>et al.</i> (2008), Gebeh <i>et al.</i> (2012)
	CB2	Expressed throughout the oviduct and unaffected by ectopic pregnancy	Gebeh <i>et al.</i> (2012)
	NAPE-PLD	Expressed throughout the oviduct and both activity and expression unaffected by ectopic pregnancy	Gebeh <i>et al.</i> (2012), Gebeh <i>et al.</i> (2013)
	FAAH	Expressed throughout the oviduct and both expression and activity reduced by ectopic pregnancy	Gebeh <i>et al.</i> (2012), Gebeh <i>et al.</i> (2013)
Uterus	AEA	Demonstrated to be produced by the mice, rat and human uterus May control decidualization, implantation and menstruation	Paria <i>et al.</i> (2001), El-Talatini <i>et al.</i> (2010), Fonseca <i>et al.</i> (2010)
	CBI	Immunoreactivity is more intense in glandular epithelium when compared with that of stroma Increase in CBI mRNA and protein expression in the secretory phase	Taylor <i>et al.</i> (2010), Resuehr <i>et al.</i> (2012)
	CB2	Evident in both glands and stroma and reaches peak in late proliferative phase	Taylor <i>et al.</i> (2010)
	NAPE-PLD	Intensively expressed in the early proliferative phase of the menstrual cycle	Taylor <i>et al.</i> (2010)
	FAAH	Glandular expression reaches a peak during menstruation Expressed primarily in stroma	Taylor <i>et al.</i> (2010)
Cervix	CB1R	Expressed cervical cancer cell lines (Caski, HeLa and CC299) in mRNA and protein	Contassot <i>et al.</i> (2004), Habayeb <i>et al.</i> (2004)
	CB2R	Expressed cervical cancer cell lines (Caski, HeLa and CC299) in mRNA and protein	Contassot <i>et al.</i> (2004), Habayeb <i>et al.</i> (2004)
	TRPV1	Expressed cervical cancer cell lines (Caski, HeLa and CC299) in mRNA and protein	Contassot <i>et al.</i> (2004)
	FAAH	Immunohistological expression in normal human cervix	Habayeb <i>et al.</i> (2004)
	AEA	Anti-tumour effect in cervical cancer cell line	Contassot <i>et al.</i> (2004)
Vagina	TRPV1	Apoptosis involving the ceramide-dependent pathway	Eichele <i>et al.</i> (2009)
	CBI	Immunohistological expression in normal human tissue primarily in the epidermal cell	Habayeb <i>et al.</i> (2004)
Vulva	FAAH	Immunohistological expression in normal human tissue primarily in the epidermal cell	Habayeb <i>et al.</i> (2004)
	CBI	Immunohistological expression in human tissue primarily in the epidermal cell	Habayeb <i>et al.</i> (2004)

2008). Furthermore, inhibition of AEA degradation via inclusion of the FAAH inhibitor (URB597) enhances AEA-mediated cytotoxicity in neuroblastoma cells (Hamtiaux *et al.*, 2011). It is proposed that lipid rafts rich in sphingolipids and cholesterol mediate AEA effects via CBI signalling (Bari *et al.*, 2005; Scuderi *et al.*, 2011). Indeed, lipid raft stabilization (Jin *et al.*, 2011), ceramide accumulation and recruitment of FAS and FAS ligand into lipid rafts facilitate the anti-proliferative and pro-apoptotic actions of AEA in cholangiocarcinoma (DeMorrow *et al.*, 2007). Cyclooxygenase (COX-2), another important cellular protein in CB-receptor-independent cell death induced by endocannabinoids, metabolizes AA to prostaglandins (PGs), and in neoplastic tissues,

elevated levels of both COX-2 and PG have been recorded. Recently, it was shown that AEA-induced cytotoxicity is mediated by the production of pro-apoptotic J-series PGs in tumourigenic keratinocytes that overexpress COX-2 (Van Dross, 2009; Kuc *et al.*, 2012). In addition, AEA stimulates COX-2-dependent cell death in apoptosis-resistant colon cancer cells (Patsos *et al.*, 2010), whilst GPR18 has recently been reported to be involved in the regulation of cytokine production (Wang *et al.*, 2014). CBD, a cannabinoid found in cannabis but with no activity at CBI or CB2 receptors and thus lacking psychotropic effects has been reported to inhibit glioma and breast tumour growth *in vitro* and *in vivo* through the induction of apoptosis and the inhibition of

