REVIEW

An update of Wnt signalling in endometrial cancer and its potential as a therapeutic target

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Abstract

Endometrial cancer is the most common gynaecological malignancy in developed nations, and its prevalence is rising as women defer or decide not to have children and as obesity rises, both key risk factors. Despite this, treatment options remain limited, particularly for advanced or refractory disease. New genomic analyses have revealed distinct mutational profiles with therapeutic and prognostic potential. Wnt signalling, which is pivotal in embryogenesis, healing and homeostasis, is of importance in the endometrium and has been linked to carcinogenesis. This review aims to update and discuss the current evidence for the role of β -catenin dependent and independent Wnt signalling, including the ROR receptors in the endometrium and its potential as a therapeutic target, in light of recent trials of Wnt-targeted therapy in multiple tumour types.

Key Words

- ▶ endometrial cancer
- ▶ uterine cancer
- ▶ Wnt signalling

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Introduction

Endometrial cancer is the most common gynaecological malignancy in the developed world, with an estimated 320,000 new cases diagnosed globally each year. It is the fourth most prevalent cancer after neoplasms of the breast, colorectum and lung among women in the West, and incidence is rising due to a combination of increased awareness and detection, population ageing and acceleration in risk factors including obesity and nulliparity (Ferlay *et al.* 2015). Endometrial cancer is also increasingly fatal, with mortality rising by 2.1% annually between 2008 and 2014 in the United States, principally attributed to the growing obesity burden. Worldwide, the mortality rate remains in excess of 20% (Ferlay *et al.* 2015).

A number of aetiological factors can increase the risk of endometrial cancer, including age, parity, oral contraceptive pill use, age at menarche and type two diabetes mellitus (Brinton *et al.* 2013). Prolonged exposure

to unopposed oestrogens is the single most important risk factor for developing endometrial cancer, particularly type one tumours (described below), and polycystic ovarian syndrome, early menarche and late menopause, anovulatory or erratic periods, use of oestrogen-only hormone replacement therapy, oral contraceptive pills or tamoxifen and nulliparity are all implicated (Grady *et al.* 1995, McPherson *et al.* 1996, Allen *et al.* 2008, Group *et al.* 2014a).

A number of previous reviews have discussed signalling pathways involved in homeostasis of the endometrium and various pathologies, and two recent reviews have examined Wnt inhibitors in pre-clinical settings but with limited discussion of implications for endometrial cancer (Eritja *et al.* 2017, Katoh & Katoh 2017). Previous reviews have focused on β -catenin-dependent Wnt signalling with no discussion of the β -catenin-independent receptors

ROR1 and ROR2 (Wang *et al.* 2009, Markowska *et al.* 2014, Kiewisz *et al.* 2015, Eritja *et al.* 2017, Katoh & Katoh 2017). Therefore, here we provide an updated discussion on possible avenues and challenges for targeted Wnt treatment in molecular and histological subtypes of endometrial cancer.

Classification

Historically, endometrial cancer was classified into two distinct subtypes known as Bokhman type one and type two (Bokhman 1983). Type one tumours originally included the endometrioid histological subtype of all grades, a class typically driven by obesity and related hormonal imbalances. These tumours account for approximately 90% of all endometrial cancers and are usually diagnosed early with good prognosis. Type two tumours included other non-endometrioid histological subtypes such as serous and clear cell and have propensity for myometrial invasion, metastasis and recurrence.

More recently, The Cancer Genome Atlas (TCGA) and others have integrated genomic, transcriptomic and proteomic analyses on large tumour cohorts to identify new molecular classification signatures with prognostic and therapeutic utility (Byron *et al.* 2012, McConechy *et al.* 2012, Talhouk *et al.* 2015). The TCGA consortium identified four discrete subtypes of endometrial cancer based on mutation profile and major genetic alterations: POLE, Microsatellite Instability (MSI), Copy Number Low (CN Low) and Copy Number High (CN High), as detailed in Table 1. The Bokhman classification does not fully align with these molecular subtypes. Underscoring the limitations of the dual classification model, the TCGA identified aggressive markers in some histologically indolent lesions (type one) or a favourable

genomic profile in other tumours assessed as having a poor outlook (type two) (Levine & The Cancer Genome Atlas Research Network 2013). One in four high-grade endometrioid endometrial cancers examined by the TCGA were reclassified from Bokhman type one to type two cancers on molecular analysis (McConechy *et al.* 2012, Church *et al.* 2013, Meng *et al.* 2014, Murali *et al.* 2014, Hussein *et al.* 2015, Talhouk *et al.* 2015). Tumour-infiltrating lymphocytes were found to be a hallmark of the POLE and MSI molecular subgroups, making them a potentially attractive target for immunotherapies, while a subset of endometrioid tumours with serous-like copy number alterations may benefit from chemotherapy rather than radiotherapy (Levine & The Cancer Genome Atlas Research Network 2013).

Genomic tumour analysis has also revealed novel avenues for therapy, targeting common pathways across organ sites. For example, serous endometrial carcinoma shares features with serous ovarian cancer and triple-negative breast cancer, while clear cell ovarian tumours resemble clear cell lesions of the endometrium, suggesting common therapeutic targets (Fadare *et al.* 2013, Levine & The Cancer Genome Atlas Research Network 2013, Hoang *et al.* 2014). By contrast, while endometrioid endometrial carcinomas appear to have a distinct mutation profile compared with endometrioid ovarian tumours, they share genetic features with colorectal carcinomas (McConechy *et al.* 2012, Levine & The Cancer Genome Atlas Research Network 2013).

