Human Reproduction Update, Vol.22, No.3 pp. 382–403, 2016
Advanced Access publication on January 5, 2016 doi:10.1093/humupd/dmv060

human reproduction update

Kinase signalling pathways in endometriosis: potential targets for non-hormonal therapeutics

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Submitted on July 31, 2015; resubmitted on November 10, 2015; accepted on December 8, 2015

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BACKGROUND: Endometriosis, the growth of endometrial tissue outside the uterine cavity, is associated with chronic pelvic pain, subfertility and an increased risk of ovarian cancer. Current treatments include the surgical removal of the lesions or the induction of a hypoestrogenic state. However, a reappearance of the lesion after surgery is common and a hypoestrogenic state is less than optimal for women of reproductive age. Additional approaches are required. Endometriosis lesions exist in a unique microenvironment characterized by increased concentrations of hormones, inflammation, oxidative stress and iron. This environment influences cell survival through the binding of membrane receptors and a subsequent cascading activation of intracellular kinases that stimulate a cellular response. Many of these kinase signalling pathways are constitutively activated in endometriosis. These pathways are being investigated as therapeutic targets in other diseases and thus may also represent a target for endometriosis treatment.

METHODS: To identify relevant English language studies published up to 2015 on kinase signalling pathways in endometriosis, we searched the Pubmed database using the following search terms in various combinations; 'endometriosis', 'inflammation', 'oxidative stress', 'iron', 'kinase', 'NF kappa', 'mTOR', 'MAPK' 'p38', 'JNK', 'ERK' 'estrogen' and progesterone'. Further citing references were identified using the Scopus database and finally current clinical trials were searched on the clinicaltrials.gov trial registry.

RESULTS: The current literature on intracellular kinases activated by the endometriotic environment can be summarized into three main pathways that could be targeted for treatments: the canonical IKKβ/NFκB pathway, the MAPK pathways (ERK1/2, p38 and JNK) and the PI3K/AKT/mTOR pathway. A number of pharmaceutical compounds that target these pathways have been successfully trialled in *in vitro* and animal models of endometriosis, although they have not yet proceeded to clinical trials. The current generation of kinase inhibitors carry a potential for adverse side effects.

CONCLUSIONS: Kinase signalling pathways represent viable targets for endometriosis treatment. At present, however, further improvements in clinical efficacy and the profile of adverse effects are required before these compounds can be useful for long-term endometriosis treatment. A better understanding of the molecular activity of these kinases, including the specific extracellular compounds that lead to their activation in endometriotic cells specifically should facilitate their improvement and could potentially lead to new, non-hormonal treatments of endometriosis.

Key words: inflammation / signalling kinase / NF kappa B / mTOR / MAPK / microenvironment / treatment / drugs / endometriosis

Introduction

Endometriosis is an estrogen-dependent condition characterized by the growth of endometrial epithelial and stromal cells outside the uterine cavity and is often accompanied by chronic pelvic pain, subfertility and an increased risk of ovarian cancer (Vercellini *et al.*, 2014). It is an extremely prevalent condition, occurring in 10% of women of reproductive age (Eskenazi and Warner, 1997) and up to 50% of women with infertility (Meuleman *et al.*, 2009) and represents a significant burden on the health care system (Simoens *et al.*, 2012). Although a number of theories have been proposed, the most widely accepted is the Sampson theory of transplantation where menstrual tissue, including viable endometrial epithelial and stromal cells, enter the peritoneal cavity via retrograde menstruation (Sampson, 1927). Once present, an innate or acquired characteristic of these endometrial cells and the inflammatory and hormonal microenvironment combine to facilitate lesion growth at multiple locations throughout the peritoneal cavity (Burney and Giudice, 2012).

Endometriosis is an extremely heterogenic condition that was originally proposed to exist as three different entities: peritoneal endometriosis, ovarian endometrioma and adenomyotic nodules of the rectovaginal septum all of which develop through distinct pathogenic pathways (Nisolle and Donnez, 1997). More recent research, however, suggests that the different clinical presentations are actually a continuum of the same disease (Vercellini *et al.*, 2000) with shared origins (Somigliana *et al.*, 2004, 2007). Superficial peritoneal endometriotic lesions represent the least severe clinical presentation, followed by endometrioma and deeply infiltrating endometriosis (DIE), the most severe (Chapron *et al.*, 2009). DIE is defined by infiltration into the *muscularis propria* (Chapron *et al.*, 2010) and is further subcategorized by the invaded organ, which may be the bladder, uterosacral ligaments, intestines and/or vagina (Chapron *et al.*, 2003b). DIE lesions are most commonly associated with strong pain (Chapron *et al.*, 2003a) and represent the most complex clinical challenge (Abrão *et al.*, 2015).

Challenges of current endometriosis management

The current European Society of Human Reproduction and Embryology (ESHRE) guidelines advocate endometriosis management via hormonal modulation with medical therapies, or the surgical removal of the lesions (Dunselman *et al.*, 2014). Both of these approaches, however, have significant shortcomings.

Hormonal modulation through medical therapies creates a hypoestrogenic environment with hormonal contraception, progestogens, anti-progestogens, gonadotrophin-releasing hormone analogues and aromatase inhibitors (Brown and Farquhar, 2014). This approach, however, is inappropriate for patients with endometriosis-associated infertility who wish to conceive normally (Dunselman *et al.*, 2014). Furthermore, symptoms reoccur once treatment has ceased (Streuli *et al.*, 2013) and up to one-quarter of patients will have intolerable side effects, or not respond (Vercellini *et al.*, 2008). An inadequate response to medical therapies is believed to be a particular problem for DIE lesions (Vercellini *et al.*, 2009), possibly due to extensive fibrosis rendering them less susceptible to hormonal modulation (Remorgida *et al.*, 2005).

Surgical intervention is the primary treatment of choice for severe forms of endometriosis (Abrão *et al.*, 2015), such as symptomatic DIE that incorporate bowel or urethra stenosis, large adnexal masses or large endometrioma (Vercellini *et al.*, 2009; Meuleman *et al.*, 2011). A reduction in pelvic pain (Jacobson *et al.*, 2009), dyspareunia (Ferrero *et al.*, 2007) and an increase in fertility (Duffy *et al.*, 2014) is achieved via surgical intervention with a significant improvement in patient wellbeing and quality of life that can be extrapolated to significant savings for the health care system (Wullschleger *et al.*, 2015). Surgery, however, can be associated with complications, particularly in complex cases. Recurrence of the lesions (Shaw, 1992) and the painful symptoms (Duffy *et al.*, 2014) is also common.

Surgical removal of DIE lesions can be complex and outcomes are highly dependent on surgical skill. A recent meta-analysis revealed the complication rates for bowel resection anastomosis of DIE lesions have been measured as 2.7% of patients for rectovaginal fistulae, 1.5% for anastomotic leakage and 0.34% for pelvic abscesses. The less aggressive techniques had slightly lower rates, but were associated with an increase in recurrence from 5.8 to 17.6% (Meuleman *et al.*, 2011). The primary reason for recurrence is unclear but an incomplete resection of the lesion (Nirgianakis *et al.*, 2014) due to the complexity of the surgery or to the presence of occult endometriosis (Khan *et al.*, 2014) are possible.

The endometriotic microenvironment

The peritoneal microenvironment is significantly altered in endometriotic women. Endometrial cells refluxed into the peritoneal cavity secrete chemokines (Lebovic *et al.*, 2001) creating a feed-forward loop (Hornung *et al.*, 2001) that stimulates the infiltration of immune cells (Halme *et al.*, 1983). Both endometriotic and immune cells (Laird *et al.*, 1993; Bersinger *et al.*, 2008, 2011) produce pro-inflammatory cytokines and prostaglandins (Badawy *et al.*, 1985; Wu *et al.*, 2005) and anti-inflammatory interleukins are suppressed (Santulli *et al.*, 2013) creating an inflammatory imbalance. Erythrocytes and menstrual debris enter the peritoneal cavity via retrograde menstruation resulting in increased iron concentrations (Arumugam and Yip, 1995; Iizuka *et al.*, 1998; Yamaguchi *et al.*, 2008) that accumulate in peritoneal macrophages (Lousse *et al.*, 2009) and mediate oxidative stress

(Defrère et al., 2008) in both the peritoneal fluid (Carvalho et al., 2012) and the endometriotic cells (Murphy et al., 1998; Oner-Iyidoğan et al., 2004; Ngô et al., 2009; Seo et al., 2010).

Once the lesions are established, local estrogen production begins through endometrial cell expression of aromatase p450 (Noble et al., 1996) and a reduction in 17β-hydroxysteroid dehydrogenase type II (Zeitoun et al., 1998). The overexpression of estrogen receptor (ER)β in endometriotic stromal cells also alters their behaviour leading to a reduction in the expression of ERα (Xue et al., 2007; Trukhacheva et al., 2009) and possibly of progesterone receptors (PRs) (Bulun et al., 2010). Finally, neuroangiogenesis leads to the infiltration of nerve fibres and blood vessels (Asante and Taylor, 2011) that supply nutrients and remove waste, as well as secreting neurogenic compounds (Sanfilippo et al., 1992) that interact with endometriotic lesions (McKinnon et al., 2013). These mechanisms create an altered endometriotic microenvironment characterized by an inflammatory imbalance, oxidative stress and increased iron concentrations that support the maintenance of the cells, while their continued growth is facilitated by estrogen production and neuroangiogenesis (Fig. 1).

The extracellular influence on endometriotic cells

The ability of this altered microenvironment to support endometriotic cells is transmitted by kinase signalling pathways. In many diseases, the dysregulation of a protein kinase leads to unchecked cellular proliferation through stimulation of neoplastic processes resulting in a kinases-dependent tumour growth (Sawyers, 2003). Pharmaceuticals targeting these kinases is proving successful in the treatment of other tumours and is increasingly being examined as potential endometriosis treatments. Whether endometriosis exhibits kinase dependency is not yet clear, although inflammation (Lee and Hung, 2007), neurogenic mediators (Azzolina et al., 2003), steroid hormones (King et al., 2010) and both iron and oxidative stress (Alvarado-Díaz et al., 2015) interact with multiple kinase signalling pathways in endometriotic cells.

