Signalling pathways in endometrial cancer

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Carcinogenesis is a multistage process, during which the activity of signalling pathways responsible for cell cycle regulation and division is disrupted which leads to inhibition of apoptosis and enhanced proliferation. Improper activation of Wnt/β-catenin and PI3K. Akt pathways play essential role in endometrial cancers (EC), mainly type I. Mutations in *APC*, *axin* or *CTNBB1* may lead to β-catenin overactivation leading to excessive gene expression. PTEN inactivation, mutations in the *PIK3CA* or *Akt* result in increased transmission in the PI3K/Akt pathway, apoptosis inhibition, intensive cell division, mTOR excitation. In non-endometrioid cancers, key mutations include suppressor gene *TP53* responsible for repairing damaged DNA or apoptosis initiation. Irregularities in gene *P16*, encoding a protein forming the p16-cyclinD/CDK-pRb have also been described.
Understanding the complex relations between specific proteins taking part in signal transduction of the abovementioned pathways is key to research on drugs used in targeted therapy.

Key words: endometrial cancer, signalling pathways, Wnt/β-catenin, Akt/PI3k pathway, p53, p16.

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Introduction

Signalling pathways are complex processes of signal transduction involving mutual activation of a protein cascade transmitting a signal from activated receptors from the cell surface to the cytoplasm and nucleus.

This mechanism involves receptor and non-receptor tyrosine protein kinases (RTKs and NTKs, respectively) that take part in many biological processes of normal and cancer cells, such as growth, differentiation, cell-cycle control, cell adhesion and transcription regulation, as well as metabolic pathway control. Signal transmission occurs in a multistage way; it involves ligand binding, receptor dimerisation, autophosphorylation and initiation of signal cascades, which leads to a change in the conformation and activity of the proteins involved in the signalling pathway. Kinases are positively activated by cyclins (a large proportion of A to T cyclins and their isoforms have been identified) and cyclin dependent kinases (CDKs), of which at least nine have been recognised (CDK1-9).

Receptors are composed of several domains that vary in function; the ligand binding site is located in the extracellular (*N*-terminal) domain. The domain is responsible for dimerisation of the ligand-receptor complex. Anchoring of the receptor occurs in the transmembrane domain, whereas the intracellular domain with the *C*-terminal fragment modulates receptor activity by binding the ATP coenzyme and the substrate.

Protein kinase activity may be repressed by CDKI inhibitors blocking ligand binding regions or by preventing active dimmers from emerging, via attachment to a receptor's exterior domain. They may also influence the function of receptors' intracellular domains, through inhibition of ATP binding sites, which blocks signal transmission. All of the mentioned signal transduction elements may be targets of targeted cancer therapy [1, 2].

Endometrial cancer (EC) is one of the most common malignant cancers of female reproductive organs. Depending on geographical region, 10–20 per 100 000 women develop this type of cancer annually. Common oestrogen-dependent type I and rarer oestrogen-independent type II are distinguished. EC occurs most often as a sporadic cancer, although women with HNPCC syndrome (hereditary non-polyposis colorectal carcinoma) may be affected. Molecular studies on the pathomechanism of the development of this cancer have been underway for more than 15 years [3].

The disruptions in Wnt/β-catenin and Akt/PI3K/mTOR pathways, together with mutations in *TP53* and *P16*, take part in endometrial cancer development. Detailed knowledge of the steps of these complex signalling transduction pathways, as well as cross connections between them, is necessary in order to understand the mechanisms of action of existing targeted therapy drugs and to discover new ones.