Treatment

Though considered a relatively good prognosis cancer, with 5-year survival of up to 90% for early stage, low-grade tumours, as many as 30% of all endometrial cases

Table 1 Molecular subtypes of endometrial cancer identified by The Cancer Genome Atlas Research Group.

Group	Mutation rate/Mb	Somatic copy number alterations	Histology	Bokhman type	Major genetic alterations	Prognosis
POLE	200 × 10 ⁶ (ultra)	Negligible	Endometrioid, high grade	T1	Similar to CN low (cellular metabolism) but with p53 mutations	Good
MSI	20 × 10 ⁶ (hyper)	+	Endometrioid, heterogeneous	T1	MSI (MLH1/Lynch)	Intermediate
MSS/CN low	2 × 10 ⁶	++	Endometrioid, low grade	T1	PTEN/PI3K-Akt, KRAS, ARID1a, CTNNB1++ (52%)	Intermediate
CN high	2 × 10 ⁶	+++	Serous, clear cell, 20% high-grade endometrioid	T2 (and T1)	P53 (cell cycle deregulation)	Poor

CN, copy number; MSI, microsatellite instability; MSS, microsatellite stable; POLE, polymerase epsilon.

are detected at regional or distant stages, and survival drops off sharply with advancing stage. Prognosis also varies significantly between histological subtypes, with endometrial cancer increasingly understood as representing a heterogeneous spectrum of diseases with divergent, and sometimes overlapping, mechanisms and therapeutic opportunities (Dellinger *et al.* 2012, AlHilli *et al.* 2014, Bakkum-Gamez *et al.* 2014, Rutgers 2015, de Haydu *et al.* 2016, Mota *et al.* 2017). Despite this, there have been few changes to the dominant treatment paradigm in recent decades. Recurrence and resistance remain significant challenges, and little is understood about who should receive particular kinds of treatment or how they will respond based on disease profile.

At present, surgery is the mainstay of management – typically consisting of hysterectomy and bilateral salpingo-oophorectomy, with lymph node dissection depending on the extent of disease (Amant *et al.* 2005, Group *et al.* 2014a,b). Critically, there are few treatment options for advanced or refractory lesions, with 5-year survival of just 19% in the context of distant metastases (Obel *et al.* 2006, Dellinger & Monk 2009, Bradford *et al.* 2015). In addition, there is presently no reliable way to differentiate hyperplasia from carcinoma prior to hysterectomy.

Targeted therapies and prognostic markers are lacking for women with endometrial cancer, particularly those with advanced disease. The advent of personalised medicine seen in lung, breast, gastrointestinal and haematological malignancies is yet to reach gynaecological cancers, and improved understanding of the molecular pathways underlying carcinogenesis holds great promise for advancing targeted therapies. Wnt signalling has been shown to have a role in tumorigenesis in all gynaecological organ systems, with the beta-catenin-independent pathway emerging as an exciting area of inquiry.

Wnt signalling

Wnt signalling is a cellular pathway consisting of an evolutionary conserved family of 19 ligands and 10 receptors. In humans, Wnt signalling is pivotal in embryonic development and tissue homeostasis, with a role in primary axis formation, organogenesis, stem cell proliferation and fate decisions. It is also one of the most extensively studied in cancer and has been implicated in majority of human malignancies, including endometrial tumours (Anastas & Moon 2012, Dellinger *et al.* 2012, van der Zee *et al.* 2013, Anastas 2015, Eskander *et al.* 2016). Wnt signalling mechanisms in the context of cancer have been extensively reviewed (Anastas & Moon 2012,

Niehrs 2012, Humphries & Mlodzik 2017, Kim *et al.* 2017, Schatoff *et al.* 2017, Tabatabai *et al.* 2017, Zhan *et al.* 2017).

Briefly, Wnt signalling comprises two distinct pathways, each with different ligands, coreceptors and functions. It is important to study both avenues, as considerable interplay and feedback exists between them. The canonical (or β -catenin dependent) Wnt signalling pathway is understood as governing proliferation and survival, achieved through accumulation of cytosolic β -catenin, which translocates to the nucleus, binding to transcription factors and activating downstream modulators. B-catenin accumulation is regulated by a 'destruction complex' consisting of APC, GSK3B and AXIN, which ubiquitinates β -catenin for degradation. Canonical ligands include WNT7, WNT3 and WNT10, which bind to FZD receptors.

Non-canonical (or β -catenin independent) Wnt signalling is linked to differentiation, cell polarity and migration (Simons & Mlodzik 2008, Sato *et al.* 2010, Clark *et al.* 2012). This arm of Wnt can be further divided into Wnt/Calcium signalling and planar cell polarity (PCP) signalling, achieved via WNT5A activation of the tyrosine kinases ROR1, ROR2 and RYK. The non-canonical pathway has the ability to antagonise Wnt/ β -catenin activity. There is significant crosstalk and overlap between the Wnt signalling arms, with growing acceptance of a Wnt network theory (Grumolato *et al.* 2010, Kikuchi *et al.* 2011, Anastas 2015, Ford *et al.* 2016).

