Expression, activation, and role of AKT isoforms in the uterus

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Abstract

The three isoforms of AKT: AKT1, AKT2, and AKT3, are crucial regulators of both normal and pathological cellular processes. Each of these isoforms exhibits a high level of homology and functional redundancy with each other. However, while being highly similar and structurally homologous, a rising amount of evidence is showing that each isoform possesses specific targets as well as preferential subcellular localization. The role of AKT has been studied extensively in reproductive processes, but isoform-specific roles are yet to be fully understood. This review will focus on the role of AKT in the uterus and its function in processes related to cell death and proliferation such as embryo implantation, decidualization, endometriosis, and endometrial cancer in an isoform-centric manner. In this review, we will cover the activation of AKT in various settings, localization of isoforms in subcellular compartments, and the effect of isoform expression on cellular processes. To fully understand the dynamic molecular processes taking place in the uterus, it is crucial that we better understand the physiological role of AKT isoforms as well as their function in the emergence of diseases.

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Introduction

AKT, a serine/threonine kinase, is widely studied in a plethora of biological phenomenon. It has emerged as one of the most critical regulators of cell fate considering its implication in cell cycle control, apoptosis evasion, and metabolic processes, among others. This versatile serine–threonine kinase is central to many human physiological processes; however, because of its fundamental role in many signaling pathways, gain of function of AKT is linked to multiple human diseases. This review focuses on the role of AKT in both normal and pathological processes in the uterus.

The uterus is composed of two major linings; the endometrial and myometrial linings. This review will focus on the former, as it is the compartment that undergoes the most drastic changes throughout the menstrual cycle and pregnancy. The endometrium is further subdivided in two layers: the functional layer and the basal layer. While the basal layer role is to spawn and subsequently support its counterpart, the functional layer is periodically built during every menstrual cycle; this process, the purpose of which is to prepare the endometrium to the possible implantation of a fertilized egg, is influenced by a plethora of factors. The successful implantation of the embryo relies on a highly coordinated interaction between the maternal endometrium and the invading trophoblast. This tightly restricted period of

receptivity, which recurs every cycle, is known as the ‘window of implantation’ and requires a very dynamic regulation of expression of several genes (Kao *et al.* 2002, Sharma & Kumar 2012); various mechanisms, which we will explain elaborately further in this review, allow fruitful implantation to take place. The uterus is also involved in many pathological conditions; endometriosis and uterine cancer, the two most prominent of these diseases, will be addressed in this review. Considering the growing body of evidence establishing AKT as a fundamental regulator of many cytokine- and hormone-driven processes, it is natural to study its involvement in the aforementioned processes and mechanisms.

The AKT protein family is constituted by three different isoforms; each of those isoforms is produced by a specific gene. The three distinct isoforms are AKT1/PKB α , AKT2/PKB β , and AKT3/PKB γ ; each of those isoforms presents a highly homologous sequence to each other, both functionally and structurally (Fig. 1; Liao & Hung 2010). The overwhelming body of evidence existing on AKT functions concerns its role in cell survival processes, apoptosis evasion, and cell proliferation (Hennessy *et al.* 2005, Manning & Cantley 2007). The cytosolic and inactive form of AKT is recruited to the plasma membrane through phosphorylation of phosphoinositides (PtdIns) at the 3-position of the inositol ring by phosphoinositide 3-kinase (PI3K), subsequently producing PtdIns3P/PIP, PtdIns(3,4)P2/PIP₂, and ultimately PtdIns(3,4,5)P3/PIP₃.

Figure 1 Structure of AKT isoforms: Schematic representation of each AKT isoform with its pleckstrin homology domain (PH domain), its catalytic domain, and its hydrophobic motif. The location of major phosphorylated residues (either T for threonine or S for serine) is also shown. Latest (as of May 2014) RefSeq NCBI protein sequence were used (NP_001014431.1, NP_001617.1, and NP_005456.1 respectively).