**Table III** Perturbators of the ECS in gynaecological cancers.

Cancers	Ligands	Classifications	Anti-cancer effect and putative mechanism of action (reference)
Cervix	Anandamide	Endocannabinoid	Cell cycle arrest (G0/G1) and death noted in three cell lines (Caski, HeLa and CC299) <a href="#">Contassot et al. (2004)</a> induced by DNA fragmentation; not dependent on CB1/CB2 Induction of apoptosis and cell death via TRPV1 <a href="#">Contassot et al. (2004)</a>
	2-AG	Endocannabinoid	Inhibition of migration <a href="#">Rudolph et al. (2008)</a>
	MethAEA and THC	Eicosanoid (synthetic) and phytocannabinoid	Decrease in cell invasion; time and concentration dependent through expression of TIMP-1 <a href="#">Ramer and Hinz (2008)</a>
	$\Delta$ -9-THC	Phytocannabinoid	Stimulation of TIMP-1 expression <a href="#">Ramer and Hinz (2008)</a>
	R (+) methAEA	Analogue of anandamide	Apoptosis activation via the PPAR- $\gamma$ - and COX-2-dependent pathways <a href="#">Eichele et al. (2009)</a> Apoptosis via induction of COX-2 expression and PGs through ceramide-dependent pathways <a href="#">Eichele et al. (2009)</a>
	WIN55,212-2 CBD Acylamido anandamide	Phytocannabinoid Analogue of anandamide	Inhibition of migration <a href="#">Rudolph et al. (2008)</a> Up-regulation of TIMP-1; involved in angiogenesis <a href="#">Ramer and Hinz (2008)</a> Selective inhibition of cell proliferation <a href="#">Burstein and Salmonsens (2008)</a>
Uterus	2-AG	Endocannabinoid	Possible imbalance in estrogen/progesterone and increase in CB2, leading to defect in mitochondrial function, inhibition of cell growth, decrease in proliferation and increased cell death <a href="#">Guida et al. (2010)</a> Reduction in cell growth through modulation of mitochondrial function, apoptosis and cell death <a href="#">Guida et al. (2010)</a>
	Anandamide	Endocannabinoid	Possible anti-tumour activity <a href="#">Schmid et al. (2002)</a> , <a href="#">Ayakannu et al. (2013a, b)</a> , <a href="#">Ayakannu et al. (2014)</a> Possible effect on cell survival/death pathways via CB2 <a href="#">Guzman et al. (2002)</a>
Ovary	2-AG	Endocannabinoid	Promotion of cell migration, invasion, survival and tumour growth through the MAGL-FFA pathways <a href="#">Nomura et al. (2010)</a>
	LPI	Endogenous ligand	Anti-proliferative effect; LPI activates Akt, increases calcium influx and increases ERK1/2 pathways <a href="#">Pineiro et al. (2011)</a>

angiogenesis and cell migration. These effects of CBD are independent of both CB and TRPV1 receptor activation ([Vaccani et al., 2005](#); [Ligresti et al., 2006](#)), but since some cannabinoids, including CBD, interfere with the ability of lysophosphatidyl-inositol (LPI) to bind to GPR55, it is thought that CBD at least uses this mechanism. LPI induces cancer cell proliferation via GPR55 stimulation by triggering ERK, Akt and calcium mobilization cascades. Furthermore, pre-treatment of breast ([Ford et al., 2010](#)) and prostate cancer cells with CBD or the CB1 receptor antagonist rimonabant prevents LPI-induced cell proliferation via GPR55 ([Pineiro et al., 2011](#)).

