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Defining characteristics of Epithelial Ovarian Clear-Cell Cancer and targets for therapy

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Ovarian clear-cell carcinomas (OCCC) accounts for 10% of the epithelial ovarian cancers, a cancer type with a high mortality rate worldwide. OCCC development is linked to endometriosis which is the growth of endometrial tissue outside the uterine cavity, what can originate from iron induced oxidative stress. In this study the characteristics, mainly based on genetic studies, of OCCC are identified and possible targets for targeted therapy are discussed. This cancer type consists of only 2 cell types, clear-cells and hobnail cells that contain much glycogen. OCCC is slowly growing and very resistant to chemotherapy. Although a number of mutated genes are commonly found in OCCC, the gene p53 is not mutated. The gene profile of this cancer type consists of more than 50 mutated genes, from which ARID1A, PP2A, HNF-1β, Emi1 and PI3K are specifically mutated in this disease and extensively researched in the last few years. A multidrug therapy designed from targets of these genes together with chemo and operative therapy would provide the best treatment. However, there is more research needed before these genes can be indicated as good targets. Finally the set of 5 specifically mutated genes can be used as biomarkers to identify OCCC in patients.

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Introduction in epithelial ovarian cancers

Subtypes of epithelial ovarian cancer
Ovarian cancer forms a serious threat to women worldwide. It is the second most common gynecologic cancer and in the North America it is fifth on the rank of casualties due to a type of cancer. Ovarian cancers can be classified on type of origin. Several types arise from the egg cells in the ovarian, called germ cell ovarian cancer, and another type derives from supporting cells. However epithelial ovarian cancer (EOC) covers approximately 90% of the ovarian cancers and it is by far the most lethal form. EOCs originate from ovarian surface epithelial cells, from epithelium within a cyst, an enlargement of the ovary filled with fluid, or from endometriotic tissue (1).

EOCs can be classified as a heterogeneous disease. Different classes within EOC are based on histopathology but also possess a different molecular background. Identified classes are low and high grade serous (OS), mucinous (OM), endometrioid (OE) and ovarian clear-cell carcinoma (OCCC) (2, 3). The different forms of epithelial ovarian cancer can also be categorized on basis of malignancy. The first type of EOC's shows a low grade slow growing stepwise progression of carcinogenesis. They arise from lesions of a cyst, called adenoma carcinomas, and often have mutations in K-RAS and an activated RAS-RAF signaling. Low grade serous, mucinous and endometroid EOCs belong to this type. The second type develops from normal epithelial ovarian tissue, called *de novo* carcinogenesis. These tumors are fast growing and often show dysfunctional mutations in tumor suppressor genes p53 and BRCA1/2. They are characterized by a high genetic instability and are very malignant due to their fast growing appearance. High grade serous EOC is a member of this type. OCCC and OE can be distinguished from other types because they are thought to arise from endometriosic lesions, but overall OCCC and OE can be classified under type one (5, 6).

For the identification of EOC subtypes multiple Histopathological techniques are used in the clinic, bimanual pelvix examination and ultrasonography of the ovaries. Also serum testing for carbohydrate antigen as a tumor marker is often used (7). Molecular or so called biomarker expression is different in every class and stable during the stage development of the subtype. A well-known biomarker example is the elevated CA125 serum detected in non serous cancer types. In high grade serous carcinomas it is always present, while it is found in only 60% of the mucinous and clear-cell subtypes. For example in high grade serous EOC WT1 and Ki-67 are prognostic factors. For every subtype of EOC a panel of tumor markers is needed to determine the EOC subtype in patients in a correct and efficient way (3, 7).

Endometriosis and it's relation to EOC
Endometriosis is defined as the presence and growth of endometrial tissue, the inner membrane of the uterus, outside the uterine cavity. It is a disorder that typically occurs during the reproductive phase, the incidence is 10-15%. The change of developing EOC is two times higher when having endometriosis, especially in relation with OCCC and OE and not with the serous and mucinous forms (6 - 10). An explanation for this can be that endometriosis and OCCC/OE share pathogenic factors such as immune alterations, excess estrogen, steroid interactions, genetic instability, angiogenic and hormonal factors (11).