This phospholipid is anchored in the plasma membrane and will subsequently activate the downstream signaling component by specific binding. Many of those proteins harbor the pleckstrin homology (PH) domain, which recognizes and binds to distinct phosphorylated PtdIns (Lemmon 2007). The presence of the PH domain can therefore be considered crucial to both activation and localization of proteins displaying it. AKT is one such protein; its PH domain binds specifically to PIP₃, recruiting and anchoring it to the plasma membrane. It must be noted that some evidences demonstrate that AKT can bind to PIP₃ as well as PIP₂, as revealed by its crystalline structure as well as *in vitro* experiments (Franke *et al.* 1997, Thomas *et al.* 2002); however, full activation of AKT requires PIP₃ (Frech *et al.* 1997, Denley *et al.* 2009, Kamimura & Devreotes 2010). Besides locating it to the plasma membrane, the binding of PIP₃ alters the conformation of AKT. This enables its activation by various kinases also present at the membrane by phosphorylation of two crucial residues. The first member of this network is the 3-phosphoinositide-dependent kinase 1 (PDK1), which also displays a PH domain (Mora *et al.* 2004, Raimondi & Falasca 2011). PDK1 will activate AKT by phosphorylating its catalytic domain's threonine residue (T308 for AKT1, T309 for AKT2, and T305 for AKT3; Liao & Hung 2010). On the other hand, the mammalian target of rapamycin complex 2, mTORC2, is the long-sought second major activator of AKT (Sarbasov *et al.* 2005). It directly phosphorylates the serine residue (S473 for AKT1, S474 for AKT2, and S472 for AKT3). This double phosphorylation fully activates AKT (Yang *et al.* 2002). One of the most crucial negative regulators of these processes is the lipid protein phosphatase tensin homolog deleted on chromosome 10 (PTEN), which antagonizes AKT activation by catalyzing the dephosphorylation of PIP₃, transforming it into PIP₂. This reduces AKT localization to the membrane, its conformational changes, and its availability for interaction with its activators (Song *et al.*

2012). Studies have shown that AKT can be transported to various cellular compartments and organelles. The mechanisms mediating this translocation are still vastly misunderstood. However, the importance of AKT localization in cellular processes, as well as the mechanisms regulating its translocation, is being documented. Recent studies have shown that AKT1 and AKT2 preferentially localized to the cytosol; however, AKT2 was found to colocalize to the mitochondria, suggesting a role in mitochondrial-induced apoptosis as well as metabolism. Finally, AKT3 was found to localize predominantly to the nucleus. These findings emphasize the need to reassess the dogma of plasma membrane activation of AKT (Gonzalez & McGraw 2009, Santi & Lee 2010).

Many studies focus on the overall role of AKT, without regards to isoform specificities; indeed, a growing amount of data show that despite the high homology found between isoforms and their resulting functional redundancy, isoforms display specific actions and have distinct roles in both normal and pathological phenomena. To understand these individual roles, many groups have endeavored to generate transgenic mice deficient in each isoforms. *Akt1*-deficient mice exhibited an increased neonatal mortality as well as a reduced body mass (Chen *et al.* 2001, Cho *et al.* 2001a); *Akt2*-deficient mice developed diabetes-like syndrome, slightly reduced body mass, but no major birth defects (Cho *et al.* 2001b, Garofalo *et al.* 2003); finally, *Akt3*-deficient mice exhibited reduced brain size due to reduced cell size and number, but did not develop glucose metabolism disorders nor body mass reduction (Easton *et al.* 2005, Tschopp *et al.* 2005). Considering the results obtained by these studies, it is evident that AKT isoforms exhibit functional redundancy, as well as non-redundant physiological activities. To further assess these differences, the next natural step was to generate double-knockout animals. Mice with combined deficiencies of both *Akt1* and *Akt2* displayed majorly impaired skin, bone, and