## The endocannabinoid system and gynaecological cancer

It is now clear that the ECS is involved in all aspects of female reproduction from oocyte production through to parturition with components of the system having been demonstrated to be present and active throughout the entire adult female reproductive tract (Table II). Impairment or dysregulation of the ECS has been associated with various pathological conditions involving these organs (Table II; Fig. 1).

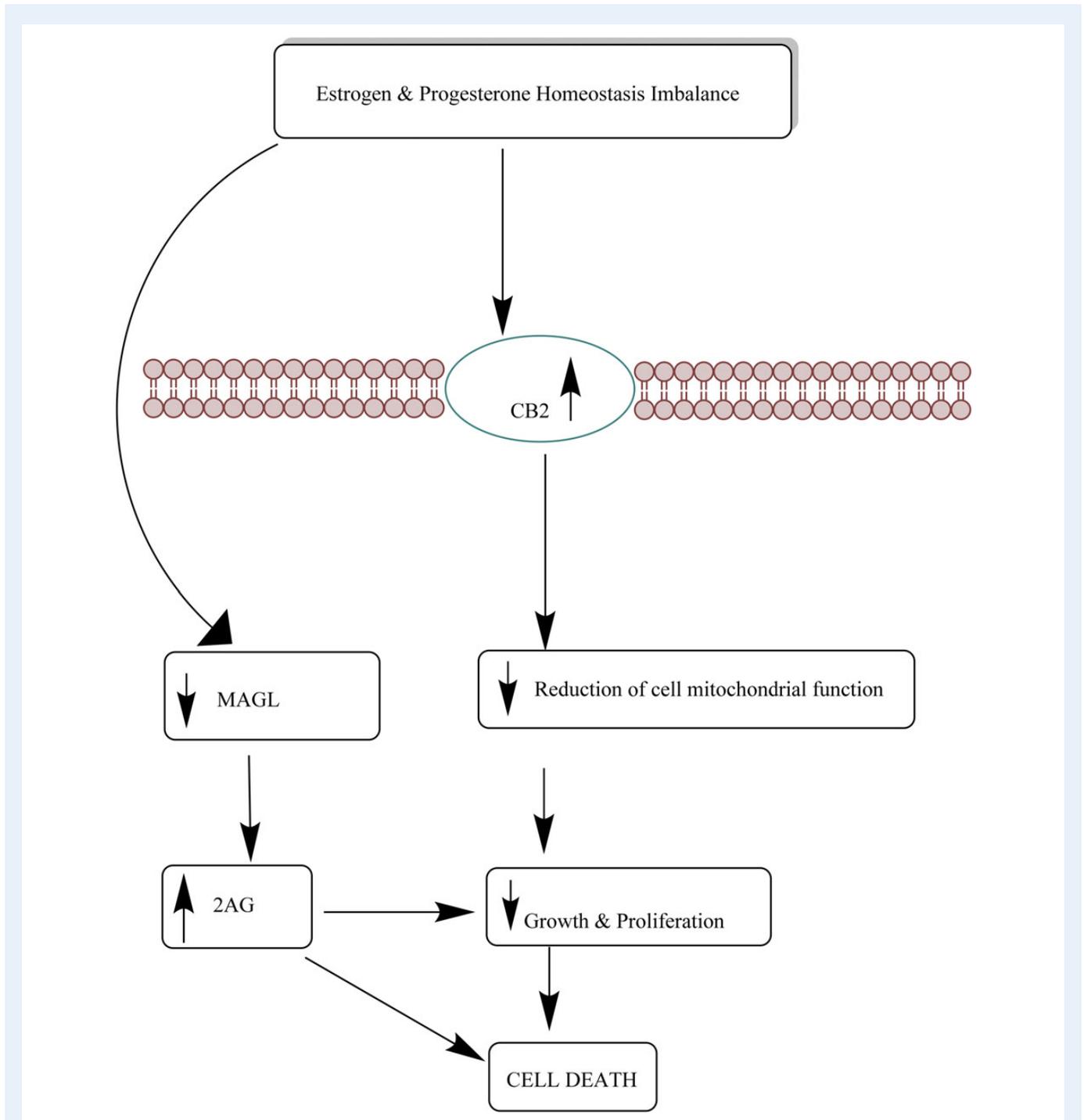
It is, therefore, not surprising that a dysfunctional ECS might be involved in the development of gynaecological cancers. The main perturbations involved are summarized in Table III.