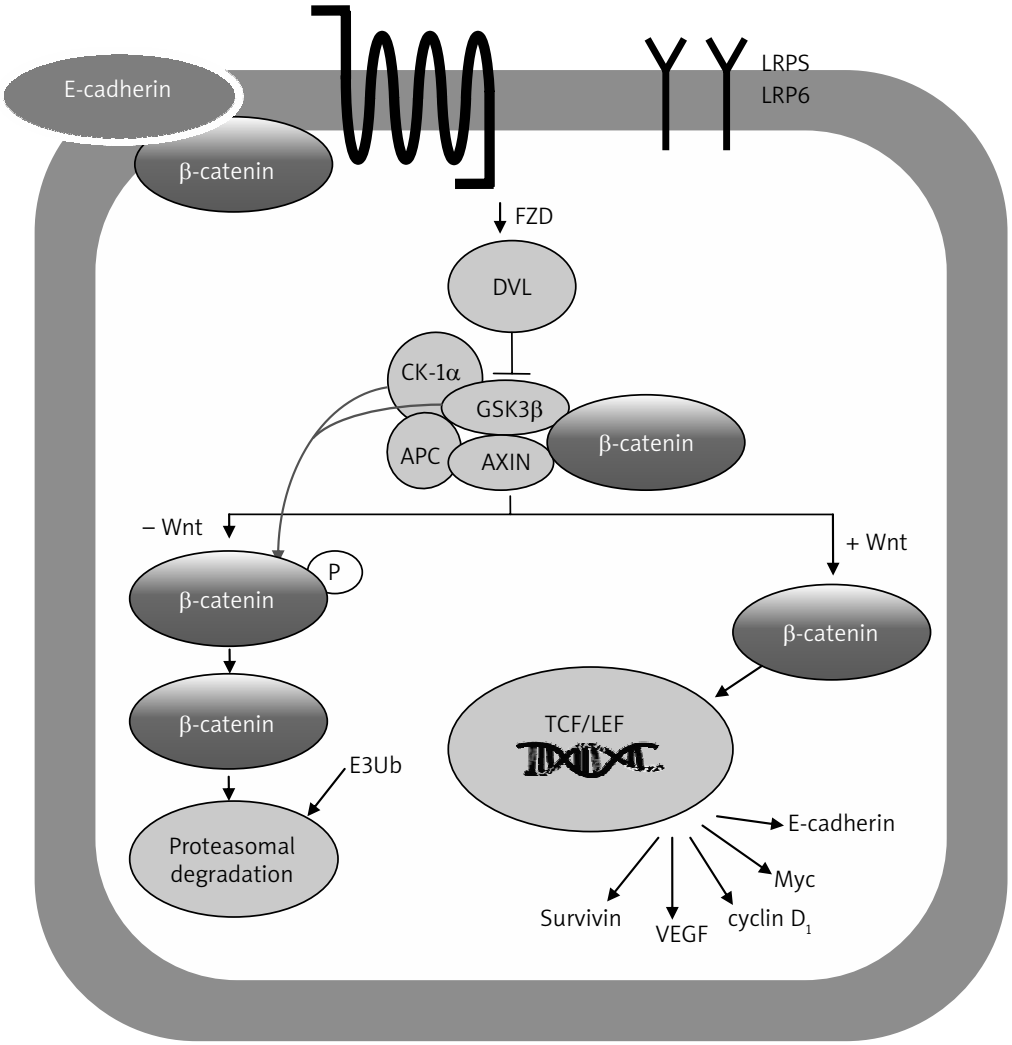
Wnt/β-catenin signalling pathway

The name comes from two acronyms: WG, *Wingless*, a mutation of the common fruit fly (*Drosophila melanogaster*), whose gene was described in 1987 and

INT-1 (integration 1), the name of a gene described in 1984, which turned out to be a homolog of the Wg gene. The gene was activated during a MMTV-induced (mouse mammary tumour virus) mouse breast cancer [4, 5]. The Wnt-dependent signalling pathway is conserved through evolution and takes part in embryo- and carcinogenesis; it is active in different species, from the common fruit fly to humans. The pathway is made up of a protein net that includes – up until now – 19 Wnt ligands and 10 members of the Frizzled (FZD) receptor family. Wnt proteins belong to the conservative family of proteins that are secreted by cells and remain in strict contact with extracellular matrix proteins. They activate various paths, including canonical (classical, evolutionary-conserved) and two noncanonical paths, one of which is calcium ion-dependent; the pathway regulates cell motility and adhesion independent of the canonical pathway [6–8].