muscle development and severely impeded adipogenesis, and died shortly after birth (Peng *et al.* 2003). Both *Akt1* and *Akt3* knockout mice died approximately at embryonic day 12 due to defects in growth as well as flawed cardiovascular and nervous system development (Yang *et al.* 2005). Finally, both *Akt2*- and *Akt3*-deficient mice exhibited impaired glucose metabolism, reduced body mass as well as reduced testis and brain weight; despite these defects, however, they were viable and healthy (Dummler *et al.* 2006). The study also demonstrated that heterozygous mice possessing a single *Akt1* at the exclusion of any other isoforms (*Akt1*^{+/-}, *Akt2*^{-/-}, and *Akt3*^{-/-}) were viable; this strongly suggests that a single functional allele of *Akt1* is sufficient for the mice to develop normally and thrive. Taken together, these findings indicate that each isoform possesses non-overlapping functions that need to be elucidated to further understand the dynamic and multiple processes in which AKT is involved. This review will focus on the role of AKT isoforms in processes such as implantation, decidualization, endometriosis, and endometrial cancer (EC; Fig. 2).

Implantation

The implantation process, by which the embryonic trophectoderm invades the maternal uterine luminal epithelium, necessitates a highly coordinated interaction between endometrial cells and trophoblastic cells. This mechanism allows the invading blastocyst to penetrate into the endometrial stroma to eventually come into contact with the maternal blood supply. First, trophoblastic cells of the trophectoderm will be exposed to the maternal endometrium; chemokines and cytokines will enable a molecular dialog between the two structures which will enable the docking and tethering of the embryo to the endometrium, not unlike leukocyte extravasation or metastatic invasion (van den Brule *et al.* 2005). The trophoblast will then invade the endometrium by penetrating into both the endometrial epithelial cells and its basement membrane; this penetration is enabled by the selective apoptosis of the endometrial epithelial cells (Gu *et al.* 1994). The invading trophoblast will then come into contact with the endometrial stroma, the uterine blood vessels, and the inner myometrium; the

Figure 2 Overview of AKT signaling in the uterus. Schematic representation of crucial regulation elements involving AKT in various physiological and pathological contexts. Green background indicates tumor suppressor proteins, whereas red background denotes potential oncogenes.

establishment of the placenta and the uteroplacental circulation will conclude this process.

In vitro experiments involving cultured blastocysts from superovulated mice demonstrated that they expressed AKT1 and AKT2 as well as phosphorylated AKT (S473), both majorly at the plasma membrane. The preimplantation embryos also displayed increased phosphorylated AKT (S473) as well as increased glucose uptake in response to insulin, an effect that was mitigated by Wortmannin as well as LY-294002 (both potent PI3K inhibitors) (Riley *et al.* 2005). This suggests that AKT is necessary for blastocysts' basic glucose metabolism, making it crucial for proper implantation in the maternal endometrium.

In a recent study, the use of cytotrophoblastic SGHPL-5 cells exhibiting features of invasive trophoblast cells that were stably transfected with shRNAmir directed against all three AKT isoforms was discussed. This experiment suggested that AKT1 and AKT3 enable trophoblast migration in response to EGF; however, none of the AKT isoforms seemed to be involved in trophoblast cell proliferation. These effects could be explained by the abrogation of EGF-induced mTORC1 phosphorylation in the absence of both AKT1 and AKT3 (Haslinger *et al.* 2013). Furthermore, we have shown that each isoform is expressed during both the pregnancy and the pseudopregnancy of the rat. However, we have evidenced that AKT3 was the only phosphorylated isoform at the time of implantation, further strengthening the idea that specific isoforms exert tight control on specific mechanisms (Veillette *et al.* 2013). However, as stated previously, double knockout mice for *Akt2* and *Akt3* did not display any birth defect except reduced body mass; the mild phenotype was not influenced by a compensatory expression of AKT1, suggesting that AKT1 and AKT3 possess a similar function in implantation processes (Dummler *et al.* 2006). This hypothesis is reinforced by the fact that *Akt1*- and *Akt3*-deficient mice were embryonically lethal because of improper placental vasculature, whereas *Akt1* and *Akt2* double-knockout mice abnormalities were more developmental in nature (Peng *et al.* 2003, Yang *et al.* 2005).