### The endocannabinoid system and endometrial cancer

CB2 expression (by immunohistochemistry) has been shown in endometrial cancer biopsies but is only weakly expressed in adjacent normal endometrial tissue ([Guida et al., 2010](#)), and furthermore, immunoblotting has

shown that CB2 receptor protein expression is significantly elevated in the endometrial cancer tissues when compared with healthy endometrial tissues ([Guida et al., 2010](#)). There are no significant differences between CB1 receptor protein expression in the endometrial carcinoma and healthy endometrial tissues ([Guida et al., 2010](#)). Analysis of ligand levels by mass spectrometry has shown significantly higher levels of 2-AG in carcinoma tissues, compared with healthy endometrium. In the same study, elevated levels of AEA and PEA in endometrial carcinoma did not reach statistical significance. Further investigation of the ECS has revealed a selective down-regulation of MAGL but not FAAH in endometrial carcinoma compared with healthy controls ([Guida et al., 2010](#)). Another study that examined the levels of AEA in endometrial cancer has shown similar results: high levels of AEA in endometrial carcinoma ([Schmid et al., 2002](#)). Furthermore, a recent study by our group examining CB receptor transcript levels ([Ayakannu et al., 2014](#)) has also demonstrated lower CB2 receptor levels in the tumour when compared with normal endometrium of age-matched controls. These data have been confirmed using immunohistochemistry ([Ayakannu et al., 2013a](#)). These findings suggest that different signalling pathways are involved in normal and malignant endometrial gland growth control and/or that non-classical cannabinoid receptors are involved in the aetiopathogenesis of endometrial cancer.

Recent work performed by [McHugh et al.](#), using the endometrial cancer cell line HEC-1B, documented that AEA,  $\Delta$ -9-THC and NAGLy all induce cell migration through CB2 and GPR18 receptor activation, an effect that is independent of CB1 receptors but involves MAPK as an intermediate ([McHugh et al., 2012](#)). By contrast, a study by [Gentilini et al. \(2010\)](#) using primary human endometrial stromal cells has



**Figure 2** Schematic representation of the possible actions of ECS in endometrial cancer. The arrows within shapes indicate the direct of the effect. Cannabinoid receptor (CB2), MAGL and 2-AG.