Overall evidence of overlapping molecular pathways in endometriosis and OCCC/OE is found to support the theory that the benign disease endometriosis can transform into a malignant EOC. The pathogenic factor estrogen is an interesting factor because it is linked to growth induction in endometriosis and ovarian cancers. Aromatase which is encoded by a single gene is the key enzyme synthesizing estrogen. In breast cancer aromatase inhibitors have been successfully used but in endometriosis and EOC their role is less clear. Aromatase expression is tissue specific due to promoters, co-activators and co-repressors that are tissue specific expressed (8). In breast cancer it is proven that prostaglandin E2 (PGE2) stimulates aromatase promoters via the cyclic adenosine monophosphate-protein-kinase A (cAMP-PKA) dependent way (12). Besides that in both endometriotic and carcinogenic ovarian epithelial cells the use of inflammatory cytokines such as interleukins, tumor necrosis factor α and growth factors such as vascular endothelial growth factor are used to enhance proliferation, evade apoptosis and escape immunoreactivity (13).

More examples of shared molecular alteration between endometriosis and OCCC/OE are known. Deactivation of the tumor suppressor gene phosphatase and tensin homologue (PTEN), which inhibits migration of cells by acting on the integrin-extracellular matrix interaction, is especially found in endometriosis and OCCC (8). In a study PTEN deletion in endometriotic cells caused progession towards these EOC subtypes (6). Also K-RAS is a shared mutated gene. It is suggested that the transformation of endometriosis to OCCC is partially induced by K-RAS mutations (14).

Enhanced excretion of matrix metaloproteinases (MMP's) is another example of shared alterations, MMP enzymes can help epithelial cells to penetrate through the basal membrane and stroma and in that way inhance invasiveness of the leasion (8).

In this review the molecular characteristics of epithelial ovarian clear-cell cancer are identified and possible targets for OCCC specific therapy are discussed.

Ovarian Clear-Cell Carcinoma characteristics

Before heading into the typical gene profile of OCCC, first some more histopathological, clinical and not directly gene-related characteristics are presented.

OCCC accounts for 10% of the ovarian epithelial cancers worldwide and is a poorly differentiated malignant type of cancer (4). In Japan, however, the occurrence of OCCC as percentage of all ovarian epithelial cancers is much higher (15-25%) than in Europe and North America (1-12%) (15). Just as each subtype of epithelial ovarian cancer, OCCC has its own characteristics. These carcinomas are very resistant to chemotherapy and are often accompanied by thrombo-embolic complications. Therefore this disease has a worse prognosis than other EOCs (3).

The pathology of OCCC shows that carcinoma tissue is rarely solid and is predominant in clear cell and 'hobnail' cells. Cells contain a lot of glycogen, what is in line with overexpressed glycogen genes in this EOC subtype. Histopathology also reveals that OCCC has a low mitotic activity, is genetically stable and as said before only contains 2 cell types in contrast with high grade serous carcinomas (see figure 1) (15-17). Development of OCCC can be categorized in 4 stages. Most OCCC's are discovered in stage 1, the stage in which the chance on survival is the highest (see figure 2). Although the disease is very resistant to chemotherapy, probably due to the low mitotic activity of carcinoma cells, nowadays platinum and paclitaxel based chemotherapy is given together with radiotherapy after surgery treatment of the carcinoma (15, 16).

OCCC can develop from healthy ovarian epithelial tissue or from an endometriotic ovarian lesion. In Japan only, woman with endometriosis have a nine-fold chance of developing OCCC (15). Most genetic changes that lead from endometriosis to OCCC are unknown, but an interesting oxidative stress pathway leading to OCCC is partially revealed (16). Carcinogenesis in endometriotic cysts
can be promoted by persistent oxidative stress induced by high concentrations of free iron that is delivered in the ovaries via retrograde transport of menstruation fluid.