Descriptive studies using human placentas have shown that all three AKT isoforms were expressed in first trimester villous and extravillous cytotrophoblasts as well as villous fibroblasts; the syncytium, however, displayed only AKT3 expression. Isoform localization was also variable: AKT1 was detectable primarily not only in the cytoplasm of both villous and extravillous cytotrophoblasts but also in the nuclei, although at a lower level; AKT2 was expressed exclusively in the cytoplasm; AKT3 was most prominent in the nuclei of cells. Experiments using SGHPL-5 cells confirmed these observations (Haslinger *et al.* 2013). It has also been demonstrated that chorionic gonadotropin, a hormone secreted by the syncytiotrophoblast, promoted trophoblast invasion through AKT-dependent upregulation of

MMP2 as well as increased motility. These effects were not dependent on increased cell proliferation (Prast *et al.* 2008). Another set of experiments, performed in mice at 5 days of pregnancy, revealed that implantation sites displayed increased PI3KCA, AKT, and RHOA expression, whereas inter-implantation site displayed increased PTEN expression; AKT was most strongly expressed in the stromal cells and luminal epithelium of the implantation site, while phosphorylated AKT (S473) was strongly expressed in stromal cells of the implantation site (Liu *et al.* 2014). Additionally, the use of contralateral intrauterine injection of LY294002 drastically reduced the number of implanted embryos. These results suggest that the phosphorylation of AKT is important in the process of implantation. Furthermore, these results were not observed when using pseudopregnant mice, allowing us to think that the crosstalk between endometrium and embryo is important in this process. In contrast to these observations, death effector domain-containing protein (*Dedd*)^{-/-} mice implantation sites exhibited decreased AKT protein levels, accompanied by a structural disintegration of the sites and implantation failures; forced expression of AKT1 partially restored polyploidy of the decidual cells, an important marker of successful decidualization, and thus, receptive endometrium (Mori *et al.* 2011). AKT has also been shown to regulate endometrial cell migration in response to chemoattractants (PDGF-BB, EGF, and FGF2) as well as 17 β -estradiol (E₂), further cementing AKT role in the dynamic process of decidualization (Gentilini *et al.* 2007). In farm animals, a very recent report has shown that IGF1 provided a crucial stimulatory signal in primary porcine trophectoderm cells (pTr), through the PI3K/Akt pathway. The study evidenced rapid activation of AKT1 upon IGF1 administration, followed by RPS6 activation, a downstream target of mTOR acting as a protein synthesis regulator. The results also suggested that the ERK1/2 MAPK pathway is dependent upon the activation of PI3K/Akt in this context (Jeong *et al.* 2014a). Another recent report concerning porcine implanting embryos suggested that vascular endothelial growth factor (VEGF; a signal protein capable of stimulating angiogenesis as well as acting as a growth factor), secreted by the receiving endometrium as well as the implanting conceptus, is responsible for the increased migration and proliferation of pTr cells through the concomitant activation of the PI3K/Akt and MAPK pathways. Again, VEGF induced rapid activation of AKT1 as well as RPS6; this activation was dependent upon PI3K activity as well as mTOR and MAPK activity, as shown by the pharmacological inhibition experiments the authors conducted (Jeong *et al.* 2014b). Finally, colony-stimulating factor 2 (CSF2), a critical cytokine responsible for proliferation and differentiation of granulocytes and macrophages, is also suggested as an activator of the PI3K/Akt pathway. CSF2 levels were highly enriched during day 10 to day 14 in the pregnant