Wnt in endometrial homeostasis

In the female adult, the endometrium undergoes structural modification in response to hormonal fluctuations of the menstrual cycle; both glands and stroma proliferate under the influence of oestrogen (proliferative phase), while progesterone drives differentiation (secretory phase) (Fig. 1 panel A). Hormone depletion at menopause causes the endometrium to atrophy and thin, with a discontinuation of normal endometrial cycling and subsequent inactivity of Wnt signalling (Fig. 1 panel B). Hyperplasia (Fig. 1 panel C) and ultimately, cancer (Fig. 1 panel D) arise when this closely controlled cycle goes awry, with epidemiological data linking type one tumorigenesis and unopposed oestrogen exposure suggesting the same pathways are involved in both cyclical proliferation and neoplasia (Kaaks *et al.* 2002).

An early *in vitro* culture study of one endometrial cell line reported that mRNA expression of Wnt ligands was unaffected by hormone levels (Bui *et al.* 1997). However, it is now well documented that most components of

Figure 1

Tracking Wnt signalling across endometrium homeostasis, menopause and tumorigenesis. Wnt signalling plays a key role in the fluctuation between proliferative, secretory and menstrual endometrium which coincides with oestrogen (yellow) and progesterone (blue) regulation (Panel A). During menopause, hormone activity decreases and there is no functioning wnt signalling (Panel B). An increase in unopposed oestrogen arising from factors such as obesity, or beta-catenin mutations, can cause re activation of Wnt signalling resulting in a thickened endometrium and hyperplasia (Panel C). Continuing signalling with the addition of non-canonical components such as the ROR receptors allows for tumorigenesis and further invasion into the myometrium (Panel D). Layers of the endometrium include the functionalis (pink), basal (light grey) and myometrium (dark grey). A full colour version of this figure is available at <https://doi.org/10.1530/ERC-18-0112>.

the Wnt system are expressed in the physiological adult endometrium, and several studies have examined Wnt signalling's role in mediating the hormonally driven regenerative balance between proliferation and differentiation (Fig. 1 panel A). In a small gene expression and cell line study, Wang and colleagues (Wang *et al.* 2009) demonstrated a regulatory relationship between oestrogen, progesterone and Wnt signalling elements. Profiling and data set pathway analysis revealed significant downregulation of β -catenin signalling in early and mid-secretory endometrium compared to proliferative tissue. Additionally, the Wnt inhibitor DKK1 was induced by progesterone and was elevated during the early secretory phase of the cycle, declining throughout the phase as supported by additional studies (Tulac *et al.* 2006). Earlier *in vitro* experiments by Oehler and colleagues (Oehler *et al.* 2002) showed that progesterone upregulated Wnt7a, suggesting a link between this ligand and the

anti-proliferative effects of progesterone in the endometrium. Coordinated upregulation of WNT5A, FZD10/9/6/4, SFRP1 and β -catenin after progesterone inhibition in patient samples suggests an important mediator role for Wnt signalling in menstruation and tissue repair (Catalano *et al.* 2007). The relationship between the Wnt system and hormones has been extensively profiled in animal studies, where induction of the Wnt/ β -catenin pathway was shown to induce endometrial hyperplasia but was not sufficient, in isolation, to trigger neoplastic transformation (Katayama *et al.* 2006, Carmon & Loose 2008, Satterfield *et al.* 2008, Jeong *et al.* 2009, Tanwar *et al.* 2009, Fan *et al.* 2012, van der Zee *et al.* 2013).

Wnt and endometrial cancer

A substantial body of literature spanning almost two decades has established a role for Wnt/ β -catenin signalling

in endometrial hyperplasia and cancer (Table 2), from the first limited study of 76 samples (Fukuchi *et al.* 1998) showing accumulation of β -catenin in 38% of endometrial cancers, to the TCGA's isolation of mutations in the β -catenin gene in 36.6% of 175 non-ultra-mutated endometrioid tumours. Estimates of the prevalence of activating mutations in β -catenin have varied depending on the study and methodology and not all have assessed the functional significance of these mutations by examining nuclear localisation of β -catenin or its correlation with clinicopathological features. Many of the immunohistochemistry studies have been on relatively small cohorts from a single clinical site and have focused on endometrioid endometrial cancers. Importantly, there has been no international study to date examining the role of Wnt/ β -catenin across a range of sites in different countries, to account for demographic influences given the significant role of obesity in pathogenesis of this disease. The more robust of the most recent estimates suggest β -catenin mutation occurs in 20–25% of endometrioid endometrial cancers (Byron *et al.* 2012, McConechy *et al.* 2014), while the more reliable – though dated – of the

immunohistochemistry studies (Moreno-Bueno *et al.* 2002, Scholten *et al.* 2003) reported nuclear accumulation of β -catenin in 12–31% of endometrioid endometrial cancers. The only larger-scale study (Saegusa *et al.* 2001) to look at both nuclear accumulation and β -catenin mutations put the latter at 23% and the former at 28%, concluding that the upper number was a more accurate reflection of prevalence. In a validation study comparing the TCGA dataset with large independent cohort, Liu and colleagues (Liu *et al.* 2013, 2014) showed overexpression in β -catenin-mutated tumours of Wnt pathway components including Wnt5a, Frizzled-10, TCF7 and LEF1, correlating this to decreased overall survival. Elegant studies in mice using lithium to mimic increased Wnt/ β -catenin signalling have shown increased atypical hyperplasia in the presence of oestrogen (Gunin *et al.* 2004) and a robust proliferative response in human endometrial xenografts (Polotsky *et al.* 2009).