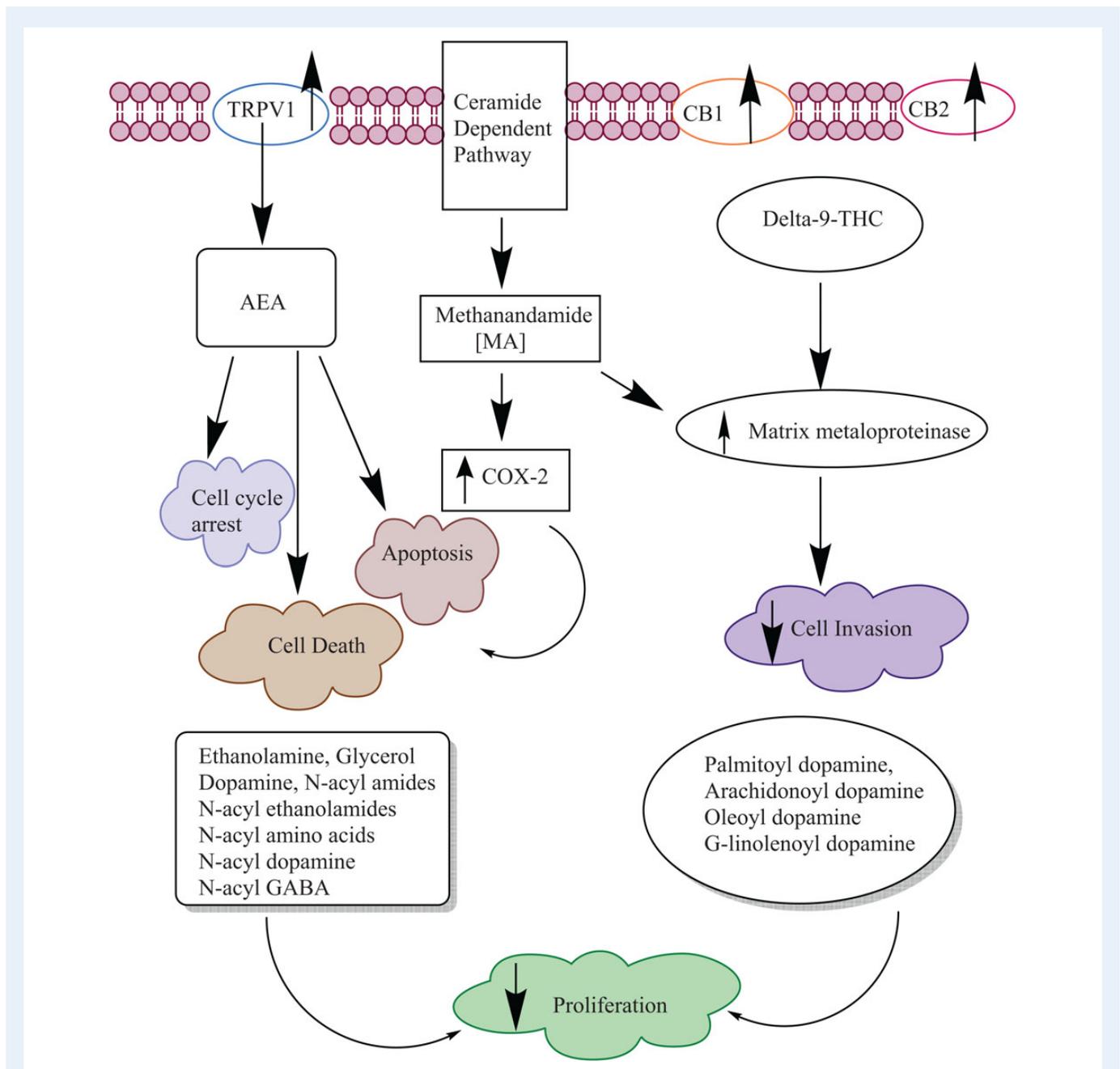
demonstrated that cell migration is increased following administration of RI-metAEA via CB1 receptor activation, suggesting that CB1 is the main signalling pathway involved in this migration process.

Nevertheless, CB2 receptors might play a vital role in the growth of endometrial cancer, probably through the control of cell survival/death pathways (Guzman *et al.*, 2002). A recent study (Guida *et al.*, 2010) has shown that the complete endogenous pathway for CB2 activation is

altered significantly in endometrial adenocarcinoma, which may be one of the underlying factors in the regulation of endometrial cancer. The marked elevation in CB2 receptor expression and 2-AG in endometrial cancer tissues might be due to the underlying imbalance in the estrogen/progesterone ratio, which is crucial for the development of endometrial cancer (Guida *et al.*, 2010). The human endometrial cancer cell line (AN3CA) used to study the possible physiological

role of the ECS, when transiently transfected with a plasmid containing the cDNA for CB2, showed a 40% reduction in mitochondrial function when compared with control cells, an effect that was not enhanced by the CB2 receptor agonist, JWH133, but was fully prevented by the CB2 receptor antagonist SRI144528. Importantly, the presence of agonist and antagonist was unable to prevent any negative effect of CB2 overexpression on AN3CA cell mitochondrial function. These data, therefore, support a potential role for the CB2 receptor in the control of

endometrial cancer cell growth through the modulation of mitochondrial function and apoptosis (Guida et al., 2010). The overexpression of CB2 receptors might, therefore, represent a novel diagnostic and therapeutic target for endometrial cancer by targeting cancer cells without damaging the surrounding normal tissues (McKallip et al., 2002). The ECS, as a whole, may be involved in the pathogenesis of endometrial cancer through various mechanisms, as summarized in Fig. 2.