porcine endometrium; using pTr cells, CSF2 has also been shown to activate the PI3K/Akt pathway, the MAPK pathway and, ultimately, RPS6 through mTOR activation (Jeong *et al.* 2014c). All the above-mentioned studies report that ERK1/2 activation was dependent upon AKT1 activation. It should also be noted that, in the porcine context, CSF2 is thought to be secreted in response to conceptus-derived estrogens. Similarly, heightened levels of CSF2 are also found in the bovine uterus during the peri-implantation period through the action of interferon tau (IFNT; Emond *et al.* 2000, Michael *et al.* 2006); conversely, the ovine uterus secretes CSF2 during the same period, which in turn enhances IFNT secretion by the trophoblast (Imakawa *et al.* 1993). These results suggest that regulation of the PI3K/Akt and MAPK pathways by CSF2 could be relevant to other species. Taken together, these results suggest that implantation mechanisms depend on a highly coordinated crosstalk between the PI3K/Akt and MAPK pathways, resulting in the pivotal activation of the mTOR pathway. Both of these networks are thus hypothesized to be crucial in the establishment of reciprocal communication between the implanting embryo and the receiving endometrium.

Decidualization

During each reproductive cycle, the endometrium undergoes extensive and crucial transformations. The window of receptivity required for implantation to occur is narrow; the endometrium needs to be aptly prepared for implantation through the intricate process of decidualization. Decidualization is initiated through the activation of the cAMP pathway (Gellersen & Brosens 2003) by the binding of various ligands to a G protein-coupled heteromeric receptor, inducing the production of cAMP in the endometrial stromal cells. Endocrine signaling such as E₂ or progesterone are both capable of inducing decidualization by their own. However, the occurrence of decidualization is considerably delayed (Brar *et al.* 1997, Brosens *et al.* 1999), when these hormones act without concomitant cAMP signaling; priming by cAMP signaling can thus be considered vital to endometrial cell sensitivity to progesterone.

This process is characterized by extensive differentiation and profound changes in cell function. The most critical change observed in this transformation is the transition of endometrial stromal fibroblasts to secretory, glycogen-filled, epithelial-like cells. This change is accompanied by the secretion of various proteins such as prolactin (PRL) as well as insulin-like growth factor-binding protein 1 (IGFBP1). PRL has been shown to be an inhibitor of apoptosis in various tissues (Al-Sakkaf *et al.* 2000, Gado *et al.* 2002, Krishnan *et al.* 2003) as well as an increasingly relevant tumor biomarker in gynecological cancers (Asai-Sato *et al.* 2005, Levina *et al.* 2009). The antiapoptotic effects of PRL on decidual cells also appear to be AKT dependent. The PRL pathway exerts its survival

effect through the activation of both MAPK and PI3K (Rao *et al.* 1995, Yamauchi *et al.* 1998), and thus, AKT. Additionally, PRL has been shown to induce phosphorylation of Akt (Ser473) as well as its transit from the cytoplasm to the nuclear compartment (Tessier *et al.* 2001). The role of AKT in decidualization is still vastly unclear and under investigation by various groups. Cultured human endometrial stromal cells undergoing decidualization through estrogen and progesterone supplementation were shown to exhibit a lowered level of phosphorylated AKT (Ser473), indicating that the phosphorylation of AKT might inhibit this process (Yoshino *et al.* 2003). Interestingly, this inhibition did not appear to be hormone dependent but cAMP dependent. We have also previously shown that both TGFβ2 and TGFβ3 could decrease AKT phosphorylation (S473) in rat decidual cells, further involving the decrease in activated AKT with the decidualization process (Caron *et al.* 2009). Finally, *Dedd*^{-/-} mice were reported to exhibit reduced decidual cell polyploidy probably due to lowered AKT stability; forced expression of AKT1 partially rescued the phenotype (Mori *et al.* 2011).