Two immunohistochemical (IHC) studies have investigated the expression, in endometrial cancer, of the canonical ligand WNT7A, which was previously touched on as a progesterone-mediated component of

Table 2 Comparison of major studies examining Wnt/beta-catenin signalling in endometrial cancer.

Study	Year	Findings	Methods
Fukuchi	1998	beta-Catenin mutation 10/76 EC samples	PCR and IHC from fresh surgical (54) or fixed (36) samples
Nei	1999	Moderate-strong beta-catenin staining nucleus in 60% of 20 EH samples; 30% of ECs	IHC, Western blot, RT-PCR
Mirabelli	1999	beta-Catenin mutations 13/29 EC samples	PCR
Schlosshauer	2000	6/32 beta-catenin mutations	PCR, Western blot
Palacios	2001	Nuclear staining of beta-catenin 10/40 EC samples	IHC
Saegusa	2001	Nuclear beta-catenin staining 55/199 EECs	IHC, RT-PCR and Southern blot, PCR and sequencing analysis, Western blot
Schlosshauer	2001	Nuclear beta-catenin staining 8/17 EECs	IHC
Machin	2002	beta-Catenin mutations in 15/59 EECs, 11 of these had Positive beta-catenin staining in the nucleus	PCR, IHC
Moreno-Bueno	2002	Nuclear beta-catenin 31.2% of 128 EECs, 3% of 33 non-EECs	IHC, PCR, laser microdissection of focal tumour areas
Ashihara	2002	Nuclear beta-catenin staining 14/25 EH and 12/20 ECs	IHC, PCR
		beta catenin mutations in 2/20	
Scholten	2003	Nuclear staining beta-catenin 29/233 ECs	IHC
Pijnenborg	2004	Nuclear staining beta-catenin 9/24 ECs	IHC, MS-PCR (for e-cadherin and APC)
Konopka	2007	9/56 EECs beta-catenin mutations	PCR
Byron	2012	beta-Catenin mutations 88/454 EECs	PCR (direct sequencing)
Cancer Genome Atlas	2013	beta-Catenin mutations in 36.6% of 175 non-ultramutated ECs; 52% of 90 mss/copy number low group	Exome, whole genome, RNA and miRNA, DNA methylation and copy number, reverse phase protein assays
Liu	2014	beta-Catenin mutations in a subgroup of 47/54 EECs in younger, obese patients	Integrated analysis of 271 EEC cases in TCGA cohort. Validation in independent cohort of 184 EECs
McConechy	2014	beta Catenin mutations in 76/307 EECs; (6/31 high grade)	Select exon capture sequencing on a gene panel Mutation and bioinformatics analysis

EC, endometrial cancer; EEC, endometrioid endometrial cancer; EH, endometrial hyperplasia.

regular endometrial cycling. The first study found that WNT7A was overexpressed in endometrial tumours (63% of patients) compared to benign controls (4.7% of patients) and correlated high WNT7A expression with increased grade, myometrial invasion and worse overall survival (Liu *et al.* 2013). This was a large cohort analysis, featuring 335 patients. The second study demonstrated a decrease in WNT7A expression in endometrial cancer (expression in only 37% of 70 patients) when compared to benign samples (expression in 88% of 70 patients), and correlated loss of WNT7A to poor survival (Peng *et al.* 2012). The use of different antibodies to detect Wnt7 expression can significantly impact the outcome of IHC studies, as we previously noted in the case of another Wnt pathway member, ROR2 (Ma *et al.* 2017). Given that WNT7A is confined to the luminal epithelium in the physiological endometrium, where it increases during gland proliferation and is inhibited by progesterone in secretory transformation (Fan *et al.* 2012), this ligand would, theoretically, be seen at increased levels during unopposed oestrogen-driven tumorigenesis.

Compared with benign endometrium, studies have revealed a downregulation of the Wnt inhibitors Dickkopf3 (DKK3) and Dickkopf1 (DKK1) and the secreted frizzled-related proteins in endometrial cancer, with a stepwise reduction in DKK1 expression from intrauterine disease to extrauterine disease and distant metastases, and correlation with high-grade disease and a poor prognosis (Hrzenjak *et al.* 2004, Risinger *et al.* 2005, Yi *et al.* 2009, Di Domenico *et al.* 2011, Dellinger *et al.* 2012, Eskander *et al.* 2016). These studies have principally examined relatively small, heterogeneous (predominantly endometrioid endometrial cancer) cohorts ($n=14-50$) from a single clinical site. *In vitro*, DKK1 knockdown by siRNA in the Ishikawa endometrial cancer cell line has been shown to increase cell invasion and migration (Yi *et al.* 2013). This is in keeping with studies showing Wnt (in particular, the signalling component glycogen synthase kinase 3- β) is one of several pivotal pathways implicated in regulation of the morphogenetic process known as epithelial-to-mesenchymal transformation (EMT). EMT is essential in embryonic development, wound healing and repair and, when activated in cancer, leads to a more invasive and metastatic phenotype (Taki *et al.* 2003, Nelson & Nusse 2004, Bachelder *et al.* 2005, Lee *et al.* 2006, De Craene & Berx 2013, Kanzawa *et al.* 2013).