The key protein of the canonical Wnt pathway is β-catenin – in humans encoded by the *CTNBB1* gene, also, in combination with E-cadherin, which takes part in cell adhesion regulation. The Wnt proteins, especially those with oncogenic potential, bind with the seven-transmembrane domain receptors FZD in the presence of co-receptors: LPRs – members of low density lipoprotein receptor family (LDL – receptor related protein) and LRP6, known as the Arrow receptor in common fruit fly [5, 6].

After the FZD receptor is activated by the Wnt proteins, the β-catenin and APC (adenomatous polyposis coli)/GSK3β (glycogen synthase 3β)/Axin complex binding is inhibited by the Dishevelled (DSH) protein. Simultaneously, β-catenin phosphorylation is suppressed, mainly by glycogen synthase kinase (GSK-3β), as well as casein kinase 1α (CK1α). The complex formed by β-catenin with APC, GSK-3β and Axin is recognised by E3 ubiquitin and degraded in proteasomes – the Wnt pathway is inactive. Free, unphosphorylated β-catenin is stabilised and translocated to the nucleus, and it assembles into a complex with transcription factors lymphoid enhancer factor (LEF) and T-cell factor (TCF), promoting expression of many target genes, including *Myc* or *cyclin D* proliferation genes, *VEGF, E-cadherin* (responsible for cells adhesion), *survivin* (inhibition of apoptosis) and many other genes involved in cancer development (including endometrial cancer) [5, 6, 8–10] (Fig. 1).



LRPS – coreceptor of the low-density lipoprotein receptor family; LRP6 – coreceptor, known as Arrow; FZD – Frizzled receptor; DVL – Dishevelled protein; GSK3β – glycogen synthase kinase-3 β; APC – adenomatous polyposis coli gene; CK-1α – casein kinase 1α; P – phosphorylation; E3 Ub – E3 ubiquitin ligase 3; TCF – nuclear transcription factor; TEF – nuclear transcription factor

Fig. 1. Wnt/β-catenin pathway

Abnormal activation of the Wnt/β-catenin signalling pathway plays a crucial role in EC onset. Around 40% of ECs show irregularities in the pathway, and most cases concern endometrioid cancer. Mutations in *APC*, *axin* or *CTNBB1* itself may lead to proteins abnormal stabilisation, translocation to the nucleus, accumulation and overactivation.

In the proliferation phase of the menstrual cycle, β-catenin is localised mainly in the nucleus, whereas in the secretion phase it is mainly in cytoplasm and the cell membrane [14–16]. It has been demonstrated in animal models that oestrogen increases Wnt4 and Wnt5, as well as the FZD-2 receptor expression, resulting in stabilisation of β-catenin in cytoplasm and the presence of active β-catenin in nucleus. This mechanism operates independently of oestrogen receptors in the nucleus [17].

Studies on stable Ishikawa EC cell lines have shown that progesterone inhibited Wnt signalling [16]. Research in EC tissue has proven that Wnt10b protein expression was significantly higher in EC tissue in comparison to hyperplastic endometrium. Additionally, there is a difference in Wnt10b concentration depending on the histological type of cancer, cell maturity, FIGO stage and lymphatic metastasis. Increased Wnt10b concentration correlated with a better prognosis in EC patients. Studies on cell lines have confirmed that Wnt10b promotes proliferation and suppresses apoptosis by activation of β-catenin and c-myc as well as APC inhibition. No effect of Wnt10a on the Wnt/β-catenin pathway was observed [18]. Studies by van der Zee *et al*. [14] demonstrated that the suppressor gene *PTEN*, crucial in many other signalling pathways, is also linked to the Wnt/β-catenin pathway; loss of its function is often associated with endometrial cancer, and a defect in the function of the APC that forms the β-catenin "destruction complex" accelerates the development of cancer stimulated by PTEN loss. Simultaneous loss of APC and PTEN was associated with an earlier onset of cancer and a more aggressive course. The data proved a synergistic effect between PTEN and Wnt/β-catenin signalling. Constitutive Wnt/β-catenin pathway activation results in squamous cell metaplasia (SCM), with no malignant transformation. Therefore, it seems that the pathway activation promotes rather than initiates the disease, whereas simultaneous Wnt/β-catenin activation and PTEN function loss is linked with development of an aggressive form of EC [14].