It has been demonstrated that the cAMP pathway is necessary in both the initiation and the maintenance of decidualization (Brosens *et al.* 1999, Tierney *et al.* 2003), through the activation of various signaling pathways. Insulin, IGF1, and IGF2 have been found to induce the phosphorylation of AKT (S473) in decidualized cells (Capp *et al.* 2011). On the other hand, decidualization has been linked to the reduced level of phosphorylated AKT and we demonstrated that phosphorylation of all three AKT isoforms is reduced during decidualization (Grenier K and Asselin E, unpublished work; Yoshino *et al.* 2003); incidentally, both MPA and progestins have been shown to reduce AKT phosphorylation. This reduction has been shown to be responsible for the increased FoxO1 translocation to endometrial stromal cell nuclei. This effect can be explained by the inhibition of AKT-dependent phosphorylation of FoxO1 which usually results in its cytoplasmic sequestration (Labied *et al.* 2006, Zhang *et al.* 2011). As FoxO1 is of crucial importance in the regulation of progesterone response in endometrial cells (Chen *et al.* 2011, Kajihara *et al.* 2013), it is plausible that the modulation of AKT activity is of crucial importance in the establishment of decidualization. AKT is further involved in the decidual cell response to progesterone by stimulating the expression of RRM2, a rate-limiting enzyme in the deoxynucleotide production for DNA synthesis, which could induce the characteristic cell proliferation found during mice decidualization (Lei *et al.* 2012). However, the specific mechanisms by which each AKT isoform acts in the process of decidualization remain yet to be elucidated.

The dichotomy between cAMP and PRL pathway effects on AKT activity in the decidualization process remains yet to be elucidated; it is possible that an overall reduction of phosphorylated AKT has been observed

because of its preferential localization to the nucleus at the exclusion of other compartments. In this context, the reduced amount of total AKT would not equate to lowered activity; the concentrated levels of AKT could signal in the nucleus despite cytosolic presence. Furthermore, as the effect of nuclear AKT is vastly unclear, we hypothesize that specific AKT isoforms, displaying distinct cellular sublocalization, possess differential targets and activation pathways.

Endometriosis

Endometriosis is a very common gynecological disorder, which is estrogen dependent as well as inflammatory in nature, prevalent in 10–15% of reproductive aged women (Olive & Pritts 2001). This disease is associated with chronic pelvic pain as well as infertility. The ectopic endometrial implants can be found most commonly on the ovaries, in the Pouch of Douglas, on the uterus, and in the fallopian tubes. The most widely accepted pathogenesis mechanism is a retrograde flow of menses, which would deposit still viable endometrial cells at anomalous locations; this process requires the endometrial cells to attach to the peritoneal mesothelial cells and establish vasculature to permit their proliferation (Sampson 1927, Nunley & Kitchin 1980, D'Hooghe 1997, Macer & Taylor 2012, Sundqvist *et al.* 2012). It is clear, however, that molecular mechanisms are integral in the disease as retrograde menstruation is common (Lucidi *et al.* 2005).

It has been shown before that the PI3K/Akt signaling pathway is abnormally activated during endometriosis and that this activation was partly responsible for the endometriotic cells' reduced decidual response (Matsuzaki *et al.* 2005, Cinar *et al.* 2009); serial analysis of gene expression revealed an increased level of both PI3K and phosphorylated AKT (S473) in endometriotic tissues, probably through the enhanced expression of GAS6, AXL, and ACTN4, responsible for enhanced proliferation and motility (Honda *et al.* 2008). On the other hand, AKT was not involved in endometriotic cells' expression of EMT markers in response to TNF α , a crucial component of inflammation in this disease (Berkkanoglu & Arici 2003, Grund *et al.* 2008). Another study highlighted the link between high levels of phosphorylated AKT (S473) an increased Survivin expression, suggesting that Akt permits cell survival and apoptosis avoidance (Zhang *et al.* 2009). According to its hormone-driven nature, E₂ was shown to promote endometriotic cell proliferation through the reduced expression of PTEN and the subsequent activation of AKT (Zhang *et al.* 2010).