The interaction of the microenvironment and the endometriotic cells may also vary based on lesion subtype. DIE lesions have a significantly different microenvironment compared with lesions from other locations as they produce significantly more inflammatory cytokine mRNA (Bertschi et al., 2013) and have higher peritoneal fluid IL-33 concentrations

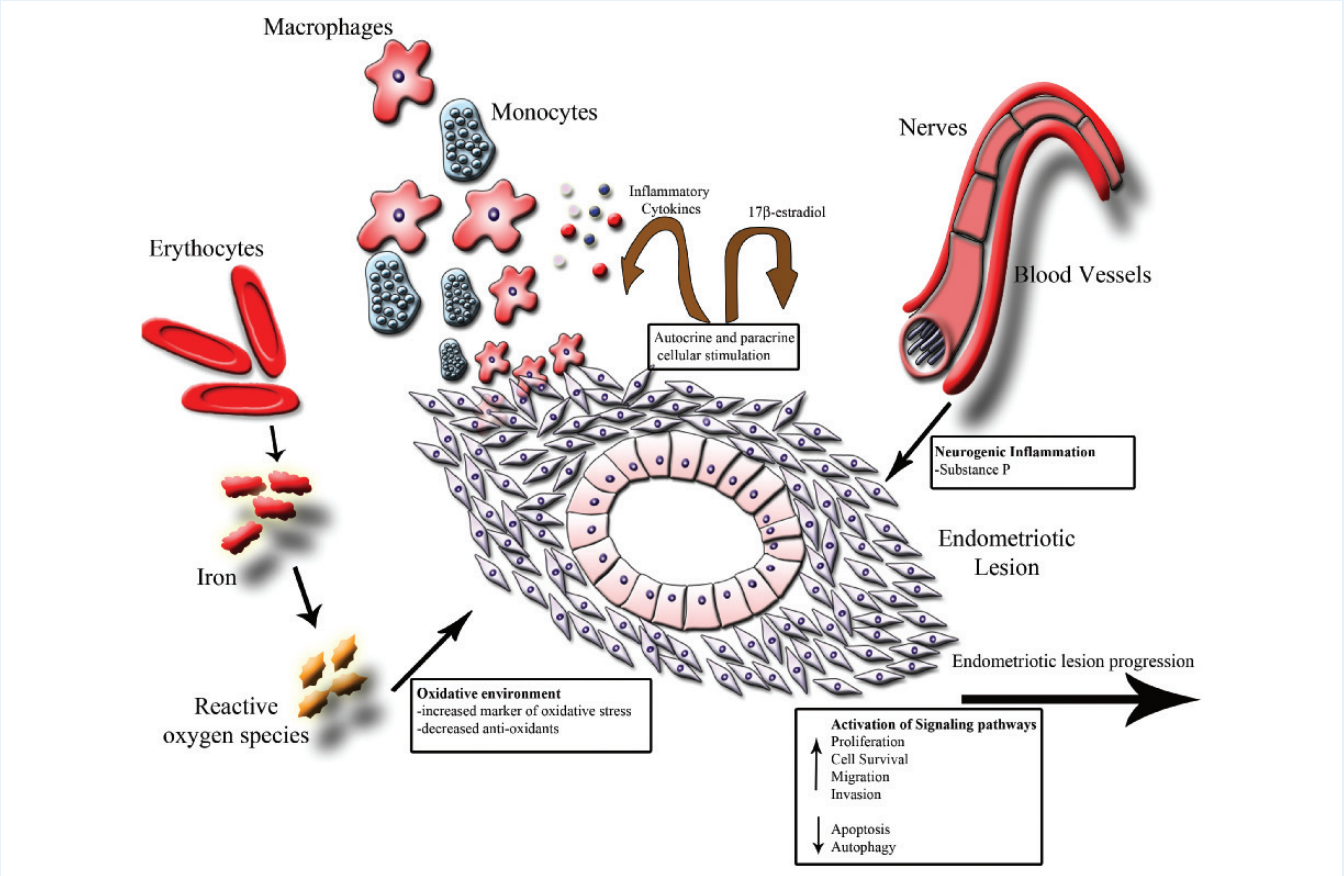

Figure 1 The endometriotic microenvironment. Endometriotic lesions exist in a unique microenvironment created by the interaction of multiple cells. Through retrograde menstruation epithelial and stromal endometrial cells, along with erythrocytes and other menstrual debris enter the peritoneal cavity. The endometrial cells attach to the underlying mesothelium and establish ectopic lesion that begin producing chemokines and hormones. These compounds can have both an autocrine and paracrine effect. Chemokines stimulate the infiltration of immune cells and hormones influence the endometriotic cells. Erythrocytes lead to increased iron concentrations, which in turn creates reactive oxygen species and an oxidative environment. The subsequent inflammatory, hormonal and oxidative environment leads to the stimulation of the signalling kinase pathways that facilitate endometriotic lesion progression.

(Santulli *et al.*, 2012) and oxidative stress markers than lesions from other locations (Santulli *et al.*, 2015a). Significantly higher concentrations of endometriosis-associated nerve fibres have also been observed in DIE lesions increasing the potential for neurogenic inflammation (McKinnon *et al.*, 2012b). Whether the extracellular environment of DIE lesions creates a specific influence is not clear, but the high concentrations of potential kinase stimulating components suggest DIE lesions may respond to kinase inhibition, as opposed to hormonal therapies.

All together this makes endometriosis a heterogeneous condition that poses a difficult clinical challenge, particularly for symptomatic DIE lesions. New therapeutic options are needed. Endometriotic lesions create a unique microenvironment capable of inducing kinase activity and potentially, a kinase-dependent lesion growth. Targeting these kinases may represent a potential novel treatment, and may also hold potential for DIE lesions. We therefore examined the relevant literature to identify published data on kinase activity in endometriotic tissue and to determine whether they were activated by components of the endometriotic extracellular environment. We focused on three specific pathways involving nuclear factor (NF)κB, mitogen-activated protein kinase (MAPK) or mammalian target of rapamycin (mTOR). We also assessed therapeutics that target these pathways and analysed their potential for future treatments.

Methods

We identified relevant English language studies published up to 2015 via a search of the Pubmed database using the following search terms in various combinations; 'endometriosis', 'inflammation', 'oxidative stress', 'iron' 'kinase', 'NF kappa', 'mTOR', 'MAPK' 'p38', 'JNK', 'ERK' 'estrogen' and 'progesterone'. Further citing references were identified using the Scopus database and current clinical trials were identified using the clinicaltrials.gov trial registry.

The NFκB pathway in endometriosis

NFκB is the nodal point of a primary inflammation stimulated signalling pathway that has a significant role in the immune response (Hayden *et al.*, 2006). The NFκB complex is assembled from two groups of proteins: the NFκB proteins, p105 and p100, which are truncated to p50 and p52, respectively, and the Rel proteins (c-Rel, REL B and p65). These proteins combine as either hetero or homodimeric complexes to form the NFκB complex of which the most common arrangement is the p50/p65 heterodimer (Ghosh *et al.*, 1998). Under resting state conditions, the dimeric NFκB/Rel complexes are bound to the inhibitor kappa beta protein (IκB). Binding between the NFκB and IκB keeps the complex sequestered to the cytosol (Fig. 2). Activation of cell surface receptors by the extracellular environment begins a cascading reaction that separates IκB and NFκB complex and allows for the translocation of NFκB to the nucleus and initiation of gene transcription. IκB removal from the NFκB complex is mediated by the IκB kinase (IKK) complex, which consists of two catalytic subunits IKKα and IKKβ and the regulatory subunit IKKγ (Smale, 2011).

Two distinct cascading reactions, each controlled by the different catalytic subunits of the IKK complex, lead to NFκB activation. The canonical NFκB pathway is characterized by activity of the IKKβ catalytic subunit removing IκB from p65 and targeting it for ubiquitin ligase-mediated degradation (Ghosh and Karin, 2002). The alternative NFκB pathway is characterized by IKKα catalytic activity that is stimulated by NFκB inducing kinase (NIK). This catalytic subunit preferentially targets IκB proteins bound to the p100-Rel B dimers stimulating a partial proteasome degradation that creates the transcriptionally active p52-Rel B dimer (Oeckinghaus *et al.*, 2011). Both the canonical and alternative NFκB pathways lead to increased transcription of different genes and therefore mediate different immune functions (Bonizzi and Karin, 2004).

NFκB may represent a potential therapeutic target due to its constitutive activation in peritoneal endometriotic lesions (Gonzalez-Ramos *et al.*, 2007). An overexpression of NFκB has been confirmed in cultured endometriotic stromal cells (Sakamoto *et al.*, 2003) and peritoneal macrophages (Lousse *et al.*, 2008) isolated from women with endometriomas. Furthermore, in ovarian endometriomas, p65 expression has been correlated with recurrence (Shen *et al.*, 2008). *In vitro* evidence raises the possibility that the constitutive activation may be due to the endometriotic microenvironment. IL-1β stimulates NFκB with a subsequent increased production of inflammatory cytokines (Veillat *et al.*, 2009), including macrophage migration inhibitory factor (MIF) (Cao *et al.*, 2006) in endometrial stromal cells, as does tumour necrosis factor alpha (TNFα) (Grund *et al.*, 2008) in the immortalized epithelial (12Z) cell line. In primary epithelial cells, 17β-estradiol stimulates NFκB nuclear translocation (Zhang *et al.*, 2010a) and progesterone withdrawal increases NFκB activity in the endometrium (King *et al.*, 2001). Interestingly, iron increases NFκB activity in endometriotic stromal cells (Alvarado-Díaz *et al.*, 2015) and it has been speculated that the alternative NFκB pathway may be responsible for the stimulation of inflammation by iron overload in endometriotic women (González-Ramos *et al.*, 2012). However, the contribution of iron to NFκB remains controversial (Hayakawa *et al.*, 2003).

There is also the significant possibility of an interaction between NFκB and peroxisome proliferator-activated receptor (PPAR)γ, a nuclear transcription factor involved in the inflammatory response (Daynes and Jones, 2002) and implicated in the pain experienced by endometriotic women (Moravek *et al.*, 2009; McKinnon *et al.*, 2010). The exact mechanism by which PPARγ agonists attenuate the inflammatory response, however, is not yet clear, but previous evidence has shown that the natural ligand for PPARγ, 15-deoxy-delta-12, 14-prostaglandin J2 (15dPGJ2) also represses NFκB (Castrillo *et al.*, 2000; Straus *et al.*, 2000), raising the possibility that some of the anti-inflammatory effects ascribed to the PPARγ agonist may be PPARγ independent. In endometrial stromal cells, both pioglitazone and ciglitazone attenuate the production of IL-6 and IL-8 in a PPARγ-independent mechanism (McKinnon *et al.*, 2012a) and pioglitazone significantly reduces the concentration of TNFα-stimulated p65 (Ohama *et al.*, 2008).

Targeting the NFκB pathway in endometriosis

As NFκB regulates numerous physiological processes and contributes to the pathology of several human diseases, there has been a great deal of interest in designing pharmacological methods to intervene in its activity (Gilmore and Herscovitch, 2006). Given the huge number of compounds already developed, we have focused only on those that have shown either *in vitro* or clinical potential in endometriosis and divided these into molecules that function prior to the removal of IκB from the NFκB complex (upstream) and those that function after the removal of IκB and the translocation of the complex to the nucleus (downstream).

Upstream modulation of NFκB activity has been trialled in endometriosis via inhibition of the catalytic subunits that mediate IκB phosphorylation and its removal from the NFκB complex and subsequent proteasomal degradation (Fig. 2). BAY 11-7085, a synthetic compound that inhibits IκB phosphorylation (Pierce *et al.*, 1997), decreased cell proliferation and DNA synthesis and induced apoptosis in endometriotic stromal cells (Nasu *et al.*, 2007). In a heterologous nude mouse model, it decreased lesion size and increased apoptotic markers (González-Ramos *et al.*, 2008). Bortezomib, a proteasome inhibitor, reduced the endometriotic lesion size in a transplanted endometriosis model using Wistar rats and decreased proliferating cell nuclear antigen (PCNA) and Ki67 expression (Celik *et al.*, 2008), whereas *N*-Tosyl-L-Phenylalanine Chloromethyl ketone (TPCK) also showed anti-NFκB activity in primary stromal cells isolated from endometrioma (Yamauchi *et al.*, 2004). Pyrrolidine dithiocarbamate (PDTC),