One transcription factor, SOX7, has been described as a strong negative regulator of the Wnt/β-catenin pathway; it can inhibit the transcriptive ability of the Wnt/β-catenin complex with TCL/LEF in the nucleus, lowering the expression of β-catenin target proteins, cyclin D1 and C-myc, involved in cell proliferation and associated with the regulation of expression of many genes [13].

Mutations in *APC* and *CTNBB1* have also been proven to have little effect on the development of recurrences and distant metastasis that occur due to lack of adhesive E-cadherin expression [19].

A number of therapies targeting the Wnt/β-catenin pathway are currently being studied. Their action consists of blocking Wnt protein binding to receptors, suppressing the DSH protein, activating E-cadherin, inhibiting proteasomal degradation of β-catenin or therapeutic use of inhibitors of the Wnt pathway, such as SFRP protein (secreted Frizzled-related protein) [5, 9, 11–13].

Last year, trials took place utilising inhibition of this pathway with a monoclonal antibody OMP-18R5 (inhibition of receptor FZD7) as well as inhibition of β-catenin binding to the TCF complex to promote expression of other genes involved in carcinogenesis [20].

Akt/PI3K signalling pathway

Activation of the Akt/PI3K pathway occurs through binding of ligands, i.e. growth factors, cytokines or insulin, to cell membrane receptor tyrosine kinases, leading to autophosphorylation of their intracellular domain. This allows for conformational changes and subsequent phosphatidylinositol 3-kinase (PI3K) activation, a heterodimeric protein consisting of a catalytic (p110) and regulatory (p85) subunit [21]. Depending on differences in structure, three classes of PI3K have been distinguished (I, II and III), with class I, being crucial in the onset of many cancers, the most thoroughly researched. PI3K enables transformation of phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3), which in turn, via phosphoinositide-dependent kinase 1 (PDK1), allows for phosphorylation and activation of the Akt protein. PTEN and INPP4B exert a contradictory effect in cells, transforming PIP3 to PIP2 and PIP2 to PIP1, respectively.

Akt, also known as protein kinase B (PKB), can also be activated as an answer to cell hypoxia, hypoglycaemia, free radical activity and many others. Three Akt isoforms have been identified: Akt1 and Akt2 are present in the majority of tissues and organs, whereas overexpression of Akt3 is limited to the brain, lungs, testicles, heart and skeletal muscles. Akt physiological activity is multidirectional; through phosphorylation of individual proteins, it regulates the cell cycle, inhibits apoptosis and promotes protein synthesis and cell proliferation as well glucose metabolism.

One of the main roles of Akt is the promotion of cell proliferation and inhibition of apoptosis. By binding phosphate radical to caspase 9 it inhibits activity of the protein, preventing the cell from entering the apoptotic pathway. Interaction with BCL2 antagonist of cell death (BAD) creates a similar effect, resulting in dissociation of BAD/Bcl2-related protein long isoform (BclXL) complex, which enables BclXL to inhibit the translocation of cytochrome c from mitochondria to cytoplasm [22].