While β -catenin dependent signalling has a documented role in endometrial cancer, little is known about non-canonical (also known as β -catenin independent) signalling and its potential therapeutic

applications. Previous reviews on Wnt signalling in the endometrium and endometrial cancer have not addressed this arm of the pathway at all. Bui and colleagues (Bui *et al.* 1997) mapped expression of Wnt ligands in a limited cohort of four endometrial cancer cell lines and four tumour samples compared with benign epithelial and stromal controls and found statistically significant downregulation of Wnt4 mRNA in the cell lines and tumours, as well as possible suppression of Wnt2, 3 and 5a mRNA. Both Wnt4 and Wnt5a act on the non-canonical pathway, and the latter has been shown to be overexpressed in cervical, aggressive basal-like breast and ovarian cancers, as well as omental metastases, suggesting that this ligand is involved in progression of gynaecological neoplasms (Jönsson *et al.* 2002, Dejmek *et al.* 2005, Klemm *et al.* 2011, Ford *et al.* 2014, Lin *et al.* 2014). siRNA knockdown of Wnt5a in ovarian cancer cell lines reduced proliferation, migration and invasion and promoted cell cycle arrest and apoptosis in a study by Chen and colleagues (Chen *et al.* 2013), while the addition of Wnt5a to ovarian cancer cells *in vitro* enhanced motility and invasiveness in a separate study by Qi and collaborators (Qi *et al.* 2014). Similar effects have been shown in metastatic melanoma (Weeraratna *et al.* 2002). Wnt5a is of particular interest because it acts as both promoter and suppressor, depending on the cancer, and is capable of signalling via all three Wnt pathways (Chen *et al.* 2013, McDonald & Silver 2009, Qian *et al.* 2007). In non-canonical signalling, Wnt5a binds to a family of receptors called receptor tyrosine kinase-like orphan receptors (RORs). RORs are emerging as an exciting area of inquiry in oncology due to their overexpression in a growing list of cancers, paucity in healthy adult tissues and location on the surface of cells (Ford *et al.* 2013).

RORs and endometrial cancer

ROR1 and ROR2 are transmembrane proteins of the receptor tyrosine kinase family first identified in a neuroblastoma cell line in 1992 (Masiakowski & Carroll 1992). At that time they were dubbed 'orphan' receptors because their ligands were unknown, but RORs have since been linked to Wnt signalling in both development and disease (Green *et al.* 2008, 2014). Expressed at high levels during embryogenesis with roles in skeletal and neuronal development, the RORs are largely repressed in the adult. ROR1 is not expressed in vital organs such as brain, heart, lung or liver, however, has been found in regions of the parathyroid, pancreatic islets and gut (Balakrishnan *et al.* 2017). ROR2 expression in adult tissues has not been

explicitly analysed, however, extrapolating from several studies, low expression of ROR2 may be found in adipose tissue, pancreas, thyroid, stomach, osteoblasts, precursor B cells, testis, lung, bladder, colon and – interestingly – the uterus, where its roles are yet to be elucidated (Al-Shawi *et al.* 2001, Matsuda *et al.* 2001, Yoda *et al.* 2003, Billiard *et al.* 2005, Baskar *et al.* 2008, Morioka *et al.* 2009, Cha *et al.* 2014). While extensive ROR1 analysis has been conducted in adult tissues, using a well-established antibody with no cross detection, such a process is yet to be undertaken for ROR2.

RORs bind Wnt5a and transduce PCP signalling. They have been shown to participate in cell migration and invasion, and cellular polarity – key tumorigenic properties – and are of interest in cancer because they tend to be associated with aggressive, poor prognosis disease (Morioka *et al.* 2009, O'Connell *et al.* 2010, Edris *et al.* 2012, Zhang *et al.* 2012a, 2014a, Ford *et al.* 2014, Henry *et al.* 2015, Henry *et al.* 2017). Aberrant ROR expression has been detected in a growing list of cancers, where it has been linked to growth, survival, motility and invasion, and where it correlates with more aggressive disease. Of note, ROR2 appears to have a dual oncogenic/suppressor role depending on the cancer and, hypothetically, whether it drives canonical or non-canonical Wnt signalling (Ford *et al.* 2016).

Only a handful of studies to date have specifically examined ROR expression in endometrial cancer, and an understanding of their role in this disease is limited. Firstly, for ROR1, a broad study of cell lines and tissue microarray using a monoclonal antibody specific for ROR1 (Zhang *et al.* 2012b), reported ROR1 expression in 28/29 (96%) uterus cancer samples – the highest of any tissue studied, though data on tumour type or histology was not available. In the same study, 78/144 (54%) of ovarian cancers stained moderately to strongly for ROR1, mostly clear cell, endometrioid and mucinous subtypes and expression was correlated with high-grade and less differentiated histology. Additionally, the authors found no detectable ROR1 expression on the 'normal' tissue counterparts. The other study examined ROR1 expression in 52 paraffin-fixed tumour samples (low stages, I-II) and blood from 26 endometrial cancer patients, correlating stronger expression to higher stage (Zhang *et al.* 2017). However, representative images from stage I and II ROR1 IHC both showed quite strong expression. Additionally, western blots representing ROR1 knockdown and overexpression in subsequent cell line models were not convincing. They used expression of C-Myc and CyclinD1 as downstream targets of ROR1, which are characterised

as canonical, β -catenin Wnt targets (instead of RhoA and Rac) (Yu *et al.* 2017, Hasan *et al.* 2018). Extending this work *in vitro* and in a murine model, the authors found that transient overexpression of ROR1 in the Ishikawa and HEC1b cell lines increased tumour volume and weight, while ROR1 knockdown had the opposite effect.