**Figure 3** Schematic representation of ECS actions on cervical cancer. Increased expression of the three membrane receptors, TRPV1, cannabinoid receptor 1 (CB1) or cannabinoid receptor 2 (CB2) or binding of ligands Delta-9-tetrahydrocannabinol ( $\Delta$ -9-THC), anandamide (AEA) or methanandamide (MA) results in the indicated events, production of new molecules and decreased cell proliferation, whilst MA via a ceramide-dependent pathway affects both COX-2 and MMP pathways to affect cell proliferation.

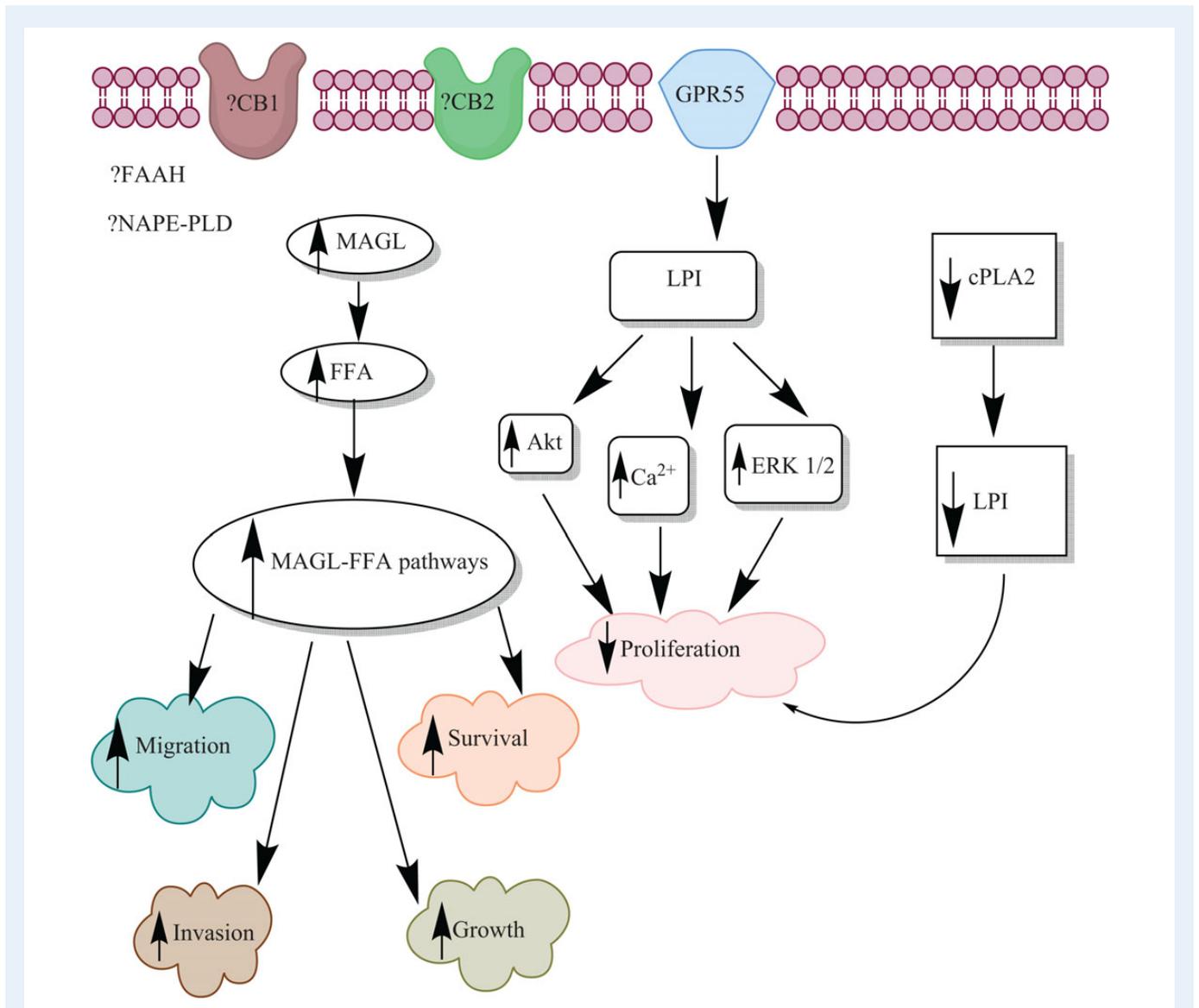
*The endocannabinoid system and cervical cancer*