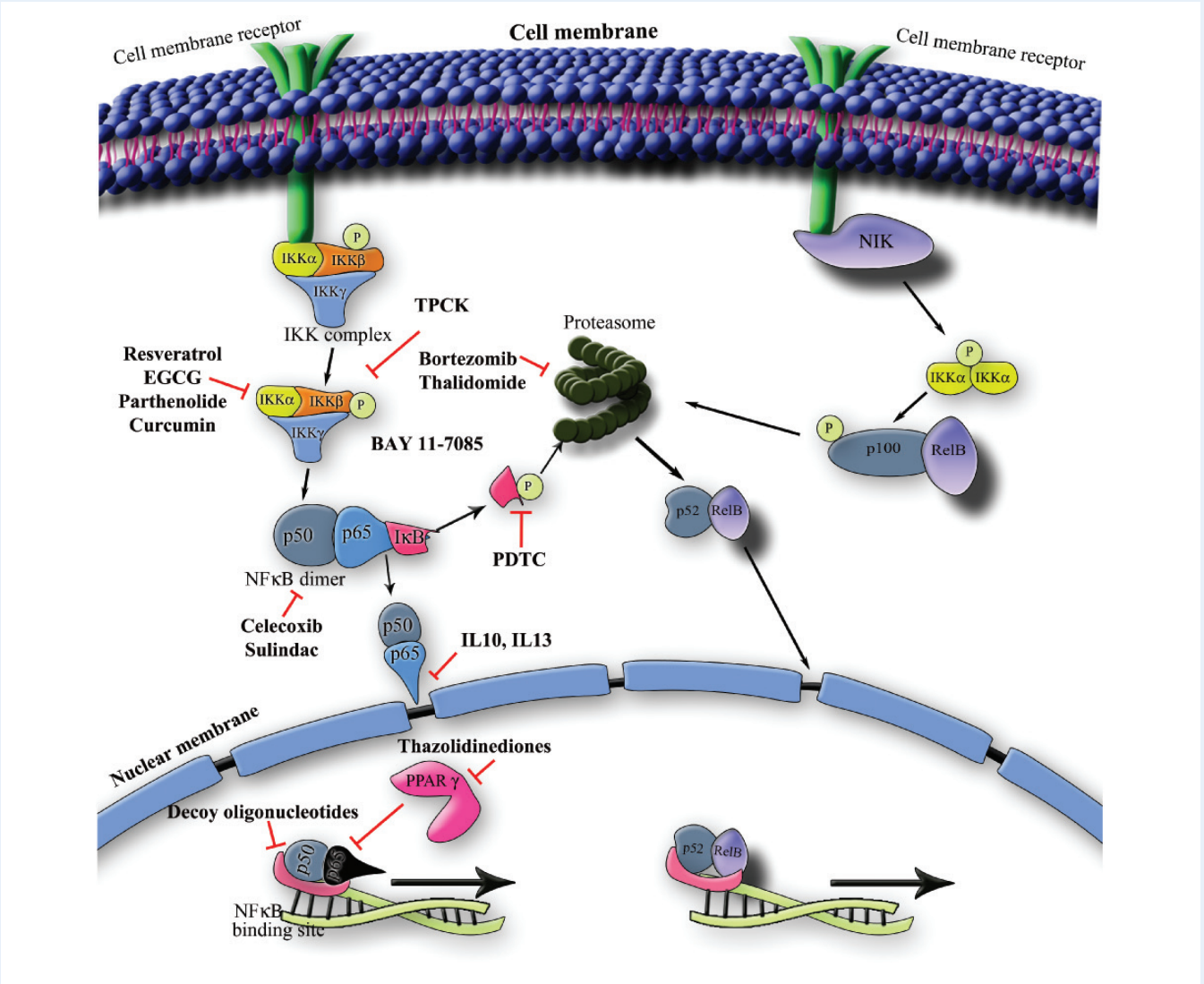

Figure 2 The NFκB signalling pathway and its inhibition in endometriosis. Binding to cell membrane receptors stimulates both the canonical and alternative NFκB signalling pathways. Stimulation of the canonical NFκB pathway leads to the phosphorylation of IKKβ. IKKβ is part of the IKK complex along with IKKα and IKKγ and an activated IKKβ phosphorylates the inhibitory protein IκB preferentially on p50–p65, removing it from the complex and targeting it for proteasomal degradation. The unbound p50–p65 complex translocates into the nucleus and stimulates gene transcription. The PPARγ nuclear transcription factor may also interact with p50–p65 complex and suppress gene transcription. Extracellular molecules, including inflammatory mediators, oxidative stress markers and iron also activate the alternative NFκB pathway. Binding of these molecules to cell membrane receptors leads to activation of the NIK, which in turn phosphorylates IKKα dimers preferentially that remove IκB from the p100-Rel B complex. Removal of the IκB protein allows a partial degradation of the p100 protein to p52 and the subsequent p52-Rel B dimer to translocate to the nucleus and stimulate gene transcription.

which functions as both an antioxidant effects and IκB-ubiquitin ligase (Hayakawa *et al.*, 2003) decreased inflammation, angiogenic factors and matrix metalloproteinases (MMP) *in vitro* in both endometrial epithelial (Zhang *et al.*, 2011) and stromal cells (Zhang *et al.*, 2010a, b), all of which were preferential in endometriotic compared with endometrial cells. Furthermore, in a heterologous transplanted endometriosis model in Wistar rats, PDTC mediated a reduction in lesion size (Celik *et al.*, 2008).

Downstream of the NFκB complex, it is also possible to inhibit the transcriptional activity of this pathway via disruption of NFκB translocation to the nucleus and the subsequent DNA binding (Fig. 2). The anti-inflammatory cytokines IL-10 and IL-13 suppress nuclear localization of NFκB and increase the IκB mRNA transcription (Lentsch *et al.*, 1997) and in endometriotic stromal cells, IL-10 treatment significantly reduces the production of TNFα-induced IL-6 but not IL-8 production (Tagashira *et al.*, 2009). Blocking the specific NFκB DNA-binding sites at promoter regions with decoy oligonucleotides is another possible strategy (Khaled *et al.*, 1998) that has been used successfully with endometriotic stromal cells *in vitro* as it was shown to suppress IL-1β-induced RANTES production and MCP-1 activity (Xiu-li *et al.*, 2009).

Pharmaceuticals with off-target effects on NFκB have also been considered for endometriosis treatment. Thalidomide inhibits NFκB through the suppression of IκB degradation (Majumdar *et al.*, 2002). Treatment of endometriotic stromal cells with thalidomide inhibited TNFα-stimulated IL-8 production and secretion (Yagyu *et al.*, 2005) and reduced the size of autologous transplanted endometriotic lesions in rat models (Azimirad *et al.*, 2014). Thiazolidinediones, ligands for PPARγ, which may have PPARγ-independent

mechanism in endometriotic stromal cells (McKinnon *et al.*, 2012a) and originally developed for diabetes treatment, reduced the size of endometriotic lesions in both rats (Lebovic *et al.*, 2004) and primates (Lebovic *et al.*, 2007). These drugs, however, also produce adverse effects on skeletal health (Bodmer *et al.*, 2009). Non-steroidal anti-inflammatory drugs (NSAIDS), such as celecoxib, inhibit cyclooxygenase (COX)-2 and also interact with NFκB in leiomyoma cells (Park *et al.*, 2014). In an *in vitro* experiment celecoxib also decreased cellular proliferation of endometrial epithelial cells (Olivares *et al.*, 2008). Sulindac also decreased RANTES through an NFκB mechanism (Wieser *et al.*, 2005). However, neither of these NSAIDs reduced the size of a surgically induced endometriotic lesion in a mouse model significantly more than any other NSAIDs (Efstathiou *et al.*, 2005).

Natural occurring compounds may also represent possible endometriosis treatments, mediated through their antioxidant effects on NFκB. Resveratrol, a compound present in red wine, modulates NFκB activity (Leiro *et al.*, 2005) and significantly reduced the size of surgically induced endometriotic lesions of nude mice (Bruner-Tran *et al.*, 2011) and reduced vascular density in a BALB/c mouse model (Ricci *et al.*, 2013). In both *in vitro* and animal models resveratrol reduced cell proliferation and increased apoptosis of endometrial epithelial cells (Ricci *et al.*, 2013; Rudzitis-Auth *et al.*, 2013) as well as reducing peritoneal fluid MCP1, VEGF (Ergenoğlu *et al.*, 2013; Ozcan Cenksoy *et al.*, 2015), IL-6, IL-8 and TNFα concentrations (Bayoglu Tekin *et al.*, 2015). Similarly, epigallocatechin-3-gallate (EGCG) a catechin found in green tea also interacts with NFκB (Khan *et al.*, 2006) and significantly reduced surgically induced endometriotic lesions in mice (Ricci *et al.*, 2013). Parthenolide, the active ingredient from the medical herb feverfew (*Tanacetun parthenium* L.), inhibited NFκB activity (Kwok *et al.*, 2001) and reduced the inflammatory response in endometriotic stromal cells isolated from endometriomas (Takai *et al.*, 2013). Curcumin, a naturally occurring polyphenol (Cao *et al.*, 2005), attenuated IL-1β induced MIF secretion (Veillat *et al.*, 2009) and TNFα-induced inflammation (Kim *et al.*, 2012) in endometriotic stromal cells, as well as reducing MMP3 expression and lesion size in BALB/c mice (Jana *et al.*, 2012).

It is possible that these compounds mediate anti-endometriotic activity. Owing to their low concentrations in the commonly consumed products of which they are found, it is unlikely, however, that they will produce lasting effects through natural consumption. However, through a manufacturing process it may be possible that the concentrations used to produce the effects observed *in vitro* and in animal models can be reproduced. Whether they will be at concentrations that are also clinically effective in human studies is not yet clear as significantly more information is still required about their bioavailability, metabolism and potential side effects.

There is also the potential for combinational therapy that incorporates an NFκB targeting compound with another molecule. The combination of celecoxib with the PPARγ agonist rosiglitazone significantly reduced the size of surgically induced lesions in mice (Olivares *et al.*, 2011) compared with the individual use of these drugs, although an antagonizing effect was observed when pairing celecoxib with the aromatase inhibitor anastrozole (Olivares *et al.*, 2013). Pycnogenol, from the bark of the French maritime pine (*Pinus pinaster*), has shown anti-NFκB activity in endothelial cells (Peng *et al.*, 2000) and used in combination with oral contraceptives showed promising results on dysmenorrhea, compared with use with contraception alone (Maia *et al.*, 2013, 2014). Resveratrol and oral contraceptives in combination also showed a greater decrease in aromatase and COX expression than in individual therapy (Maia *et al.*, 2012).

The adverse effects associated with modulating such a ubiquitously employed pathway may also limit therapeutic targeting of the NFκB pathway. Given the importance of NFκB to immune regulation, it may not be feasible to inhibit this pathway long-term as it may suppresses the host-immune response and leave the patient vulnerable for infection, an effect observed in animal models (Lavon *et al.*, 2000). Furthermore, a number of the targeted mechanisms in this pathway are regulatory proteins that control numerous other functions within the cell and thus their inhibition may also lead to other unwanted side effects (Yamamoto and Gaynor, 2001).

Both embryotoxicity and teratogenicity are also important considerations given the demographic characteristics of endometriotic women. Of the drugs that interact with the NFκB pathway, thalidomide has a dire history and will be unlikely to have a useable reputation for women with endometriosis. PDTC has also shown some teratogenicity on zebrafish models (Tilton *et al.*, 2006) and the thiazolidinediones are categories C class pregnancy drugs and are currently not indicated during pregnancy. Sulindac also produced cleft palates in mouse models (Montenegro and Palomino, 1990) and high concentration of resveratrol was toxic in chick embryo toxicity assays (Venturelli *et al.*, 2013). Lastly, the parthenolide like compounds have recently been indicated as possibly embryotoxic (Amorim *et al.*, 2013).

Summary

A constitutive activity of NFκB has been observed in endometriotic cells both *in vivo* and *in vitro*. Furthermore, inflammation, oxidative stress and hormones stimulate this pathway in endometriotic tissue and it therefore represents a potential target for endometriosis treatment. Given the central role of NFκB in mediating the immune response however, it is a concern that targeting its activity might also impair the body's natural ability to remove ectopic tissue. Targeting this pathway successfully therefore requires a balance between the suppression of the immune response and the induction of its apoptotic activity. Both upstream and downstream modulation of NFκB are viable approaches with particular promise in targeting proteasomal degradation of IκB. Such a balance may be achievable by combining a moderate inhibition of NFκB through naturally occurring compounds with additional targets, similar to other drugs that have off-target effects on this pathway. However, problems with reputation (thalidomide) and adverse side effects (thiazolidinediones) of some compounds will most likely limit their clinical applications. The ability of naturally occurring compounds to inhibit NFκB and their minimal side effects may provide the opportunity to combine these compounds with other drugs.