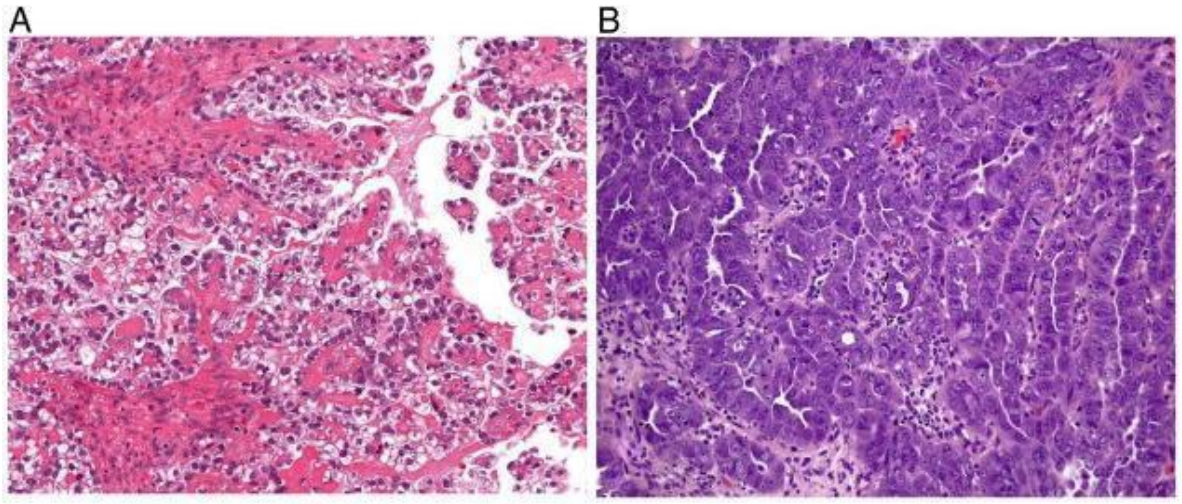

Figure 1, histology samples of clear cell carcinoma (A) and high-grade serous carcinoma (B). The clear cell carcinoma contains only 2 cell types, hobnail and clear cells, contains a lot of glycogen (white blobs in the image) and has a low mitotic activity. In contrast the high-grade serous carcinoma tissue contains a lot more cell types and has no glycogen rich cells (5).

There is a pro and anti oxidative balance in cells in OCCC. Oxidative stress is induced by reactive oxygen species (ROS) such as free iron. They consist of a superoxide anion, hydrogen peroxide and hydroxyl radicals. Overproduction of ROS can exceed the capacity of antioxidant mechanism in the cell and lead to oxidative stress. Oxidative stress results in protein, lipid, DNA and cell membrane damage, but also DNA mutations. These consequences of oxidative stress are carcinogenic (18). ROS also has a second messenger function in signal transduction, fibrinolysis and ECM turnover and can injure cells. Besides the role of ROS in cancer it is also involved in diseases like AIDS, diabetes, fibrosis and aging (19). The oxidative stress factor free iron in OCCC development from endometriosis is reduced from ferric Fe^{3+} to ferrous Fe^{2+} in the presence of superoxide radicals and hydrogen peroxide. Now, the formation of hydroxyl radicals is catalyzed what can promote DNA damage and carcinogenic events such as oncogene activation, tumor suppressor gene inactivation, mutagenesis and lipid peroxidation (20). In the ovaries, pro-oxidant factors containing free iron can be delivered by retrograde menstruation, when menstruation fluid is moving towards the ovaries instead of moving out of the body. The theory that there is a relation between iron, endometriosis and development of OCCC is supported by a study in which ovarian surface epithelial cells were exposed to free iron or contents of endometriotic cysts during 14 days. The exposure resulted in up and down regulation of a pack of OCCC signature genes (see figure 3) (2). It could be that DNA damage catalyzed by iron is not random, but certain areas in the DNA are more vulnerable, although more is not known about this aspect. Besides in OCCC development also in hepatitis B and C tumorigenesis via free iron involvement is observed (6). Oxidative stress can also induce the overexpression of anti-oxidative proteins in OCCC, such as glutathione S-transferase, peroxidases and superoxide dismutases. Activation of protein protective genes, the heat shock proteins also takes place. Expression of these factors can contribute to chemotherapy resistance. OCCC cells show a high survival rate under oxidative stress what suggests that endometriotic cyst cells acquire high oxidative stress resistance after being exposed to such an environment (2). So besides inducing apoptosis in the majority of endometriotic cells, on the long term oxidative stress can also prevent cell death by initiating anti-oxidative responses that on their turn could initiate cell proliferation or angiogenesis leading to tumor progression (5).