Recent studies using high-throughput methods have shown that AKT1 expression was highly elevated in early stages of endometriosis while moderate in later stages of the disease. Higher expression of AKT1 was also linked to dramatically reduced fertility in normal patients. AKT1 was noted to be overwhelmingly located in the

cytoplasm of both glandular and stromal cells (Laudanski *et al.* 2009). Interestingly, studies have linked endometriosis to infertility due to progesterone resistance and failure of endometrial cells to properly decidualize (Bulun *et al.* 2006). As AKT activation is a hallmark of endometriosis and the inhibition of PI3K and AKT allowed for increased response to MPA and cAMP, it is possible that AKT plays a role in this process; abrogating AKT phosphorylation may reinstate normal localization of FoxO1 and allow for proper decidualization, enhancing fertility of endometriosis stricken women (Engelman 2009, Samartzis *et al.* 2013).

Increased and decreased expression of phosphorylated AKT (S473) is usually concomitant with similar changes in the activity of mTORC1, leading us to think that one of the primary effect of AKT in endometriosis is the activation of the mTOR pathway (Lucidi *et al.* 2005, Honda *et al.* 2008, Zhang *et al.* 2009). The NF κ B pathway also seems to be entwined in the hormonal response as well as survival mechanisms in which AKT is involved (Grund *et al.* 2008, Zhang *et al.* 2010, Capp *et al.* 2011). It seems highly plausible that these proteins are all part of a complexly intertwined signaling network as links have been extensively demonstrated among the mTOR complex, the PTEN/PI3K/Akt axis, and the NF κ B signaling pathway.

Cancer

Multiple malignant tumors can develop in the uterus. However, considering that EC is the most common gynecological malignancy in North America, it will be the focus of this section of the review. Multiple risk factors are linked to the development of this type of tumors, namely obesity, use of tamoxifen adjuvant therapy for the treatment of breast cancer, a family history of polycystic ovarian syndrome, nulliparity, and increased age; 95% of cases will occur in women aged over 40 years. These risk factors are also distinctly linked to each subtype of EC; this will briefly be discussed further in this section (Arora & Quinn 2012).

EC is usually detected at early stages, enabling prompt and effective treatments using a combination of surgery, radiotherapy, and chemotherapy; however, more than 25% of primary endometrial tumors exhibit metastasis at the moment of detection. This aggressive subtype of tumors presents the most important therapeutic challenge, as they are disseminated and frequently chemoresistant (Muss 1994, Humber *et al.* 2007). Endometrial carcinomas are generally classified following Bokhman's categorization specifying two major types of endometrial carcinomas, type 1 and type 2 tumors (Bokhman 1983). Type 1 malignancies are generally adenocarcinomas of endometrioid nature and represent the wide majority of endometrial tumors (~80%); they usually arise in younger perimenopausal women displaying an atypical hyperplasia, are linked

to the presence of high levels of estrogens, and do not display highly invasive phenotypes. Type 2 tumors, however, regroup clear cell, squamous, and undifferentiated carcinomas (Ko *et al.* 2014). These various diseases do not share the risk factors associated with type 1 tumors and present a very bleak prognosis. Finally, while type 2 tumors are often associated with TP53 mutations, type 1 tumors are overwhelmingly associated with PTEN/PI3K pathway mutations (Bansal *et al.* 2009, Wild *et al.* 2012). Increased activity of the AKT/PI3K/mTOR pathway has also been shown to be linked to poor prognosis, regardless of the histological type (Salvesen *et al.* 2009). Considering its larger incidence and the scope of this review, we will focus thereafter on type 1 EC.

Considering the very high proportion of ECs displaying *PTEN* and *PI3K* mutations, we are allowed to think that the PI3K/PTEN/Akt axis is of primordial importance in the initiation of the disease (Fig. 3). However, recent data obtained from high-throughput mass spectroscopy and RPPA approaches revealed that *Akt1* was mutated in only 1% of ECs, while no mutation hotspots were

detected for both *Akt2* and *Akt3*. This suggests that AKT's effect in EC initiation and progression does not emerge from gain-of-function mutations but rather from the activation of upstream proteins. However, while the great majority of ECs display multiple mutations in the PI3K/PTEN/Akt pathway, mutation in *Akt1* has been demonstrated to be sufficient for oncogenic transformation (Shoji *et al.* 2009).