The MAPK pathways in endometriosis

The MAPK pathways encompasses a collection of kinase signalling pathways, organized in a three tier hierarchical structure (1st-MAPK, 2nd-MAP2K and 3rd-MAP3K) with abundant crosstalk, that play a significant role in linking the extracellular environment with fundamental cellular responses. The MAPK signalling kinases are subdivided into the three families (Fig. 3): extracellular signal-regulated kinase (ERK), p38 and c-Jun-N terminal kinase (JNK) (Yoshino *et al.*, 2004). Within these subfamilies, six distinct terminal MAPKs have been characterized; ERK1/2, ERK3/4, ERK5, ERK7/8, which comprise the ERK family, JNK1/2/3, which make up the JNK family, and the p38 subunits α/β/γ/δ, which comprise the p38 family (Dhillon *et al.*, 2007). The extracellular environment activates all three pathways with ERK predominantly activated by inflammation and growth factors and JNK and p38 predominantly activated by stress and inflammation. Once activated, the MAPKs initiate a cellular response via nuclear transcription factors.

The ERK1/2 pathway

The ERK pathway is the most comprehensively studied of the mammalian MAPK pathways and was once synonymous with cell proliferation, although is now known to regulate other cellular responses (Dhillon *et al.*, 2007). At the cell membrane receptor, tyrosine kinases associate with small guanosine triphosphate proteins (GTPases) known as Ras (H, K and N-Ras). Once activated, these Ras GTPase mediate the tertiary Raf kinases, which in turn activates the secondary kinases MEK1/2 and subsequently the terminal kinase ERK1/2 (Little *et al.*, 2013; Fig. 3). The downstream effects of ERK pathway activation is the regulation of over 160 proteins, most of which are nuclear and alter gene expression (Yoon and Seger, 2006).

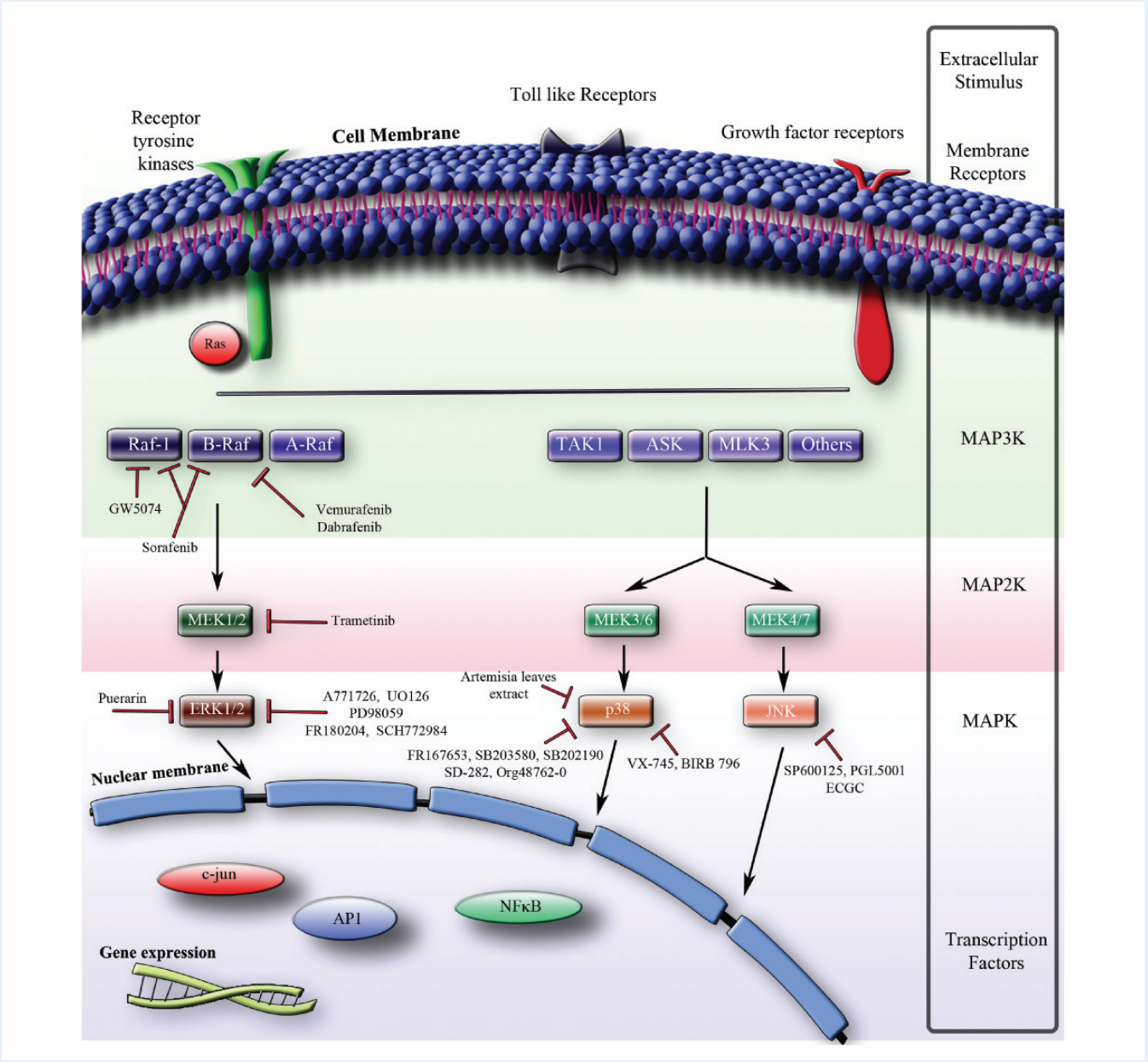

Figure 3 MAPK pathways and their inhibition in endometriosis. The MAPK pathways is a collection of signalling pathways organized in a three tier structure. Through a series of membrane receptors, including cytokine receptors, toll-like receptors and growth factor receptors, the MAPK pathways are stimulated by many components of the endometriotic microenvironment. These membrane receptors stimulate a series of MAP3K signalling molecules that transmit this signal to the secondary MAP2K kinases, followed by the MAPK kinases. The ERK1/2 pathway is predominantly activated upstream by the Raf kinases (Raf-1, B-Raf and A-Raf), which have become a significant target for pharmaceutical modulation. These kinases signal through MEK1/2 to activate ERK and initiate nuclear translocation. The p38 and JNK pathways share a number of common upstream molecules in the MAP3K level that include TAK1, ASK, MLK3 level but diverge at the secondary MAP2K level with MEK3/6 mediating p38 activation and MEK4/7 mediating JNK activation. Once activated all three MAPK translocate into the nucleus and bind to transcription factors. These pathways can be targeted at numerous levels and the pharmaceutical compounds that have been trialled in endometriosis are marked at their location of action.

The increased ERK activation in endometriotic tissue suggests that it may have a role in endometriosis pathogenesis. Increased phosphorylated ERK has been reported in primary eutopic epithelial cells (Yotova *et al.*, 2011; Matsuzaki and Darcha, 2015), as has a prolonged phosphorylation of ERK in endometrial stromal cells from women with endometriosis compared with women without endometriosis (Velarde *et al.*, 2009). Furthermore, in both epithelial and stromal cells *in vitro* there is a significantly increased phosphorylation of ERK in cells derived from endometriomas (Ngô *et al.*, 2010) and DIE (Leconte *et al.*, 2011) than in cells derived from normal endometrium. The factors that lead to a constitutive activation of ERK in endometriosis are not yet resolved, although one possibility that presents an attractive hypothesis is the reduction in the inactivating enzyme dual-specificity phosphatase (DUSP2) (Wu *et al.*, 2011), through a hypoxia induced expression of miRNA-20a in endometriotic tissue (Lin *et al.*, 2012).

The endometriotic microenvironment may stimulate increased ERK activity in ectopic cells. Both TNFα and IL-1β activate ERK and induce the expression of IL-8 and IL-6, although only IL-1β induced-IL-8 secretion and COX2 production could be attenuated by the ERK1/2-specific inhibitor PD98059 (Yoshino *et al.*, 2004). Another study, however, found that ERK inhibition had no effect on the IL-1β-mediated COX2 expression in endometriotic stromal cells, but that it was rather through p38 activation (Huang *et al.*, 2013). TGFβ-induced ERK activation through a Raf-dependent pathway has also been identified in endometrial epithelial and stromal cells (De La Garza *et al.*, 2012). The chemokine MCP1 also elicits a significant induction of PGE2 (Carli *et al.*, 2009) as well as VEGF, IL-8 and MCP-1 via an ERK-specific pathway in human endometriotic cells (Veillat *et al.*, 2010), and PGE2 in turn activates ERK in ectopic endometrial stromal cells (Sun *et al.*, 2003).

Oxidative stress may also contribute to ERK activation. H_2O_2 induces ERK phosphorylation in endometriotic stromal cells (Yoshino *et al.*, 2004) with a stronger induction compared with stromal cells from women without endometriosis (Andrade *et al.*, 2013). An increase in oxidative stress markers was observed in stromal and epithelial cells derived from women with endometriosis in a similar pattern to phosphorylated ERK levels, however, no direct relationship between oxidative stress and pERK activation was confirmed. Endocrine disruptors, such as diethylhexyl phthalate (DEHP) have also been linked with a possible pathogenesis of endometriosis through the induction of oxidative stress and stimulation of ERK activity (Cho *et al.*, 2015).

Estrogen also regulates ERK activation in endometriosis. Treatment with 17β-estradiol increases phosphorylated ERK expression in eutopic epithelial cells from women with and without endometriosis at similar rates between all cell types (Zhang *et al.*, 2010a). Treatment of ESC with E2 conjugated to bovine serum albumin (E2-BS) also increases phosphorylated ERK expression in a dose-dependent manner (Cheng *et al.*, 2012), indicating the effects are mediated at the cell membrane, as E2-BS cannot penetrate cells. This effect may also occur on immune cells with 17β-estradiol stimulating the release of MCP1 through activation of ERK in monocytes isolated from an endometriotic pelvic cavity (Lee *et al.*, 2012). Furthermore, in endometrial epithelial cells, TNFα-induced activation of ERs mediates an increase in ERK activation (Gori *et al.*, 2011).

The p38 pathway

Environmental stress stimuli including heat, osmotic shock and inflammatory cytokines influence the p38 MAPK pathway (Zarubin and Han, 2005). This diverse range of stimuli is indicative of the numerous tertiary level (MAP3K) kinases that participate in p38 activation (Fig. 3). These tertiary kinases include, but are not limited to TAK1 (Taniguchi *et al.*, 2009), ASK1, DLK/MUK/ZPK (Zarubin and Han, 2005). Many MAP3Ks stimulate both p38 and JNK, resulting in a convergence of the two pathways. Divergence of these two pathways occurs at the secondary kinase level with the activation of MEK3 and MEK6 kinases leading to the phosphorylation of p38 at a conserved amino acid sequence, threonine–glycine–tyrosine. Four isoforms of p38 have been characterized; α,β,γ,δ, of which p38α is the best characterized. Upon activation p38α translocates into the nucleus and activates nuclear transcription factors (Fig. 3).

At present, there is little data to confirm an over-activation of p38 in endometriotic cells. The endometriotic microenvironment, however, contains high concentrations of numerous molecules that activate this pathway, suggesting that constitutive activation in ectopic endometrial cells is possible. It has been suggested that in normal endometrium p38 activity is stronger in epithelial than in stromal cells (Seval *et al.*, 2006), although most of the current data has been collected in stromal cells. In endometriotic stromal cells, IL-1β, TNFα and H_2O_2 stimulate p38 phosphorylation, while its suppression attenuates IL-1β-induced IL-6, IL-8 (Yoshino *et al.*, 2004) and VEGF secretion (Huang *et al.*, 2013), as well as COX2 mRNA production (Yoshino *et al.*, 2004). MIF induces VEGF, IL-8 and MCP-1 secretion through p38 activation (Veillat *et al.*, 2010), as well as reduced COX2 expression, which may be specific to p38 (Carli *et al.*, 2009). In the immortalized *in vitro* epithelial model of peritoneal endometriotic cells (12Z), TNFα induces activation of p38 and concurrent treatment with specific inhibitors blocks IL-8, IL-6, MCP-1 and granulocyte macrophage colony-stimulating factor (GMCSF) secretion, as well as N-cadherin mRNA production (Grund *et al.*, 2008).