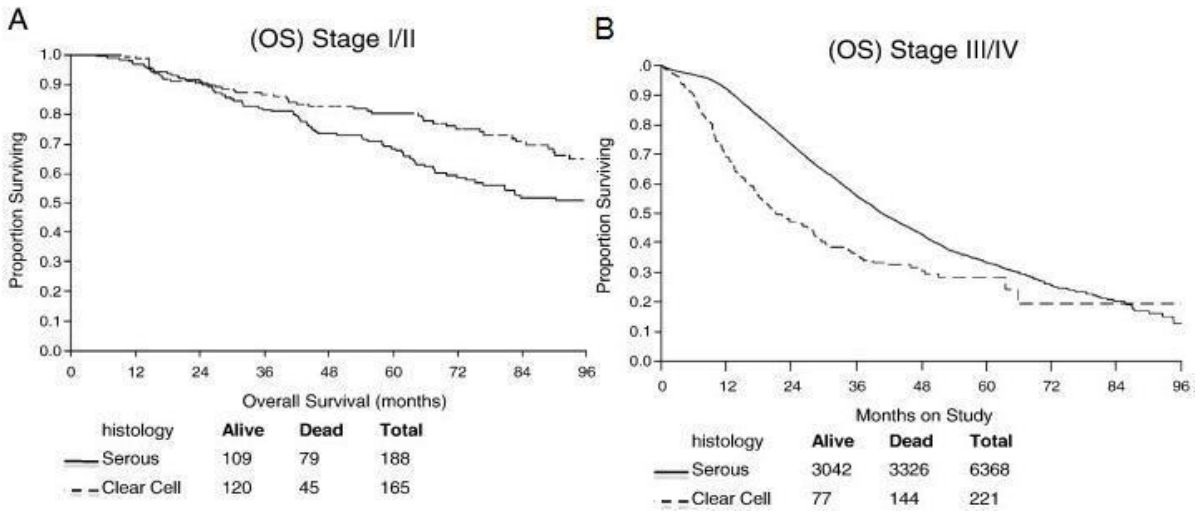

Figure 2, clear cell carcinoma patients show a better survival prognosis when the disease is discovered in stage 1 or 2 compared to serous carcinoma, the cancer type with the highest prevalence among ovarian cancers (A). When discovered in the 3th or 4th stage, clear cell carcinomas have a worse prognosis compared to serous (B) (5). The vertical axis shows the proportion of researched patients that survive OCCC a certain amount of months (horizontal axis).