The expression of endocannabinoid receptors (CB1, CB2) and the endovanilloid receptor (TRPV1) in different cervical cancer cell lines (Caski, HeLa and CC299) has been described using RT-PCR and Western blot analyses (Contassot et al., 2004). RT-PCR analysis has revealed strong evidence for CB1, CB2 and TRPV1 transcripts in all analysed cervical cancer biopsies (Contassot et al., 2004).

The effects of AEA in respect of cervical cancer have been investigated using Caski, HeLa and CC299 cells. These three cell lines demonstrated similar dose-dependent sensitivities to AEA resulting in significant cell cycle arrest and cell death (Contassot et al., 2004). AEA-induced DNA fragmentation of cervical cancer cells started 24 h after the addition of AEA, with an increasing proportion of cells staying in sub-G0/G1 (cell

cycle phase) (Contassot et al., 2004). The AEA-induced death of cervical cancer cells was not mediated through the CB1 and CB2 receptors because selective antagonists of CB1 (SR141716A) and CB2 (SR144528) enhanced the toxic effects of AEA, suggesting that CB1 and CB2 receptors play a protective role against AEA-induced cell death, in marked contrast to other malignancies (Contassot et al., 2004). The role of TRPV1 in the AEA-induced cell death was investigated using the TRPV1-selective antagonist capsazepine (CZ) and in contrast to CB1 and CB2 antagonists, the addition of CZ protected cells against AEA, suggesting that TRPV1 is involved in the mechanism of AEA-induced apoptosis in cervical cancer cell lines (Contassot et al., 2004).

Products such as ethanolamine, glycerol, dopamine, *N*-acyl amides, *N*-acyl ethanolamides, *N*-acyl amino acids, *N*-acyl dopamine and *N*-acyl



**Figure 4** Schematic representation of ECS actions on ovarian cancer. Cannabinoid receptor 1 (CB1), cannabinoid receptor 2, (CB2), GPCR55 (GPR55), fatty acid amide hydrolase (FAAH), *N*-acyl-phosphatidylethanolamine phospholipase D (NAPE-PLD), mono-acylglycerol lipase (MAGL), free fatty acid (FFA), lysophosphatidylinositol (LPI), extracellular signal-regulated kinase (ERK) 1/2, intracellular calcium (Ca<sup>2+</sup>) and cytoplasmic phospholipase A2 (cPLA2). Those parts of the ECS not yet investigated in human ovarian cancer are indicated with a question mark.

GABA, may also play a role in the proliferation of cervical cancer cell lines. A series of dopamine–fatty acid analogues such as palmitoyl dopamine, arachidonoyl dopamine, oleoyl dopamine and  $\gamma$ -linolenoyl dopamine have been shown to significantly inhibit HeLa cell proliferation (Burstein and Salmonsén, 2008).

The role of COX-2 in apoptosis caused by the FAAH-resistant endocannabinoid analogue MA has been investigated in HeLa and C33A cervical carcinoma cells (Eichele et al., 2009). MA induced COX-2 expression and subsequent PG synthesis in HeLa cells. Such cells were less sensitive to MA-induced apoptosis when COX-2 was suppressed by a selective COX-2 inhibitor (NS-398) or siRNA knock-down of COX-2 expression. It has also been demonstrated that MA-induced COX-2 expression and apoptosis are not mediated through either CB receptors or TRPV1 receptors but instead via a mechanism involving a ceramide-dependent pathway (Eichele et al., 2009), which mediates apoptosis in glioma cells (Hinz et al., 2004). Inhibition of MA-induced apoptosis was also evident by siRNA targeting of lipocalin-type PGD synthase (L-PGDS) or PPAR  $\gamma$  (Eichele et al., 2009).