GSK3 phosphorylation and inactivation by Akt leads to inhibition of phosphate radical binding to cyclin D and E as well as transcription factors c-Jun and c-Myc and thus protects them from proteolytic degradation. As a consequence, active forms of the above-mentioned proteins accumulate in the cell, which pushes the cell from G1 to S phase giving a "green light" to further cell division [23, 24]. Akt also inhibits cyclin-dependent kinase inhibitors p21 and p27, limiting their transfer from cytoplasm to the nucleus, which is conducive to cell proliferation [25, 26]. Moreover, Akt regulates carbohydrate balance via an increase in expression of the glucose transporters GLUT1 and GLUT3, and it facilitates GLUT4 translocation to the

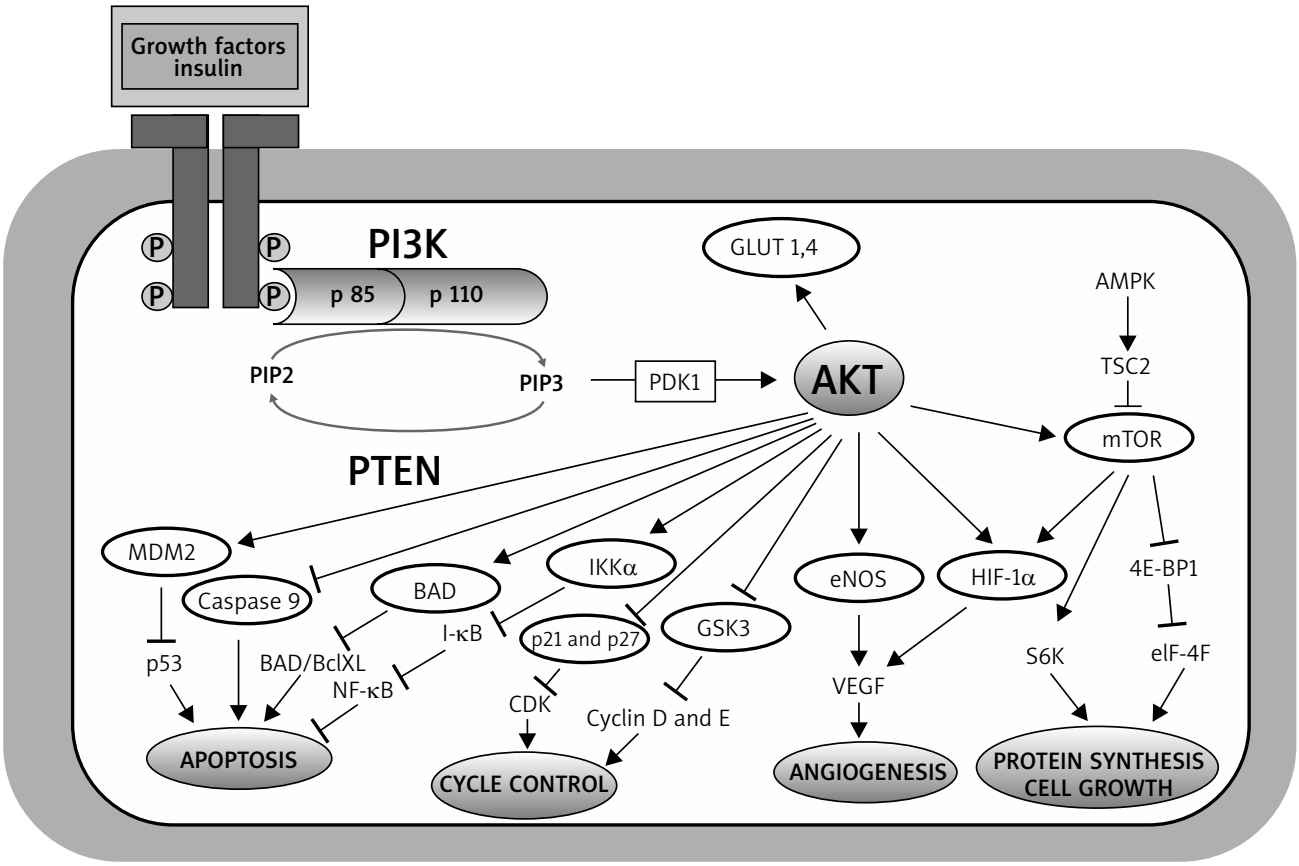
cell membrane, contributing to an amplified influx of glucose to the cell.