Two studies have examined ROR2 broadly in endometrial cancer, but neither focused explicitly on this subject. The first investigated leiomyosarcoma, a rare non-epithelial endometrial tumour, which is clinically distinct from endometrial adenocarcinoma, as part of ROR2 screening in a host of soft tissue sarcomas (Edris *et al.* 2012), and the second largely extrapolated findings from endometrioid ovarian cancer on CD55-ROR2 signalling (Saygin *et al.* 2017).

The second ROR2 study, examining cancer stem cells in the endometrioid subtype of ovarian and endometrial cancers, reported a link to stemness and the complement protein CD55 mediated via ROR2-JNK signalling (Saygin *et al.* 2017). However, the majority of the experimental work in this study was performed on ovarian TOV112D and A2780 cell lines and the findings extrapolated to endometrioid endometrial cancer on the basis of CD55-ROR2 immunoprecipitation in an endometrial model and levels of CD55 in the HEC1a cell line. (Saygin *et al.* 2017).

Our recent study investigated both ROR1 and ROR2 in a small clinical cohort of 77 endometrioid endometrial tumours and 10 serous endometrial tumours and identified distinct roles for these sister receptors (Henry *et al.* 2018). ROR2 overexpression correlated with better survival, and when silenced in the endometrial RL95-2 line, cells increased their ability to migrate and invade. It is worth noting the discovery of a potential tumour suppressor role for ROR2 in colorectal carcinogenesis, given the genetic link (clinically referred to as Lynch syndrome) between this cancer and some type one endometrioid endometrial tumours (Ma *et al.* 2016). By contrast, we found stronger ROR1 expression in the serous subtype of endometrial cancer, with silencing of this receptor in the KLE cell line decreasing migration and invasion. Future investigations should include larger cohorts, so that more patient data for less common subtypes such as serous can be analysed.

Given the potential overlap of genetic features and therapeutic paradigms between endometrial cancer and breast or ovarian tumours (Fadare *et al.* 2013, Levine & The Cancer Genome Atlas Research Network 2013, Hoang *et al.* 2014), studies examining RORs in this context are of relevance. Several large-scale IHC studies (100–300 patients) have examined ROR expression in breast cancers (Zhang *et al.* 2012a, Henry *et al.* 2015, Chien *et al.* 2016).

Of particular note due to the similarities between triple-negative breast cancer and serous endometrial tumours, Chien and colleagues (98) found a correlation between strong ROR1 staining in 47/210 samples of this breast tumour type and shorter disease-free, metastasis-free and overall survival. We previously (95) demonstrated ROR2 expression in 256/295 breast tumours and linked this to decreased disease-specific survival and lymphatic invasion. Knockdown studies by the authors in triple-negative breast cancer cell lines resulted in increased proliferation and migration, suggesting involvement of both β -catenin dependent and -independent signalling pathways. Studies (Zhang *et al.* 2012a, Chien *et al.* 2016) identified ROR1 expression as a feature of triple-negative breast cancer cell lines, and similar silencing studies resulted in reduced proliferation and increased apoptosis. ROR1 was also shown in breast adenocarcinomas to be linked to EMT-like gene signatures and higher rates of relapse and metastasis in a major study of 582 tissue microarray samples and 16 breast cancer cell lines (Cui *et al.* 2013). In serous ovarian cancer, both ROR1 and ROR2 have been demonstrated as overexpressed compared with benign controls. ROR1 has been correlated with disease-free and overall survival and presence of metastases (Zhang *et al.* 2014a), stem cell-like gene signatures, higher rates of relapse and shorter median survival (Zhang *et al.* 2014b). *In vitro*, we have demonstrated (Henry *et al.* 2016) that overexpression of both RORs results in increased invasion, while their knockdown inhibits migration and invasion and sensitises chemotherapy resistant cell lines to cisplatin in ovarian cancer. In a cohort of 178 patient samples with matched benign, primary and metastatic lesions, both receptors were abnormally expressed in both epithelium and stroma – ROR2 most prominently in early stage, low-grade endometrioid ovarian tumours and in the stroma of the serous subtype (Henry *et al.* 2017). Therefore there is evidence for the potential of ROR1 and ROR2 as therapeutic targets in the related endometrial tumours.

In light of the established role for Wnt signalling in endometrial development, and median age of endometrial cancer diagnosis being 61 years (Hüsing *et al.* 2016), the effect of menopause on ROR1 and ROR2 expression warrants investigation. In our recent study of ROR1 and ROR2 (Henry *et al.* 2018), we did not have access to the menopause status of our patient cohort, but future studies should seek this information for both benign controls and cancer patients.

In sum, these findings indicate that ROR1 and ROR2 hold promise as prognostic markers and potential

therapeutic targets in endometrial cancer, the most common gynaecological malignancy in the developed world and a growing problem as obesity rises.