The activation of p38 may have a significant role in the regulation of non-endometriotic cells in the peritoneal microenvironment. MCP1 release from monocytes after treatments with peritoneal fluid is attenuated by a specific p38 inhibitor (Lee *et al.*, 2012), although this occurs equally in cells from women with and without endometriosis. IL-1β stimulates the thymic stromal lymphopoietin expression in Th2 cells by p38 inhibitors (Urata *et al.*, 2012). CCL20-induced Th17 cell recruitment to the peritoneal cavity of endometriotic women is regulated by p38 and other MAPK pathways (Hirata *et al.*, 2010). In a feed-forward mechanism, the Th17 cells in turn secrete IL-17 which induces IL-8 secretion through p38 and other MAP kinases pathways in endometriotic stromal cells (Hirata *et al.*, 2008). Lastly, p38 activation occurs in sensory nerve cells of the rostral–ventromedulla in a BALB/c mouse with surgically induced endometriosis (Chen *et al.*, 2015), suggesting a possible role for this pathway in inflammation-mediated endometriotic pain (McKinnon *et al.*, 2015).

Estrogen may also regulate p38 in endometriosis. Estradiol treatments of endometrial stromal cells increase p38 phosphorylation within two minutes and can be inhibited by ER antagonists (Seval *et al.*, 2006). 17β-estradiol stimulates p38 activation via ERβ in endometrial stromal cells (Chen *et al.*, 2014) and, in combination with the endocrine disruptor 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), influences macrophage polarization into an M2 phenotype, which is reduced by p38 inhibition, but not via JNK or ERK inhibition (Wang *et al.*, 2015). Another endocrine disruptor diethylhexyl phthalate increases the generation of ROS and decreases antioxidant enzymes through both ERK and p38 (Cho *et al.*, 2015), indicating the possible influence of environmental factors on this pathway.

Given the complexity of the microenvironment, it is not surprising that negative feedback loops exist to limit the influence of chronic inflammation and the p38 pathway may have a significant role in this negative regulation. Lipoxin A4 (LXA4) activates biochemical pathways necessary for the resolution of acute inflammation (Serhan *et al.*, 2008). LXA4 attenuates inflammation, angiogenic markers and estrogen metabolism as well as the endometriotic lesion itself in a surgically induced C57BL/6J mouse model (Kumar *et al.*, 2014) and importantly this effect of LXA4 is mediated through the p38 pathway in endometriotic stromal cells (Wu *et al.*, 2014). Sheddases also function in a feedback mechanism by cleaving receptors from the cell membrane. Sheddases activate MAPK pathways inducing A disintegrin and Metalloproteinases (ADAM)-10 and 17 that influence the receptor and ligand composition at the membrane, resulting in constitutive action of compensatory pathways, including p38 (Miller *et al.*, 2013).

The JNK pathway

Environmental stimuli for the JNK pathway include cytokines, growth factor deprivation, and G protein coupled receptors and stress signalling (Weston and Davis, 2002). In this pathway, the JNK protein represent the terminal (MAPK) kinase with up to 10 isoforms of JNK identified through alternative splicing of three different genes (*jnk1*, *jnk2* and *jnk3*). JNK can be activated upstream via the MEK4 and MEK7 kinases, which in turn are activated by several MAP3Ks that share similarities with those of p38, including TAK1 (Taniguchi *et al.*, 2013), MEKK1-4, MLL2/3, YTpl-2, DLK, TAO1/2 (Dhillon *et al.*, 2007). Stress signalling pathways feature a large number of MAP3K, reflecting the many possible molecules that can mediate a stress response. Once activated, the terminal kinase JNK translocates to the nucleus and activates transcription factors, of which c-Jun is a major target, enhancing AP-1 transcriptional activity (Adler *et al.*, 1992). JNK and NFκB often operate

in opposition, as anti-apoptotic effects of TNFα stimulation are mediated by NFκB-induced genes that suppress JNK activity (Javelaud and Besançon, 2001; Tang et al., 2002; Fig. 3).

Similar to p38, there is currently little data on whether there is an over-activation of the JNK pathway in endometriotic cells. Additionally, p38 and JNK share many activating molecules and upstream regulators. IL-17 (Hirota et al., 2005), IL-4 (OuYang et al., 2008) and IL-1β (Urata et al., 2012) mediate JNK phosphorylation, as does IL-1β, TNFα and H_2O_2 (Yoshino et al., 2004). One extracellular molecular that may be specific to JNK activation is indoleamine 2,3-dioxygenase-1 (IDO1), as treatment of endometrial stromal cells with this compound stimulates a phosphorylation of JNK, but not of ERK or p38, and is able to stimulate an increase in proliferation, p53 expression and COX2 and MMP9 production (Mei et al., 2013). Estrogen may also play a role, as stimulation of TSLP by estrogen induces JNK phosphorylation and the subsequent secretion of IL-8 and MCP-1 (Chang et al., 2014). It has also been confirmed from miRNA profiling that some miRNAs in endometriotic tissue interact with downstream targets of the JNK pathway, such as c-jun (Teague et al., 2010).

Targeting the MAPK pathways in endometriosis

Given the upstream convergence of the three MAPK pathways (ERK1/2, p38 and JNK) attempts have been made to target shared upstream mediators. Specific inhibitors of B-raf, vemurafenib and dabrafenib, have been approved for use in melanoma, however, significant side effects, including the development of cutaneous squamous-cell carcinomas, exist (Su et al., 2012). Similar side effects have also been observed for the MEK inhibitor trametinib (Menzies et al., 2015), although at a lower frequency than for dabrafenib. Raf-1 represents another upstream mediator of ERK activity and inhibition with GW5074 attenuated EM42 and primary stromal cells proliferation and invasion (De La Garza et al., 2012).

Sorafenib is a multi-kinase inhibitor which has activity on the MAPK pathway at both Raf-1 and B-RAF and also has activity on receptor tyrosine kinases VEGF receptor 1, 2 and 3, platelet-derived growth factor receptor β (PDGFR-β) and c-Kit (Wilhelm et al., 2004). A significant decrease in endometrial stromal cell proliferation, as well as a reduction in surgically induced endometriotic lesions in a heterologous nude mouse model, was observed with high concentration treatments of sorafenib (Leconte et al., 2015). Sorafenib has also been associated with numerous side effects, the most common of which include palmoplantar erythrodysesthesia which occurs in 76.3% of patients, diarrhoea (68.8%), alopecia (67.1%), rash (50.2%), fatigue (49.8%), weight loss (46.9%), hypertension (40.6%) and anorexia (31.9%) (Krajewska et al., 2015). In a phase III clinical trial on thyroid cancer patients, these side effects lead to dose interruptions, reductions and withdrawals in 66.2, 64.3 and 18.8% of patients, respectively, over a 28 day treatment cycle (Brose et al., 2014).

Additional teratogenic and embryogenic effects should also be considered with the MAPK targeting drugs. Vemurafenib can cross the placenta in rat models, although no tetragenic effects were observed (Grunewald and Jank, 2015). In humans, its use during pregnancy was documented in one patient who experienced fetal growth retardation during gestation with a subsequent recovery after birth (Maleka et al., 2013). Dabrafenib on the other hand has shown reproductive toxicity in rats and dogs (Grunewald and Jank, 2015). Data from clinical trials on reproduction, however, are limited due to ethical concerns and while animal studies have been performed, the significant variations between the reproductive systems of different animals make it difficult to draw effective conclusions from these studies.

It is possible that a reduced side effects profile may be achievable if further downstream targets with an over-activity in endometriotic cells are identified. At the tertiary kinase level several small molecular weight inhibitors have been specifically designed to target ERK, p38 or JNK. The inhibition of ERK in endometriosis-derived cells with A771726 (Leconte et al., 2011), UO126 (Matsuzaki and Darcha, 2015) and higher concentrations of PD98059 (Ngô et al., 2010) decreased cell proliferation. Some of these have reached the stage of animal and clinical trials for other chronic inflammatory conditions and may be worth investigating in endometriosis. FR180204 alleviates clinical arthritis and hypersensitivity elicited by an inflammatory reaction in collagen induced arthritis in a DBA/1 mouse model (Ohori et al., 2007) and SCH772984 has been successful in preclinical testing in cell lines that were BRAF and MEK inhibitor-resistant (Morris et al., 2013).

Small molecular weight inhibitors have also been developed for p38 and trialled both *in vitro* and in animal studies for use in endometriosis. The subcutaneous injection of 30 mg/kg FR167653 mediated a reduction in endometriotic lesion size and reduced both IL-6 and MCP-1 in the peritoneal fluid of BALB/c mice after a surgical transplantation of endometriotic lesions (Yoshino et al., 2006). SB203580 reduced IL-1β secretion and endometriotic lesion size in endometriotic stromal cells (Huang et al., 2013), as well as reducing TNFα, IL-1β, MMP3 and MMP9 mRNA and protein concentrations in cells isolated from the peritoneal cavity of an induced mouse model of endometriosis (Zhou et al., 2010). SB202190 attenuated cell proliferation of endometriotic stromal cells (OuYang et al., 2008). However, p38α inhibitors are plagued by liver toxicity that suggests specific on-target effects (Xu et al., 2008) that may significantly limit their potential use. Both VX-745 and BIRB 796 failed phase II clinical trials due to high liver toxicity (Dambach, 2005). The inhibition of p38α may also antagonize the JNK-c-jun pathway, as judged by a conditional deletion in mice (Hui et al., 2007).

The utility of targeting JNK in endometriosis is yet to be fully realized, as it is the least characterized pathway. SP600125 is a small molecular weight inhibitor developed to specifically target JNK (Bennett et al., 2001) and initial studies in both mouse models and in *in vitro* analysis of human synoviocytes, as a model of rheumatoid arthritis, it was capable of reducing the inflammatory response (Han et al., 2001). SP600125 also attenuated IL-1β induced inflammation in endometriotic stromal cells (Yoshino et al., 2004). The bentamapimod, PGL5001 is registered for a Phase IIa clinical trial in the treatment of endometriosis although there is very little publicly available information on the effectiveness of this compound *in vitro* (clinicaltrails.gov registry number; NCT01630252). However, similar to p38 inhibitors, it is possible that JNK inhibitors may be plagued by adverse effects as specific *jnk* mouse knockout models spontaneously develop intestinal tumours (Tong et al., 2007). Therefore, as long-term therapy is required to treat chronic inflammation, global inhibitors of JNK1 and p38α by orally applied kinase inhibitors at this stage appear unlikely candidates (Gaestel et al., 2009).

Finally, some naturally occurring substances interact with the MAPK pathways and may be beneficial for endometriosis treatment alone, or in combination. Puerarin, a phytoestrogen, was shown to inhibit E2-BSA mediated proliferation, although not as strongly as the ERK inhibitor UO126 (Cheng et al., 2012). EGCG from green tea had a moderate effect on JNK phosphorylation with a concomitant effect on VEGFC, which may mediate the angiogenic potential of endometriotic lesions (Xu et al., 2011). Artemisia leaves (APE) induced apoptosis of 12Z and 11Z endometriotic epithelial cells, which could be attenuated by the specific p38 inhibitor, SB203580 (Kim et al., 2013).

Summary

The MAPK pathways represent a series of pathways and interconnecting kinases that are influenced by the endometriotic microenvironment. The strongest evidence for constitutive activity in endometriotic tissue is available for ERK; however, this may simply be due to it being the most extensively studied. Importantly, all three pathways are influenced not only by inflammation, but also by oxidative stress and hormones. It is also possible that the MAPK pathways, and in particular JNK, have a significant role in the feedback mechanisms that limit the overexpression of other pathways activated in the endometriotic environment and thus combination targeting could be considered. Current strategies for targeting this pathway have focused on upstream

molecules, but appear associated with significant side effects that are not tolerable for endometriosis treatment. Downstream targeting of kinases that are dysregulated in endometriosis may reduce the adverse effects; however, for the p38 and JNK pathways liver toxicity and other side effects may represent a problem. Therefore in conclusion, while a dysregulation of this pathway in endometriotic microenvironment may occur, more specific targeting is required.