Furthermore, MA and  $\Delta$ -9-THC decrease cervical cancer cell invasion (HeLa C33A) in a time- and concentration-dependent manner associated with an increased expression of TIMP-1 (Ramer and Hinz, 2008). Pre-treatment of cervical cancer cell lines with CBI or CB2 antagonists, with inhibitors of MAPKs and with an antagonist to TRPV1 resulted in the reversal of TIMP-1 expression and suppression of cell invasion. The knock-down effect of cannabinoid-induced TIMP-1 expression through siRNA reverses cannabinoid elicited a decrease in cervical cancer (HeLaC333A) cell invasion (Ramer and Hinz, 2008).

The actions of the ECS on cervical cancer development are represented schematically in Fig. 3.

#### *The endocannabinoid system and ovarian cancer*

Immunohistochemical investigations of normal ovarian tissues for CBI, CB2, FAAH and NAPE-PLD have revealed a widespread pattern of immunostaining in the ovarian cortex and medulla (Table II; El-Talatini et al., 2009). Functional proteomic analysis of ovarian (aggressive [SKOV3] and non-aggressive ovarian cancers [OVCAR3]) data has demonstrated that aggressive cancer cells display highly elevated monoacylglycerol hydrolytic activity and most, if not all, of this activity originates from the MAGL enzyme (Nomura et al., 2010), which degrades 2-AG (C20:4 MAG). MAGL and its FFA products were found to be elevated in aggressive ovarian cancer cell lines, as well as in high-grade primary human ovarian tumours. These data suggest that the MAGL-FFA pathway regulates a fatty acid network enriched in oncogenic signalling lipids that promote cell migration, invasion, survival and *in vivo* tumour growth. The overexpression of MAGL in non-aggressive cancer cells increases their pathogenicity, and this effect is reversed by MAGL inhibitor (Nomura et al., 2010).

The orphan GPCR 55 (GPR 55) has also been investigated in ovarian cancer cell lines. Western blot analysis revealed an abundant GPR55 expression in the ovarian cancer cell lines OVCAR3 and A2780 (Pineiro et al., 2011). Furthermore, a quantitative reverse transcriptase PCR investigation revealed the expression of GPR55 mRNA in OVCAR3 cells (Pineiro et al., 2011). GPR55 mediates the effects of the endogenous ligand LPI in ovarian cancer cells, where LPI activates Akt and increases intracellular  $\text{Ca}^{2+}$  levels and extracellular signal-regulated kinase (ERK) 1/2 expression. Moreover, its down-regulation using siRNA and

pharmacological blockade strongly inhibits OVCAR3 cell proliferation (Pineiro et al., 2011). It is, therefore, possible to speculate that this anti-proliferative effect is attributable to the concomitant reduction of cytosolic phospholipase A2 (cPLA2), which is responsible for the synthesis of LPI and ATP binding cassette transporter ABCC1, thus decreasing the levels of LPI (Pineiro et al., 2011).

The actions of ECS on the ovarian cancer cell are summarized in Fig. 4.

## Conclusion

Currently available data suggest that the ECS may be targeted to restrain the development and progression of gynaecological cancers. The system exerts a variety of interesting effects that are dependent on the cell type and/or malignancy. The ECS is implicated in cancer cell proliferation, angiogenesis, metastasis and apoptosis. The evidence suggests that an imbalance in endocannabinoid homeostasis may promote cancer development, proliferation and migration; therefore, the ECS is an attractive target for pharmacological intervention in the fight against cancer. The ECS is complex, involving different signalling pathways including activities mediated via CBI, CB2, TRPV1 and GPR55 receptors and other as yet uncharacterized receptors, and also through receptor-independent pathways. These complex signalling pathway interactions, both in normal and cancer tissues, offer a great challenge to researchers. However, the need for a better understanding of the role of the ECS in gynaecological cancer means a need for further research to unravel the complex interplay between the ECS, its signalling pathways and carcinogenesis.

## Authors' roles

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