Targeting the Wnt pathway

Current clinical trials which include endometrial cancer patients and targeted therapies are limited, as detailed in Table 2. Challenges documented with Wnt inhibitors in clinical trials include low recruitment (PRI-724 β -catenin inhibitor, NCT01302405 and Frizzled 10 inhibitor, NCT01469975), withdrawn funding (Wnt974, NCT02649530) and correction to dosage (RXC-004, NCT03447470). In Australia, there are no current Wnt therapies, but four endometrial-specific trials are underway for the use of immunotherapies, including a PD-L1 checkpoint inhibitor and a CTLA4 inhibitor. Although available for all advanced endometrial cancer patients eligible for chemotherapy, only POLE or MMR-instable tumour subtypes may respond. However, no study requires molecular screening for eligibility – the minimum is available blocks for MMR protein and PD-L1 IHC testing. A recent review of the PD-L1 inhibitor trials results from the KEYNOTE-028 study reported no benefit to patients if their PD-L1 IHC score was low (Kurnit & Jazaeri 2017). Only two patients in the cohort exhibited POLE or MMR mutations, and both achieved a significant and partial response respectively. Therefore, for current immunotherapy trials in endometrial cancer to be successful, eligibility criteria should include screening for those with molecular subtypes which would respond best.

Globally, other endometrial cancer-targeted treatments are under phase I clinical trials, including ERK, VEGF and mTOR inhibitors. Interestingly there is only one study targeting the Wnt pathway that includes endometrial cancer patients: DKN01, a DKK1 inhibitor which actually restores active Wnt signalling. As previously discussed, DKK1 is reduced in endometrial cancer progression to invasive and metastatic disease, and the rationale to include endometrial cancer patients in a DKK1 inhibitor trial is, therefore, unclear. A controversial role for DKK1 and Wnt signalling in carcinogenesis was explored at length in a recent review (Kagey & He 2017), but there was no evidence drawn from their analysis of the literature for DKK1 in endometrial cancer progression, and the review did not consider studies which found DKK1 downregulation. However, interesting conclusions were drawn on DKK1's role in alternative signalling pathways such as immune cell and tumour evasion. DKK1 can be released by cells of the tumour microenvironment,

resulting in local immunosuppression. It may be important to analyse DKK1 across the molecular subtypes of endometrial cancer to better select patients for trial eligibility, as treating those with already-activated Wnt signalling may have unwanted side effects.

A number of small molecule canonical Wnt inhibitors are currently in phase I/II clinical trials for various cancer types, currently limited to tumours of the breast, colon and pancreas. If canonical Wnt signalling is indeed driving progression of endometrioid, type one tumours in approximately 30% of women with endometrial cancer, these trials could be extended to include these patients, with the caveat that targeting Wnt components can have unintended adverse effects via crosstalk to other parts of the network.

Wnt ligands are processed by the Porcupine protein before secretion, a key step in lipid modification and ligand-receptor binding activity. Therefore, to disrupt oncogenic Wnt signalling, a number of Porcupine inhibitors have been generated and are now in clinical trial (Table 1, LHK974, SMO9502, CGX1321, ETC-1922159). Porcupine modifications affect all Wnt ligands, irrespective of canonical or non-canonical signalling. Therefore, removal of all Wnt ligands in cancer cells may have unknown and undesired effects; for example in colorectal cancer, the non-canonical Wnt ligand ROR2 is methylated and silenced, allowing increased canonical β -catenin activity. When ROR2 is expressed and bound with Wnt5a in this context, feedback mechanisms result in canonical inhibition. If some

Figure 2

Current Wnt inhibitors in clinical trial. Inhibitors are shown in grey boxes with red arrows indicator target molecule. β -catenin-dependent signalling is shown on the left, while independent signalling through ROR1, ROR2 and Wnt5a is on the right. Drug compounds are listed in Table 3. A full colour version of this figure is available at <https://doi.org/10.1530/ERC-18-0112>.

subtypes of endometrial cancer are genetically similar to colorectal cancer, it follows that this mechanism may also apply, and removing all Wnt ligands may actually have an activating or unknown effect, necessitating caution with this approach.

RNF43 is a tumour suppressor that reduces Wnt frizzled receptor expression (Tsukiyama *et al.* 2015). It has been shown that those patients with RNF43 mutations and hyperactivated Wnt signalling have the greatest response to Porcupine inhibitors, meaning screening for patients with these characteristics may be key in anti-Porcupine clinical trials (Ho & Keller 2015, Bhamra *et al.* 2017).