The PI3K/AKT/mTOR pathway in endometriosis

The PI3K/Akt/mTOR pathway regulates cell growth, proliferation, differentiation and apoptosis in response to both intra- and extracellular signals including nutrients, energy and oxygen levels, inflammation and growth factors (Hennessy *et al.*, 2005). mTOR exists as either the mTOR complex 1 (mTORC1) or complex 2 (mTORC2). In mTORC1, the most extensively studied complex, mTOR is bound to four additional proteins; regulatory-associated protein of mTOR (raptor), mammalian lethal with Sec13 protein 8 (mLST8), proline rich AKT substrate (PRAS40) and DEP-domain-containing mTOR interacting protein (Deptor) and represents an important nodal point in this pathway. Upstream, the most common mediator of mTOR activity is the membrane-bound phosphoinositol 3 kinase (PI3K), a membrane-bound phospholipid that together with AKT, forms the core of the PI3K/AKT/mTOR pathway (Fig. 4).

Stimulation of the PI3K/AKT/mTOR pathway begins once PI3K is activated leading to the phosphorylation of phosphatidylinositol-4,5-biphosphate (PIP2) to phosphatidylinositol-3,4,5 triphosphate (PIP3). Proteins with a pleckstrin homology domain, such as phosphoinositide-dependent kinase 1 (PDK1) and AKT are co-recruited to PIP3 and their subsequent proximity results in AKT phosphorylation by PDK1 (Cantley, 2002). Phosphatase and tensin homolog deleted on chromosome ten (PTEN) functions as a negative regulator of this reaction by dephosphorylating PIP3, back to PIP2. Once phosphorylated, AKT subsequently regulates downstream activation of mTOR via an interaction with tuberin sclerosis complex (TSC)2 (Manning, 2004). TSC2 exists as a heterodimer with TSC1 and this complex is a negative regulator of mTOR activity through their interaction with GTPase Ras homology enriched in brain (Rheb) (Li *et al.*, 2004). Downstream targets for mTOR are predominantly proteins involved in the translational machinery and ribosomal recruitment to mRNA (Hay and Sonenberg, 2004; Fig. 4).

Crosstalk with other kinases is common in the PI3K/AKT/mTOR pathway. IKKβ interacts with TSC2 and influences mTOR mediated protein synthesis (Lee *et al.*, 2007; Fig. 4) and conversely AKT can influence both IKKβ and phosphorylate the p65 subunit of NFκB (Nidai Ozes *et al.*, 1999; Sizemore *et al.*, 1999). Interactions are also possible between the PI3K and MAPK pathways. The upstream mediator of the MAPK pathways, Ras-GTP, can bind and activate PI3K (Rodriguez-Viciana *et al.*, 1994) and an ERK mediated phosphorylation of TSC2 also occurs (Roux *et al.*, 2004). Importantly, however, these phosphorylation sites are different to that mediated by AKT phosphorylation. An interaction between p38 and mTOR has also been reported with the downstream target of p38 activation MK2, phosphorylating TSC2 at serine 1210 altering mTOR activity (Li *et al.*, 2003).

mTOR maintains cellular viability by striking a balance between the anabolic and catabolic processes, such as protein synthesis and autophagy. Protein synthesis is regulated through the activation of the mTOR substrates S6K and 4EBP-1, which translate a subset of messenger RNAs that promote cell growth and proliferation in a phospho-specific manner. When 4E-BP1 is dephosphorylated, it sequesters the eIF-4F cap-binding protein and inhibits its assembly into the eIF-4F cap-binding complex attenuating cap-dependent translation (Pause *et al.*, 1994). S6K is also able to mediate protein translation through multiple substrates, such as S6K1aly/REF-like target (SKAR), programmed cell death 4 (PCD4), eukaryotic initiation factor 4B (eIF4B) and ribosomal protein S6 (Ma and Blenis, 2009). Under growth promoting conditions, the S6 protein, a component of the 40S ribosomal unit, is primarily responsible for stimulating high rates of protein synthesis (Gressner and Wool, 1974).

Autophagy is a catabolic process whereby the cell liberates intracellular stores of nutrients by degrading cytoplasmic proteins in lysosomes. During periods where nutrition and growth factors are in abundance, mTOR inhibits autophagy. If nutrients and growth factors are withdrawn or oxidative stress occurs, inhibition of mTOR allows autophagic process to increase, resulting in the production of amino acids that function as a feedback loop to again activate mTOR and attenuate the autophagic response (Yu *et al.*, 2010). Given the presence of oxidative stress in the endometriotic microenvironment, the potential for activation of mTOR mediated autophagy should be an important consideration.

At present, little is known about the function of the PI3K/AKT/mTOR pathway in endometriosis, although there is some evidence of a dysregulation. Mutations in the PTEN gene have been identified in 21% of endometriomas (Sato *et al.*, 2000). Phosphorylated AKT has been observed in ovarian endometriosis of post-menopausal women (Yagyu *et al.*, 2006), and increased pAKT is present in eutopic and ectopic endometrial cells of women with endometriosis, compared with those from women without (Cinar *et al.*, 2009). An increased pAKT has also been observed in stromal cells from endometrioma compared with cells from the endometrium of women without endometriosis (Yin *et al.*, 2012). The over-activation of AKT may also lead to decreased PR expression in endometriosis (Eaton *et al.*, 2013). Phosphorylated mTOR is increased in ectopic lesions compared with the eutopic endometrium of women with endometriosis (Guo *et al.*, 2015) and increased mRNA expression of both AKT1 and 4EBP1 has also been observed in the eutopic endometrium of women with endometriosis compared with women without endometriosis (Laudanski *et al.*, 2009).

As a key regulator of the nutrient and growth factor levels, mTORC1 also contributes to glucose homeostasis, the regulation of iron-free radicals and oxidative stress. Although much of this work is still in its infancy, some relationships have been identified. Inhibition of PI3K/mTOR reduces the GLUT1 membrane localization in lung adenocarcinoma (Makinoshima *et al.*, 2015) and in cervical cancer the inhibition of AKT/mTOR significantly inhibits GLUT1 and GLUT4 membrane transport (Rashmi *et al.*, 2014). We have previously shown an altered regulation of GLUT1 and GLUT4 receptors in ectopic tissue (McKinnon *et al.*, 2014) and it is therefore possible that this may be mediated through a dysregulation in the mTOR mechanism, although it is yet to be investigated in endometriosis. mTOR has also recently been implicated in iron homeostasis (Bayeva *et al.*, 2012; Guan and Wang, 2014) and the modulation of iron uptake through regulation of the transferrin receptor (Galvez *et al.*, 2007). A dysregulation of the mTOR pathway in ectopic tissue could provide a means for iron overload within the endometriotic cells and a stimulation of oxidative stress.

Over-activation of the mTOR pathway may also be a function of the microenvironment. IL-8 increases AKT phosphorylation and the induction of the anti-apoptotic Bcl-2 and survivin proteins (Li *et al.*, 2012) in endometriotic stromal cells. In the immortalized epithelial 12Z cell line, TNFα stimulates AKT phosphorylation that is inhibited by wortmannin, a PI3K-specific inhibitor (Grund *et al.*, 2008) and 17β-E2 decreases PTEN expression in both normal and endometriotic cells (Zhang *et al.*, 2010a). In endometrial tissue from normal women, the menstrual cycle progression induces an autophagic response that does not occur in endometriotic women (Choi *et al.*, 2014) and markers of autophagy are increased in ovarian endometriomas, as is the oxidative marker heme oxygenase 1 (Allavena *et al.*, 2015). Platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and fibroblast growth factor 2 (FGF2) all stimulate a phosphorylation of AKT and cell migration in endometrial stromal cells (Gentilini *et al.*, 2007). Furthermore, the hyper-proliferative phenotype observed in DIE lesions is associated with

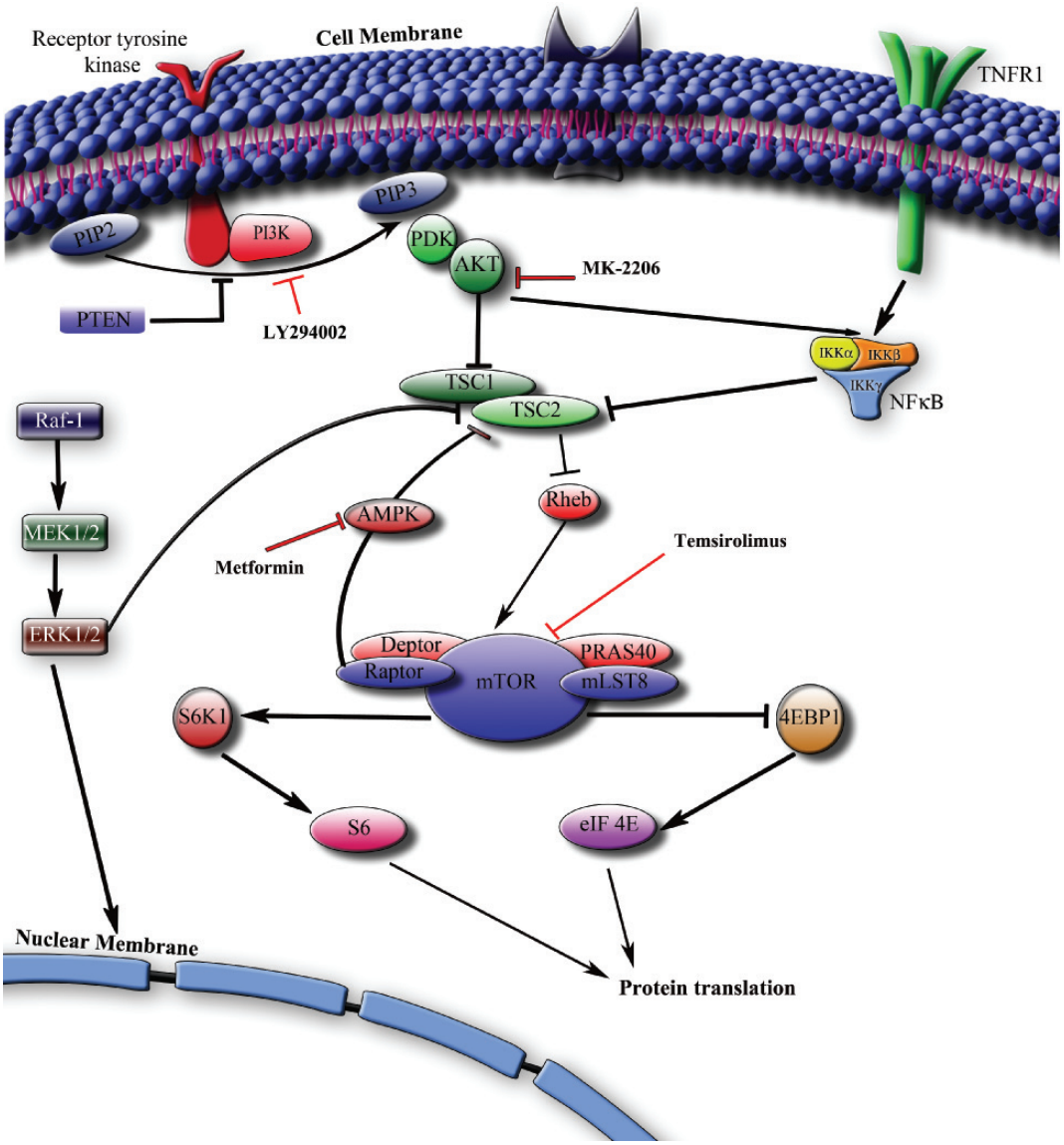

Figure 4 The PI3K/AKT/mTOR signalling pathway and its inhibition in endometriosis. The mTOR pathway is activated by multiple extracellular stimuli through numerous cell membrane receptors, including receptor tyrosine kinases and cytokine receptors. Binding to these receptors stimulates PI3K to mediate the phosphorylation of PIP2 to PIP3, leading to an association between PDK and AKT. PTEN serves as an inhibitory protein in this reaction. The physical proximity between PDK and AKT leads to the phosphorylation of AKT and subsequent inhibition of TSC1. TSC1 exists as a heterodimer with TSC2 and through the Rheb GTPase has an inhibitory function against mTORC1, which exits in a complex with four additional proteins bound to mTOR, including Deptor, Raptor, PRAS40 and mLST8. Activation of mTORC1 leads to the activation of S6K1 and the downstream ribosomal S6 protein, as well as the inhibition of 4EBP1 that subsequently stimulates eIF4E and cap-dependent translation of mRNA and the translation of selected proteins. The mTOR pathway interacts with both the NFκB pathway and the ERK1/2 MAPK pathway through an interaction with TSC2. A negative feedback loop also via AMPK also connects mTOR with TSC2. Numerous pharmaceutical compounds modulate mTOR activity at different locations some of which have been trialled in endometriosis.

increased levels of endogenous oxidative stress and activation of the mTOR/AKT pathway (Leconte *et al.*, 2011).