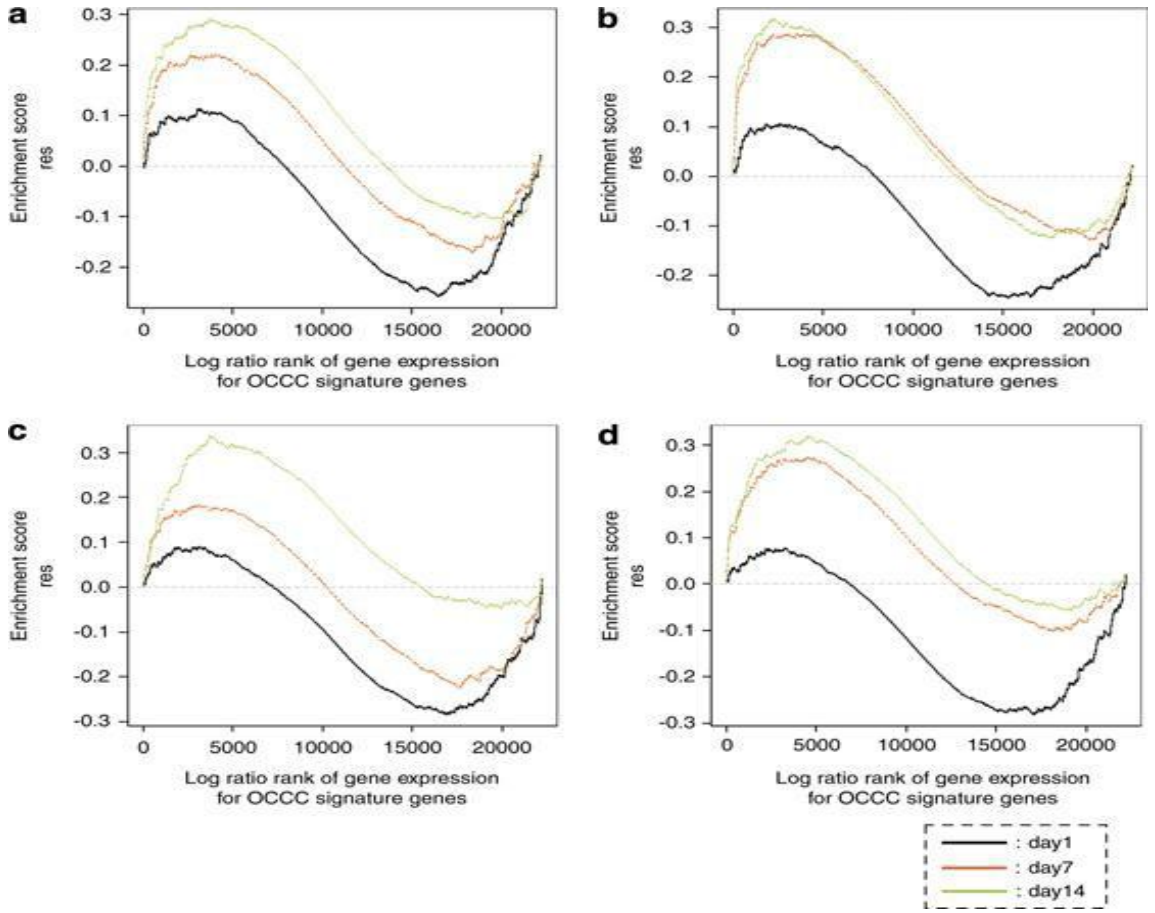

Figure 3, the induction of OCCC gene signature in vitro is shown by treatment with two different endometriotic cyst contents (A) and (B), and free iron in 5 nM (C) or 20 nM (D). Measurements were taken at day 1, 7 or 14 during treatment. Vertical axis shows enrichment scores and horizontal the log ratio of expression of OCCC signature genes (2).

Defining the ovarian clear-cell carcinoma gene profile

In this chapter the results of recent gene profile studies in OCCC is presented to create an overview of the mutated genes in this disease.

Most human carcinomas share a subset of mutations that are identified as inducers of cancer. Dysfunctional mutations in the tumor suppressor gene p53, a regulatory protein involved in signaling resulting in cell cycle arrest and apoptosis, overexpression of the anti-apoptotic Bcl-2 gene and reduced expression of the pro-apoptotic factor Bax are some examples of this subset. For some of these factors it is not clear whether it are mutations or not. In OCCC mutations in p53 are seldom found what gives this carcinoma type a very unique profile of mutated genes (8). Until recently the molecular profile of this subtype of EOCs was understudied, but in the last years a lot of research revealed a part of it.

Gene profiling studies revealed that OCCC is a genetically stable form of ovary carcinoma and that the profile shares similarities with clear-cell carcinomas in the kidney and endometrium (16). This finding suggests that molecular events leading to clear-cell carcinomas in these three locations in the body may be similar (21). The change in gene profile in OCCC is characterized by stress-responsive genes, what correlates with the fact that cells in the endometriotic cyst face high oxidative stress (2). A positive alteration in genes involved in regulation of the Mitogen-activated protein kinase (MAPK) pathway occurs in OCCC cells. The MAPK pathway is known as a stress signaling route. Also gene activity regulating cytokine biosynthese is positively changed, indicating a boost in inflammation. Moreover, genes involved in glycoprotein biogenesis are up regulated. This is in line with the previous discussed histopathological finding that in OCCC, cells store a lot of intracellular glycogen. A fourth finding is that genes involved in coagulation, blood clotting, are altered. This result is consistent with the discussed symptom that OCCC patients experience systematic thrombo-

embolic complications, a sort of thrombosis (2). In conclusion the difference between OCCC and other epithelial ovarian cancers is that in OCCC especially oxidative stress and inflammation related genes are highly upregulated (5).

Multiple studies described a part of the gene profile of OCCC. In one study 18000 genes have been researched in OCCC tumor cells of 8 patients. An average of 20 mutations per tumor was found. In most tumors the genes PI3K, KRAS, PPP2R1A and ARID1A were mutated. In a larger group of 42 tumors these genes were mutated respectively in 40%, 4,7%, 7,1% and 57% of the tumors. Mutations of these genes were found to be caused by base substitution, insertion or deletion of a base (4). This indicates that PI3K and ARID1A are genes typically mutated in OCCC. These genes will be further discussed as possible targets for therapy in the next chapter.

In a different study 66 genes were found to be mutated. Hepatocyte Nuclear Factor-1β (HNF-1β), cyclin dependant kinase inhibitor 1A, also called p21 which is a factor inducing slow growth of the OCC carcinoma, HIF-1a, IL-6 and STAT3 which is involved in inflammation associated carcinogenesis, were the most important mutated factors found. An overview of these genes is presented in figure 4. The gene HNF-1β will also be discussed as possible therapeutic target in the next chapter (2).