Upon Akt activation, growth factors (PDGF, VEGF and IGF-1) and insulin block the TSC2 (tuberous sclerosis complex 2) complex, which in turn inhibits the mTOR protein (mammalian target of rapamycin). A lack of the TSC2 inhibiting effect results in mTOR activation, which regulates growth processes, increases protein synthesis and angiogenesis and promotes cell division via its effector proteins, ribosomal protein S6 kinase (S6K) and eukaryotic initiation factor 4E-binding protein (4E-BP1). Metformin, a biguanide commonly used in the treatment of diabetes type 2, activates AMP-activated protein kinase (AMPK), crucial for proper cell energy balance, via LKB1 (liver kinase B1), leading to TSC2 phosphorylation and subsequent mTOR inhibition [27, 28] (Fig. 2). Many clinical trials indicate metformin as being highly effective in cancer prevention among diabetic patients and show its beneficial effect in supporting oncological treatment [29, 30]. Preliminary results of research on different rapamycin analogues also seem promising.

Increased PI3K/Akt pathway activity is diagnosed in many human cancers as a result of overexcitation at the receptor level, loss of inhibiting function of PTEN as well as amplification or mutation in *PI3K* or *Akt* genes.

Irregularities in the *PTEN* suppressor gene, leading to its inactivation, are one of the best recognised genetic aberrations in EC. Somatic point mutations are the most common; however, germinal mutations of the gene are also described – these are present in Cowden syndrome and result in a 10% risk of endometrial cancer. Lowered PTEN expression may also stem from promoter deletion or its hypermethylation. PTEN inactivation is thought to affect 37–61% of endometrial cancer of endometrioid type [31] and occurs at an early stage of carcinogenesis, as evidenced by lowered expression in endometrial hyperplasia. Only a small percentage of type II endometrial cancers (up to 10%) show abnormalities in the gene. Phosphatase and tensin homologue deleted from chromosome 10 inactivation leads to an inability to disengage phosphate radicals from PIP3 and Akt overactivation, resulting in apoptosis inhibition and increased cell proliferation. The results of clinical trials assessing the effect of irregularities in the *PTEN* gene on patient prognosis remain ambiguous.

The *PIK3CA* gene encodes the catalytic subunit PI3K (p110α); its mutations, which lead to overactivation of Akt, are commonly observed in endometrial cancer. Genetic disorders in the *PI3K* region are considered typical for endometrioid cancer and may be present in as much as 26–36% of cases [32]. The mutations are mostly located in exon 9 and 20, with endometrial cancers of higher histological malignancy more often demonstrating mutation in exon 20 [33]. A study by Oda *et al.* [34] confirmed



PTEN – phosphatase and tensin homologue deleted from chromosome 10; PI3K –phosphoinositol 3-kinase; IKKα – IκB kinase α; MDM2 – mouse double minute 2 homolog; BAD – BCL2 antagonist of cell death; GSK3 – glycogen synthase kinase-3; eNOS – nitric oxide synthase; HIF-1α – hypoxia inducible factor 1α; GLUT 1, 4 – glucose transporters 1, 4; AMPK – 5' adenosine monophosphate-activated protein kinase; TSC2 – tuberous sclerosis gene 2; VEGF – vascular endothelial growth factor; BAD/BclXL – Bcl2 related protein long isoform; CDK – ; S6K – S6 kinase; eIF-4E – eukaryotic initiation factor-4E; 4E-BP1 – eukaryotic initiation factor-4E binding protein; PDK1 – phosphoinositide-dependent kinase; PIP2 – phosphatidylinositol-3,4-bisphosphate; PIP3 – phosphatidylinositol-3,4,5-triphosphate; mTOR – mammalian target of rapamycin, NK-κB – nuclear factor κB

Fig. 2. PI3K/Akt signalling pathway

a higher percentage of *PIK3CA* mutations in endometrial cancer samples in the simultaneous presence of the *PTEN* gene mutations (46% vs. 24% of cancers with normal *PTEN* gene). Simultaneous assessment of the presence of *p53* and *PIK3CA* mutations in endometrial cancer samples confirmed that the coexistence of both mutations is more common in endometrioid cancers of low cell maturity in comparison to clear cell and serous carcinomas [33]. Recent studies demonstrate a high rate (about 43%) of type I endometrial cancers with diagnosed mutations in *PIK3RI*, a gene encoding the p85α PI3K subunit. Such mutations are found in 12% of type II endometrial cancers [35].