Targeting the PI3K/AKT/mTOR pathway in endometriosis

In vitro evidence indicates that disrupting the PI3K/mTOR pathway reduces the proliferation of endometriotic epithelial and stromal cells. Estrogen down-regulates nometastatic gene 23-H1 (NME1) expression, which mediates a subsequent elevation in expression of PCNA, survivin and integrin (Li *et al.*, 2013) as well as VEGF and IL-8 (Chang *et al.*, 2013), all of which could be attenuated by LY294002, a specific PI3K inhibitor. Temsirolimus, a specific mTOR inhibitor, blocked proliferation of endometriotic cell proliferation *in vitro* and in a heterologous nude mouse model (Leconte *et al.*, 2011). The inhibition of AKT phosphorylation by MK-2206 in stromal cells reduced the levels of a target protein p(S256)-forkhead box O1 and decreased the viability of cells from women both with and without endometriosis (Kim *et al.*, 2014) (Fig. 4).

Some compounds already in use also exert off-target effects on mTOR pathway regulation. Metformin, an oral anti-diabetic drug (Stumvoll *et al.*, 1995) activates 5′ adenosine monophosphate-activated protein kinase (AMPK), mediating the drug's effects in muscle, adipose, liver (Zhou *et al.*, 2001) and breast cancer cells (Zakikhani *et al.*, 2006). AMPK is a negative upstream regulator of TSC2, which exerts inhibitory effects on mTORC1 (Inoki *et al.*, 2003). A recent clinical study on metformin in endometriosis found a significant reduction in the symptomatic cases, increased chance of pregnancy, and a decrease in the levels of serum cytokines, suggesting an anti-endometriotic potential (Foda and Aal, 2012). Other studies had previously documented this treatment effect in rat models (Oner *et al.*, 2010; Yilmaz *et al.*, 2010).

A principle drawback of targeting the mTOR pathway is that the substantial crosstalk, as well as critical roles performed by this pathway increases the likelihood of unwanted side effects. The mTOR inhibitor temsirolimus, which has shown promise in reducing endometriotic lesions in *in vitro* and animal models (Leconte *et al.*, 2011), is currently approved for treatment of renal cell carcinoma and through this use, the class-specific toxicities of these drugs are emerging. Adverse effects commonly include an impact on the haematological, pulmonary and dermatological systems (Hutson *et al.*, 2008; Eisen *et al.*, 2012) and while these can be unpleasant they can be medically managed with close patient monitoring and early intervention with a return to normal after cessation of therapy (Bellmunt *et al.*, 2008). However, the immunosuppressive effects of temsirolimus have also been linked to an increase in infection of cancer patients (Kaymakcalan *et al.*, 2013) that one study linked to an increase in fatal adverse effects (Choueiri *et al.*, 2013). Similar to MAPK inhibitors, there is a suggestion that this class of drugs may be teratogenic, although limited evidence has been obtained due to ethical concerns. Whether these adverse effects and the need for their medical management have a sufficiently limited impact to warrant the use of temsirolimus in a non-life threatening condition, such as endometriosis, will need be carefully considered against the symptomology of the patient, the technical difficulty of surgical removal of the endometriotic lesion and the patients' response to traditional therapies (Table I).

Summary

The mTOR pathway plays a significant role in integrating signals from the extracellular environment into cell viability and proliferation and a number of kinases within this pathway may be over-active in endometriotic cells. This pathway therefore represents a potential treatment option for endometriosis. At present, however, even though there are numerous compounds that modulate this pathway, only a few of these have been trialled in endometriosis. While unwanted side effects still occur, the majority of these are non-life threatening, medically manageable and dissipate after cessation of treatment, particularly for temsirolimus. Therefore, although at present there are no clinical trials currently underway, they may have significant potential if their class-specific toxicities can be better delineated.

Conclusion

Endometriosis treatment represents a complex clinical challenge and new therapies are needed. The peritoneal environment of endometriotic women is significantly altered which can lead to an over-activation of kinase signalling pathways in endometriotic tissue. In this manuscript, we reviewed three pathways: NFκB, MAPK and PI3K/AKT/mTOR in endometriotic cells. Increased activity of the NFκB pathway in endometriotic cells and *in vitro* and animal data supports its potential as a target. Less data were available on the MAPK pathway activation, although targeting ERK may have potential. Similarly, the PI3K/AKT/mTOR pathway also displays promising *in vitro* results in an endometriosis models. There is therefore the potential for targeting these and perhaps other pathways in endometriosis if current limitations and challenges can be overcome.

Limitations and challenges

Although an increase in the activity of many kinases in endometriotic cells has been identified, a specific kinase dependency for endometriotic lesions, through an activating genetic mutation is yet to be confirmed. It is possible, however, that a kinase dependency may stem from the extracellular environment. Kinase-dependent tumours without activating mutations, but with an overexpression of kinase ligands have previously been identified (Simon *et al.*, 1997; Shimizu *et al.*, 1999), as has the influence of the extracellular environment on the clinical efficacy of kinases targeting drugs (Jänne *et al.*, 2009). Identifying the kinase dependency for endometriosis will be key to creating an effective kinase inhibiting therapeutic.

A lack of a specific, single kinase dependency may also present challenges in regards to acquired drug resistance. Tumour cells are adept at creating drug resistance by inducing mutations in other kinase signalling pathways when challenged (Zhang *et al.*, 2009). The ability of the extracellular environment to stimulate multiple signalling pathways could mean the extracellular environment has multiple possibilities to mediate tumour growth and that targeting a specific kinase will result in the over-activation of a compensatory pathway. Therefore, to successfully treat endometriosis through inhibition of these pathways, more information on kinase activation, the extracellular environment in endometriosis and the effects of interrupting this interaction is needed.

Management of the associated toxicity profiles is the most immediate challenge presented by the use of these drugs with both on-target and off-target effects responsible for their toxicity. Off-targets effects are inherent to the high degree of conservation of the ATP biding sites across the human kinome, whereas the on-target effects are due to the central role these kinases play and are cell specific. The off-target effects may be addressed by drug design strategies and improved binding site specificities in next generation kinase inhibitors. Careful selection of dosage is also critical. The specificity of kinase inhibitors decreases as concentrations increase and there is little justification for concentrations above those required for maximal inhibition of the specific target, a consideration that should also be important during both *in vitro* and clinical studies. On-target effects present a more significant problem and will need to be assessed from a disease-specific point of view and thus more studies in endometriosis-specific models are needed.

Future directions

While the adverse effects associated with these drugs limits their usefulness in endometriosis at present, well-designed clinical strategies could open the door to their clinical use in the future. As recently proposed by Santulli *et al.* (2015a, b) for MAPK inhibitors, the current generation of drugs could find a use in more severe cases of symptomatic DIE lesions (Santulli *et al.*, 2015b). These lesions have extracellular environments that predispose them to increase kinase activation are more likely resistant to hormonal modulation and represent complicated surgical procedures. If proven to be cytoreductive, these drugs could be used for short-term treatment prior to surgery to reduce the size and depth of a lesion. Furthermore, women with strong symptoms may also be more willing to tolerate the adverse effects short-term. An important consideration, however, is the potential embryotoxic and

Table I Pharmaceutical compounds that interact with kinase signalling pathways and trialled in endometriosis treatment.

Signalling pathway	Compound	Iupac name	Target	Function	Reference	Functional target validation
NFκB	BAY 11-7085	(E)-3-(4-tert-butylphenyl)sulfonylprop-2-enenitrile[b]	IκB	Inhibits IκB phosphorylation	Pierce *et al.* (1997)	Endometriotic stromal cells
				Decreases cell proliferation and DNA synthesis. Induces apoptosis	Nasu *et al.* (2007)	
				Decreases lesion size Increases apoptotic markers.	González-Ramos *et al.* (2008)	Heterologous nude mouse model
	BORTEZOMIB	Mannitol boronic ester, [(1R)-3-methyl-1-[[(2S)-3-phenyl-2-(pyrazine-2-carbonylamino)propanoyl]amino]butyl]boronic acid[b]	Proteasome	Reduces endometriotic lesion size Decreases PCNA and Ki67 expression	Celik *et al.* (2008)	Transplanted endometriosis in Wistar Rat
	TPCK	*N*-Tosyl-L-Phenylalanine Chloromethyl ketone, *N*-[(2S)-4-chloro-3-oxo-1-phenylbutan-2-yl]-4-methylbenzenesulfonamide[b]	NFκB	Anti-NFκB activity	Yamauchi *et al.* (2004)	Endometrioma stromal cells
	PDTC	Pyrrolidine dithiocarbamate, 2-acetamido-3-sulfanylpropanoic acid;pyrrolidine-1-carbodithioic acid[b]	IκB	IκB-ubiquitin ligase	Hayakawa *et al.* (2003)	Jurkat T-cells
			NA	Decreases inflammation, angiogenic factors and MMPs	Zhang *et al.* (2010a, b)	Endometriotic stromal cells
					Zhang *et al.* (2011)	Endometriotic epithelial cells
			NA	Reduces in lesion size	Celik *et al.* (2008)	Transplanted endometriosis in Wistar Rats
	THALIDOMIDE	α-Phthalimido glutarimide, 2-(2,6-dioxopiperidin-3-yl)isoindole-1,3-dione[b]	IκB	Suppression of IκB degradation	Majumdar *et al.* (2002)	NA
			NFκB	Inhibits TNFα-stimulated IL-8	Yagyu *et al.* (2005)	Endometriotic stromal
			NA	Reduces endometrial implants	Azimirad *et al.* (2014)	Autologous endometrial implant in Sprague-Dawley rat
	THIAZOLIDINEDIONES	1,3-Thiazolidine-2,4-dione[b]	PPARγ	Reduces endometriotic lesion size	Lebovic *et al.* (2004)	Autologous endometrial implant in Sprague-Dawley rat
					Lebovic *et al.* (2007)	Primates
	CELECOXIB (NSAID)	4-[5-(4-Methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide[b]	COX-2	Interacts with NFκB	Park *et al.* (2014)	Leiomyoma cells
				Decreases cellular proliferation	Olivares *et al.* (2008)	Endometrial epithelial cells
	SULINDAC (NSAID)	2-[(3Z)-6-fluoro-2-methyl-3-[(4-methylsulfinylphenyl)methylidene]inden-1-yl]acetic acid[b]	NA	Decreases RANTES through NFκB mechanism	Wieser *et al.* (2005), Efstathiou *et al.* (2005)	Normal and endometriotic stromal cells
						C57BL/6J mice
	RESVERATROL[a]	3,5,4′-Trihydroxy-trans-stilbene, 5-[(E)-2-(4-hydroxyphenyl)ethenyl]benzene-1,3-diol[b]	NA	Reduces surgically induced endometriotic lesions	Bruner-Tran *et al.* (2011)	Nude (NCr) mice