Because non-canonical Wnt signalling may have a more prominent role in the development of serous endometrial tumours, ROR-targeting therapies or Wnt5a inhibitors may be of most benefit to these patients. However, there is presently only one non-canonical ligand target drug in clinical trials: Foxy-5 (NCT02655952), a Wnt5a mimetic, which activates non-canonical signalling (Canesin *et al.* 2017). A dose-escalating study of Foxy-5 in breast, colon or prostate cancer patients was recently completed in mid-2017, but results are yet to be published. Pre-clinical *in vivo* models reported that Foxy-5 inhibited metastasis of prostate cancer cells without affecting tumour cell proliferation (Canesin *et al.* 2017). Foxy-5 was used on low-expressing Wnt5a tumour cells, with the theory that increased ROR2-Wnt5a signalling inhibits β -catenin activation of metastatic target genes (Ying *et al.* 2008). However, for the current Foxy-5 clinical trial, there is no inclusion or exclusion criteria to single out patients with low Wnt5a expression. As we and others

have shown, Wnt5a can bind to both ROR1 and ROR2, initiating a multitude of complex downstream cascades, which may be involved in cancer progression, depending on tumour type (Weeraratna *et al.* 2002, Da Forno *et al.* 2008, Fukuda *et al.* 2008, Ren *et al.* 2011, Kanzawa *et al.* 2013, Ford *et al.* 2014, Qi *et al.* 2014, Hasan *et al.* 2018). In addition, recent work has demonstrated a role for two distinct Wnt5a isoforms, Wnt5a-short and Wnt5a-long, in colorectal cancer progression and active Wnt signalling (Huang *et al.* 2017). It is unknown what isoform Foxy-5 is based upon, and prudence is required, particularly for breast cancer patients where an increase of Wnt5a and ROR receptors has been shown to have oncogenic properties (Henry *et al.* 2015, Han *et al.* 2018, Kobayashi *et al.* 2018).

A number of therapies have entered development as evidence builds for the non-canonical receptors, ROR1 and 2 across tumour types. The most advanced of these is Cirmtuzumab, a first-in-class antibody that targets ROR1, which is presently in phase I/II trials in chronic lymphoid leukaemia (CLL; Choi *et al.* 2015) and breast cancer (NCT03420183, NCT02776917). In results that have only just come to press, this world-first ROR1-directed therapy demonstrated no dose-dependent toxicity, a long half-life and inhibition of stem cell-like expression signatures in CLL (Choi *et al.* 2018). Following this successful trial, a phase II study has commenced to investigate the efficacy of cirmtuzumab in combination with Ibrutinib (BTK inhibitor) in B-cell malignancies (NCT03088878).

In addition to cirmtuzumab, a number of other anti-ROR1 therapies are also in pre-clinical development

Table 3 Current clinical trials involving Wnt targets and/or endometrial cancer.

Target	Drug	Clinical trial inclusion	Clinical trial ID
Australia: Endometrial only			
PD-L1	Durvalumab	Advanced endometrial ca	ACTRN12617000106336
CTLA4	AGEN1884	Advanced endometrial ca	NCT03495882
PD-L1	Rucaparib	Advanced endometrial ca	NCT03101280
PD-L1	CBT-501	Advanced endometrial ca	NCT03053466
mTOR	MLN0128	Advanced endometrial ca	NCT02725268
USA/Europe: Endometrial only			
ER	Fulvestrant	Recurrent, persistent or met endo ca	NCT00006903
MEK/ERK	Selumetinib	Recurrent, persistent or met endo ca	NCT01011933
VEGF	Cediranib	Recurrent, persistent or met endo ca	NCT01132820
EZH2	Tazemetostat	Recurrent, persistent or met endo ca	NCT03348631
mTOR	Temsirolimus	Recurrent, persistent or met endo ca	NCT01068249
Multiple kinases	Nintedanib	Recurrent, persistent or met endo ca	NCT02730416
DKK1	DKN-01	Recurrent, persistent or met endo ca	NCT03395080
Wnt inhibitors			
Porcupine inhibitor	LGK974	Pancreatic, colorectal, melanoma, TNBC, head and neck SC, cervical SC, oesophageal SC, lung SC	NCT01351103
Unknown Wnt inhibitor	SM08502	Advanced solid tumours	NCT03355066

Ca, cancer; CLL, chronic lymphocytic leukaemia; Endo, endometrial; met, metastatic; SC, squamous cell; TNBC, triple-negative breast cancer.

(Hojjat-Farsangi *et al.* 2017, Yin *et al.* 2017, Hassannia *et al.* 2018). Of these, ROR1 chimeric antigen receptor (CAR)-T cell therapy has attracted interest. CAR-T cell therapy involves gene transfer of receptors into T cells to induce cancer-specific immune responses. Because ROR1 is known to be expressed specifically on cancer cells, it is an attractive target for T cell therapy. *In vivo* models have shown positive responses to this type of therapy (Berger *et al.* 2015); however, of the two current phase I trials investigating toxicity of these methods, one was terminated due to unavailability of reagents and as it had no enrolments (NCT02194374). Recruitment is ongoing for the other, a basket design, dose-escalating study including ROR1-positive tumours such as breast cancer, lymphoma, leukaemia and lung cancer (NCT02706392). As we have demonstrated opposing roles for ROR1 and ROR2 in endometrial cancer (Henry *et al.* 2018), it will be critical to monitor the effect on ROR2, when targeting ROR1 in endometrial cancer.

Current Wnt-targeting therapies and which components of the pathway they target are shown in Fig. 2.

Conclusion

Despite its prevalence, endometrial cancer remains understudied and the dominant treatment paradigm entails significant morbidity. Advances on the molecular frontier could be life-changing for patients, and Wnt signalling may prove a fruitful avenue for targeted therapies. Further investigation into Wnt activity, internal signalling interactions, and what influence hormonal fluctuations and metabolic factors have on this complex but therapeutically promising signalling network is required in endometrial cancer, where treatments remain limited despite growing prominence due to obesity and childbearing later in life or not at all.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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