Common abnormalities in the PI3K/Akt pathway, found in many types of cancerous cells, were gave rise to the launch of trials to find particles that could block overexcitation. Additionally, knowing that most endometrial cancers develop from hyperplasia due to oestrogen/progesterone imbalance, attempts have been made to utilise the drugs' positive effect in the promotion of progesterone receptor synthesis. A study by Pant *et al.* [36] in an animal model found increased PRβ progesterone receptor expression after administration of the Akt inhibitor MK-2206 and a reduction in tumour volume after progestogen treatment. Increased PR expression in Ishikawa cells was also obtained after administration of LY294002, a particle inhibiting PI3K [37]. Currently, a number of stage I and II clinical trials are underway that assess the effectiveness of therapies utilising PI3K/Akt pathway inhibitors in women with advanced or recurrent endometrial cancer.

p53 and p16

Apoptosis disorders are among the many mechanisms significant in carcinogenesis. One of the most extensively researched genes that takes part in apoptosis is the *TP53* gene located on the short arm of chromosome 17 (17p13), also known as "the guardian of the cell", which reflects its crucial role as a "guardian" of genome stability. The signalling pathway is activated in response to stress, including hypoxia, telomere shortening and DNA and spindle apparatus damage [38, 39]. Abnormal p53 protein production due to gene mutation leads to an inability to repair the damaged DNA and as a result, via complex mechanisms, to uncontrollable cancer cell growth. Mutations affect more often non-endometrioid cancers, most commonly serous carcinoma, as confirmed by Lax *et al.* and Ragni *et al.* [40–42]. The presence of mutations in gene *TP53* occurs in 17–61% of endometrioid cancers and 93–100% of serous type. Mutations in the *TP53* gene are associated with statistically significant shorter patient survival [43, 44].

P53 is a suppressor protein involved in *IGF-IR* gene expression regulation via Sp1 transactivator protein. The cell lines under study were characterised by varied expression of p53 protein: the USPC-1 line demonstrated a high expression of p53, the USPC-2 line a low one. It was found that expression of Sp1 protein was much higher in USPC-2 cells. The dependencies described are related to the possible use of cixutumumab, a human antibody against IGF-IR. The results of clinical trials show a significant decrease in USPC-1 cell line proliferation in comparison to the control group, a phenomenon not noted in the USPC-2 line [44].

The *P16* gene, also known as *MTYS1* or *INK4A*, has also been attributed to playing a significant role in the development of endometrial cancer. It seems that in the early stages of carcinogenesis of endometrial cancer, the p16-cyclin D/CDK-pRB signalling pathway also plays a role. The p16 protein is responsible for cell proliferation in the G1 phase. The cyclin D/CDK4 and cyclin D/CDK6-dependent phosphorylation of retinoblastoma protein (pRb) influences expression of the p16 protein: pRb phosphorylation leads to its inactivation and, consequently, to an increase in p16 expression [45–47]. Tsuda *et al.* showed that irregularities in the p16-cyclin D/CDK-pRB signalling pathway were found in 51.4% of endometrial cancers [48]. Netzer *et al.* in turn [49] discovered p16 overexpression in 78% of serous carcinomas and in 36% of endometrioid cancers.

Summary

Wider knowledge on abnormalities at specific stages of signal transduction in the pathways responsible for regulation of cell growth, proliferation and apoptosis allows for a better understanding of the complexity of carcinogenesis. Unfortunately, some elements in this complex puzzle still remain unknown or inconsistent and require investigation. Understanding the sophisticated relationships between these components is necessary for further research on the drugs used in targeted therapy, directed at specific proteins of this intricate net of connections.

The authors declare no conflict of interest.

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