Continued

Table I *Continued*

Signalling pathway	Compound	Iupac name	Target	Function	Reference	Functional target validation
				Reduces cell proliferation Increases apoptosis	Ricci *et al.* (2013), Rudzitis-Auth *et al.* (2013)	Endometrial epithelial cells
				Reduces cytokine concentrations	Ergenoğlu *et al.* (2013), Ozcan Cenksoy *et al.* (2015), Bayoglu Tekin *et al.* (2015)	Peritonal from Rat models
	EGCG[a]	Epigallocatechin-3-gallate, [(2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromen-3-yl] 3,4,5-trihydroxybenzoate[b]	NA	Interacts with NFκB Reduces surgically induced endometriotic lesions	Khan *et al.* (2006) Ricci *et al.* (2013)	NA mice
	PARTHENOLIDE[a]	(1a*R*,7a*S*,10a*S*,10b*S*)-1a,5-dimethyl-8-methylene-2,3,6,7,7a,8,10a,10b-octahydrooxireno[9,10]cyclodeca[1,2-*b*]furan-9(1a*H*)-one	NA	Inhibits NFκB activity Reduces the inflammatory response	Kwok *et al.* (2001) Takai *et al.* (2013)	NA Endometriotic stromal cells BALB/c mice
	CURCUMIN[a]	(1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione[b]	NA	Attenuates cytokine secretion and inflammation	Veillat *et al.* (2009), Kim *et al.* (2012), Jana *et al.* (2012)	Endometriotic stromal cells BALB/c mice
	IL-10	Interleukin 10	DNA binding	Attenuate cytokine secretion	Lentsch *et al.* (1997), Tagashira *et al.* (2009)	Endometriotic stromal cells
	Decoy Nucleotides	Nucleotide sequences Forward; 5′-CCTTGAAGGGATTTC CCTCC-3′ Reverse; 3′-GGAACTTCCCTAAAGGGAGG-5′	DNA binding	Attenuate inflammation	Xiu-li *et al.* (2009)	Endometriotic stromal cells
MAPK	VEMURAFENIB	*N*-[3-[5-(4-chlorophenyl)-1H-pyrrolo[2,3-b]pyridine-3-carbonyl]-2,4-difluorophenyl]propane-1-sulfonamide[b]	B-raf	NA	Su *et al.* (2012)	Approved for use in melanoma
	DABRAFENIB	*N*-[3-[5-(2-aminopyrimidin-4-yl)-2-tert-butyl-1,3-thiazol-4-yl]-2-fluorophenyl]-2,6-difluorobenzenesulfonamide[b]				
	TRAMETINIB	*N*-(3-{3-Cyclopropyl-5-[(2-fluoro-4-iodophenyl)amino]-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydropyrido[4,3-d]pyrimidin-1(2H)-yl}phenyl)acetamide[b]	MEK	NA	Menzies *et al.* (2015)	NA
	Sorafenib	4-[4-[[4-Chloro-3-(trifluoromethyl)phenyl]carbamoylamino]phenoxy]-*N*-methylpyridine-2-carboxamide	Raf-1, B-Raf, VEGFR1, 2, 3, PDGFR-β, c-KIT	Inhibits cellular proliferation. Decreases lesion size	Leconte *et al.* (2015)	Endometrial stromal cells Nude mouse model
	GW5074	(3Z)-3-[(3,5-dibromo-4-hydroxyphenyl)methylidene]-5-iodo-1H-indol-2-one[b]	Raf-1	Inhibits cell proliferation and invasion	De La Garza *et al.* (2012)	Epithelial EM42 cells and primary stromal cells
	A771726	(Z)-2-cyano-3-hydroxy-N-[4-(trifluoromethyl)phenyl]but-2-enamide[b]	ERK	Decrease cell proliferation	Leconte *et al.* (2011)	Epithelial and stromal cells from eutopic and ectopic lesions
	UO126	(2Z,3Z)-2,3-bis[amino-(2-aminophenyl)sulfanylmethylidene]butanedinitrile[b]			Matsuzaki and Darcha (2015)	
	PD98059	2-(2-Amino-3-methoxyphenyl)-4H-chromen-4-one[b]			Ngó *et al.* (2010)	
	FR180204	5-(2-Phenylpyrazolo[1,5-a]pyridin-3-yl)-2H-pyrazolo[3,4-c]pyridazin-3-amine[b]		Alleviate clinical arthritis	Ohori *et al.* (2007)	DBA/1 mouse model

	SCH772984	(3R)-1-[2-oxo-2-[4-(4-pyrimidin-2-ylphenyl)piperazin-1-yl]ethyl]-N-(3-pyridin-4-yl-1H-indazol-5-yl)pyrrolidine-3-carboxamide[b]		NA	Morris *et al.* (2013)	Preclinical testing in BRAF and MEK inhibitor-resistant cell lines
	FR167653	1-[7-(4-Fluorophenyl)-1,2,3,4-tetrahydro-8-(4-pyridyl)pyrazolo[5,1-c][1,2,4]triazin-2-yl]-2-phenylethanedione sulphate monohydrate	p38	Reduces endometriotic lesion size. Reduces peritoneal fluid IL-6 and MCP-1	Yoshino *et al.* (2006)	BALB/c mice
	SB203580	4-[4-(4-Fluorophenyl)-2-(4-methylsulfinylphenyl)-1H-imidazol-5-yl]pyridine[b]		Reduces IL-1β secretion and endometriotic lesion size	Huang *et al.* (2013)	Endometriotic stromal cells
				Reducing TNFα, IL-1β, MMP3 and MMP9 mRNA and protein concentrations	Zhou *et al.* (2010)	BALB/c mice; cells isolated from peritoneal cavity
	SB202190	4-[4-(4-Fluorophenyl)-5-pyridin-4-yl-1,3-dihydroimidazol-2-ylidene]cyclohexa-2,5-dien-1-one[b]		Attenuates cell proliferation	OuYang *et al.* (2008)	Endometriotic stromal cells
	VX-745	5-(2,6-Dichlorophenyl)-2-(2,4-difluorophenyl)sulfanylpyrimido[1,6-b]pyridazin-6-one[b]		NA	Dambach (2005)	Failed phase II clinical trials due to high liver toxicity
	BIRB 796	Doramapimod, 1-[5-tert-butyl-2-(4-methylphenyl)pyrazol-3-yl]-3-[4-(2-morpholin-4-ylethoxy)naphthalen-1-yl]urea[b]				
	SP600125	1,9-Pyrazoloanthrone, Dibenzo[cd,g]indazol-6(2H)-one	JNK	Reducing the inflammatory response	Han *et al.* (2001)	Mouse models and human synoviocytes as a model of rheumatoid arthritis
					Yoshino *et al.* (2004)	Endometriotic stromal cells
	PGL5001	Doramapimod		NA	Clinicaltrails.gov NCT01630252	Phase IIa clinical trial in the treatment of endometriosis
	Puerarin[a]	7-Hydroxy-3-(4-hydroxyphenyl)-8-[(2S,3R,4R,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]chromen-4-one[b]	NA	Inhibits E2-BSA mediated proliferation	Cheng *et al.* (2012)	Endometriotic stromal cells
	ECGC[a]	Epigallocatechin-3-gallate, [(2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromen-3-yl] 3,4,5-trihydroxybenzoate[b]	JNK	Moderate effect on JNK phosphorylationand VEGFC	Xu *et al.* (2011)	Heterologous mouse models
	Artemisia leaves[a]	NA	NA	Induces apoptosis	Kim *et al.* (2013)	12Z and 11Z epithelial cells
PI3K/AKT/mTOR	LY294002	2-Morpholin-4-yl-8-phenylchromen-4-one[b]	PI3K	Decreases PCNA, surviving, integrin, VEGF and IL-8 expression	Li *et al.* (2013), Chang *et al.* (2013)	Endometrial stromal cells
	TEMSIROLIMUS	(1*R*,2*R*,4*S*)-4-{(2*R*)-2-[(3*S*,6*R*,7*E*,9*R*,10*R*,12*R*,14*S*,15*E*,17*E*,19*E*,21*S*,23*S*,26*R*,27*R*,34a*S*)-9,27-dihydroxy-10,21-dimethoxy-6,8,12,14,20,26-hexamethyl-1,5,11,28,29-pentaoxo-1,4,5,6,9,10,11,12,13,14,21,22,23,24,25,26,27,28,29,31,32,33,34,34a-tetracosahydro-3H-23,27-epoxypyrido[2,1-c][1,4]oxazacyclohentriacontin-3-yl]propyl}-2-methoxycyclohexyl 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate[b]	mTOR	Inhibits proliferation	Leconte *et al.* (2011)	Heterologous nude mouse model
						Endometriotic cells *in vitro*
					Behbakht *et al.* (2011)	Phase II trial in ovarian cancer patients

Continued

Table I *Continued*

Signalling pathway	Compound	Iupac name	Target	Function	Reference	Functional target validation
	MK-2206	8-[4-(1-Aminocyclobutyl)phenyl]-9-phenyl-2H-[1,2,4]triazolo[3,4-f][1,6]naphthyridin-3-one[b]	AKT	Reduces FOXO1 protein. Decreases cell viability	Kim *et al.* (2014)	Endometriosis and endometrial stromal cells
	METFORMIN	3-(Diaminomethylidene)-1,1-dimethylguanidine[b]	NA	Activates AMPK kinase	Zhou *et al.* (2001)	Muscle, adipose, liver
					Zakikhani *et al.* (2006)	Breast cancer cells
				Increased chances of pregnancy. Decreases serum cytokines	Foda and Aal (2012)	Endometriosis patients
				NA	Oner *et al.* (2010), Yilmaz *et al.* (2010)	Wistar rat model

[a]Natural compounds.
[b]IUPAC name.

teratogenic effects of these compounds and thus treatments should be performed in combination with contraception allowing for at least 6 months post therapy wash-out. It should also be noted that due to the influence on CYP3A4-mediated metabolism, the plasma concentrations of hormonal contraceptives could vary and caution on their effectiveness during this period should be considered.

Future treatment strategies for kinase inhibitors could also incorporate the heterogeneity of endometriosis and target-specific kinases based on individual patient profiles. Through the use of robust and reproducible genome wide association studies, the genetic basis of endometriosis is increasingly being elucidated (Rahmioglu *et al.*, 2014) as are the peripheral changes and the extracellular environment that influence the disease progression and symptomology (Morotti *et al.*, 2014; McKinnon *et al.*, 2015). A better understanding of their biochemical basis and inflammatory profiles of endometriotic subtypes and the contribution of specific kinase pathways to individual endometriotic lesions may soon provide more information on the kinase dependency of specific lesions and the opportunity for personalized treatment.

Endometriosis research is gradually advancing the understanding of the disease pathogenesis; the task now is to translate these discoveries into novel therapeutics. An over-activation of kinases in endometriotic tissue has been observed and thus the targeting of kinase signalling pathways represents a valid treatment option. In the near future these drugs may find applications for short-term use in more severe cases, but at present more information is needed on the dysregulation of these pathways in endometriotic tissue. Looking further ahead the outlook is promising, early studies suggest these drugs can be cytoreductive and the development of new kinase inhibitors is increasing and thus so is the likelihood of improvements in their specificity and side effects profiles. A reduction in the adverse effects, combined with more knowledge on which patients to match to particular drugs through an understanding of endometriosis heterogeneity and kinase dependency could make them tolerable and efficacious for endometriosis patients.

Authors' roles

B.D.M. conceived, designed and prepared the manuscript and figures. V.K. contributed to section about biological bases of signalling pathways. K.N. contributed to clinical and treatment sections. N.A.B. contributed to section on the endometriotic microenvironment. M.D.M contributed to the concept, intellectual content and revision the manuscript.

Funding

This review was supported by the Swiss National Science Foundation (320030_140774).

Conflict of interest

All authors declare there are no conflicts of interest.

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