Also a set of transcription factors are part of the gene profile of OCCC. The most important might be the earlier addressed Hepatocyte Nuclear Factor-1β (HNF-1β), a transcription factor involved in cell proliferation, glucose homeostasis, is anti apoptotic and promotes anti oxidative mechanisms. But also Polo-Like Kinase (PLK) could be a crucial transcription factor mutated in OCCC. It can be up regulated by HNF-1β, and is involved in DNA-damage checkpoints what makes it critical for mitosis progression under oxidative stress. At last Octamer-Binding (Oct) transcription factor, involved in the cellular stress response, was found to be part of the OCCC gene profile (5). Overall the profile of altered genes in OCCC consists of more than 50 genes involved in detoxification, proteases, cell signaling, adhesion, transcription, metabolism, cell cycle and others, which are functions associated with chemo resistance and apoptosis (see also figure 5) (5).

Inactivation of tumor suppressor genes from the OCCC gene profile can be caused by allelic loss, which occurs by homozygous deletion (LOH), mutations, or methylation of the genes. Approximately one third of the endometriotic cysts and OCCC does contain homozygous deletions. Loss of the earlier described tumor suppressor gene PTEN due to homozygous deletions in OCCC is noted 40% in early stages of the disease. This suggests PTEN loss is an early event in OCCC development (21). But the presence of PTEN deletions in OCCC is contradictive; a more recently publicized research article suggests PTEN deletions are not involved in OCCC (2). Other tumor suppressor genes possibly inactivated via homozygous deletions in OCCC are the growth suppression associated gene p73, cycle dependant negative cell cycle regulator kinase inhibitor CDKN2 and genes encoding for superoxide dismutase (21).

Taken together, the OCCC gene profile consists of a complex pattern of inactivated tumor suppressor genes such as PTEN, p73 and CDKN2, mutated kinases such as PI3K, transcription factors such as HNF-1β and a variety of genes from which the function is not clear and the mutations are possibly induced via reactive oxygen species like free iron.

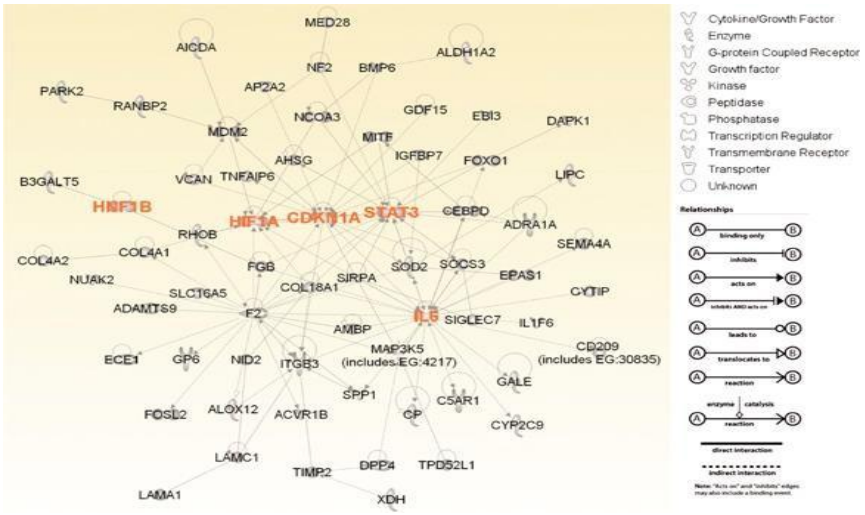

Figure 4, this scheme visualizes a network of genes involved in OCCC development from a study. The red highlighted genes, HNF-1β, HIF-1A, CDKN1A, STAT3 and IL-6 are the most important in development of this disease (2).