Progesterone has been long known as a crucial antagonist of EC progression through a variety of mechanisms, notably through cell cycle arrest and apoptosis induction (Yang *et al.* 2011). However, progestin resistance is a major hurdle in the treatment of EC. The PI3K/Akt pathway has been shown to be activated in progesterone receptor (*PGR*)-deficient cell lines following MPA treatments, an effect that was reversed when reinstating *PGR* expression. These data suggest that the loss of *PGR* could potentially induce progesterone-induced apoptosis resistance and instead promote a *PGR*-independent, Akt-driven survival pathway (Gu *et al.* 2011). Further data have confirmed

Figure 3 *PTEN* and *PIK3CA/PIK3CB/PIK3CD/PIK3CG* are highly mutated in endometrial carcinomas: the histogram shows the frequency of mutations, deletions, amplifications, and multiple alterations in endometrial cancer. An exceedingly high number of tumors display mutations in one of the five genes studied. The histogram was generated using cBioPortal and is produced from 69 distinct studies.

AKT involvement in this pathway; the use of MK-2206, a novel AKT inhibitor, has been shown to increase progesterin response in EC (Pant *et al.* 2012). On the other hand, estrogen is of prime importance in the development of EC; its unopposed use is a primary risk factor of this disease. Evidences have shown that E₂ induced the phosphorylation of AKT (S473) through PI3K as well as estrogen receptor alpha (ER α ; Guo *et al.* 2006). Furthermore, loss of *PTEN* has been shown to activate AKT, which in turn is responsible for the phosphorylation of ER α on S167; this phosphorylation subsequently activates it (Vilgelm *et al.* 2006).

As stated previously, chemoresistance is a major challenge in the treatment of EC. Studies using chemoresistant cell lines and RNAi have been conducted to assess the effect of individual AKT isoforms on drug resistance. Results have shown that while all three isoforms were necessary for optimal cell proliferation, apoptosis evasion, and cell survival, AKT2 was most crucial in chemoresistance (Girouard *et al.* 2013); this is in accordance with previous studies which suggested that elevated AKT2 activation following cisplatin treatment was responsible for Bcl2 activation and reduced apoptosis (Rouette *et al.* 2012). Finally, we have previously shown that AKT regulates PTGS2 expression in EC through the activation of the NF κ B pathway; this, in turn, enables enhanced angiogenesis, tumor invasiveness, and potentially chemoresistance (St-Germain *et al.* 2004a,b, Chaudhry & Asselin 2009).

The role of nuclear AKT in cancer has not been extensively investigated as yet. Furthermore, studies showing a link between nuclear accumulation of AKT and cancer progression have been focused on pan-AKT, without regard to isoform specificity (Martelli *et al.* 2012). Using tissue microarrays, we have recently demonstrated that the nuclei of normal endometrial cell were highly enriched with AKT3, as well as AKT1, albeit at a reduced level; this nuclear enrichment was inverted, with AKT1 being the predominant isoform at the nucleus, upon grade progression of endometrial carcinomas; nuclear localization of AKT was altogether abrogated at a high grade. We could not detect nuclear AKT2 (Fabi F and Asselin E, 2014, results not shown). These results suggest that localization of AKT isoforms may be involved in grade progression of ECs; the nuclear exclusion of AKT3 might lead to dysregulation of normal signaling cascades while the nuclear localization of AKT1 could induce abnormal growth and migratory signals.

Conclusion

In this review, we have highlighted the major known roles in various reproductive and pathological processes involving AKT isoforms in the uterus. We believe that we have outlined the need for further studies in the expression and activation of AKT isoforms in reproductive process; the interaction between AKT and its partners is

still widely unexplored. The role of AKT in the complex network of hormonal signaling is also still unclear. The functional redundancy of AKT isoforms is beginning to emerge, but the distinct targets and partners enabling isoform-specific mechanisms are still unclear. We believe that a deeper understanding of this crucial network could allow for better treatments against infertility, endometriosis, and EC.

Declaration of interest

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

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