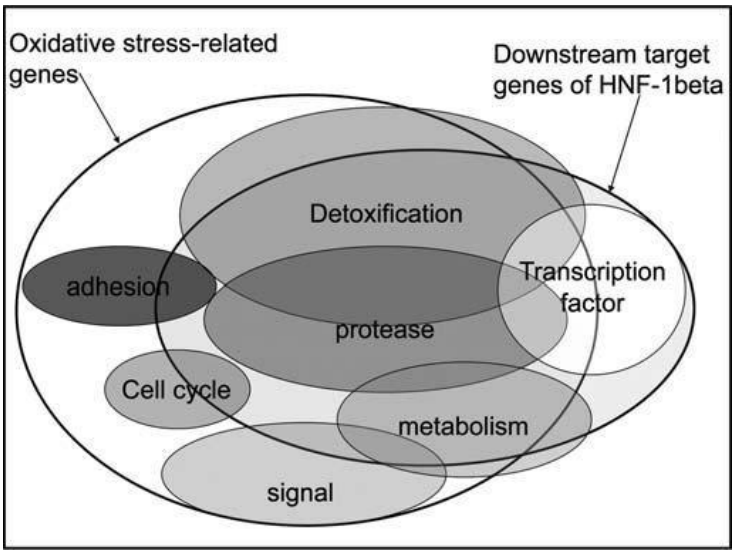

Figure 5, an overview of the functions involved in the gene profile of altered genes in OCCC. Downstream targets of transcription factor HNF-1β are processes involved in cell metabolism, such as glucose homeostasis and proliferation, but also anti apoptotic and oxidative mechanisms (15).

ARID1A and other possible targets for OCCC therapy

In this chapter the most promising targets in the OCCC mutated gene profile are explained on basis of their normal gene function and how therapy directed at these targets might help treating the disease.

ARID1A

In the last few years research on OCCC revealed that mutations in the gene AT-rich interactive domain-containing protein 1A (ARID1A) is specific for this disease. Previous results showed mutations in 57% of 42 OCCC tumors. The mutations were found throughout the encoding region of the gene in both alleles, resulting in formation of a stop-codon, insertion or deletion. This indicated that ARID1A is a tumor suppressor gene since mutations inactivated the gene product called BAF250a (4). Because ARID1A mutations are specific for OCCC, these mutations seem to be pathogenic and not random. Deletion of ARID1A in just one allele resulted in embryonic lethality in mice (23). Now researchers focus on the function of ARID1A to look if it may be used as a target for therapy in OCCC treatment.

The gene product of ARID1A, BAF250a, is part of the adenosine triphosphate-dependant chromatin remodeling complex switch/sucrose-nonfermentable (SWI/SNF) (24). The gene is located on chromosome 1p46, and encodes for a nuclear protein that has interactions with other proteins. These proteins include core ATPase and BRG and BRM who are responsible for moving the protein complex along DNA strands in an ATP dependent way. Together these proteins form the SWI/SNF chromatin remodeling complex. This complex consumes ATP in order to mobilize nucleosomes. Now it can regulate the accessibility of promoters to activate transcription. The complex is involved in methylation of nucleosomes. In other words, this complex induces chromatin modeling which can force epigenetic changes that influence the activity of many genes included genes involved in cancer. In this way it is part of regulation of cellular differentiation, development, proliferation, DNA repair and tumor suppression. It can act as a control protein complex to prevent excessive proliferation and suppress tumorigenesis (25-28).

The subunit encoded by ARID1A is one of the two ARID1 subunits. It is non catalytic and able to regulate target and ATPase activity. ARID1A is necessary for specificity in regulating gene expression. Mutations in ARID1A are formed in the AT-rich interactive domain 1A. These mutations lead to misfolded proteins of ARID1A subunit proteins. These misfolded proteins are vulnerable to u biquitination and degradation via the proteasomal pathway (16, 25).

It is thought that mutations in ARID1A lead to tumor initiation and are not drivers of further development of the carcinoma, because no link between progression of the OCCC tumor and loss of ARID1A was found (25). It is found that the SWI/SNF remodeling complex interacts with the earlier addressed hypoxia and cytokine related transcription factors HIF-1a and STAT3, what is in line with the tumor initiating role of ARID1A loss (15). Corresponding with this theory, in endometriosis, the precursor of OCCC, loss of ARID1A is often present (29). Moreover, ARID1A loss is found to be typical for uterine endometrioid carcinomas as well (30, 31).

It is now clear that ARID1A is involved in the accessibility to other genes via nucleosome methylation. In this way it can have a huge influence on the expression of all sorts of genes and it can be involved in the cell processes like differentiation, development, proliferation, DNA repair and tumor suppression. The high prevalence of ARID1A mutations in OCCC could make it a good biomarker to detect the disease in an early phase because of its tumor initiating properties. ARID1A could be a very interesting target for specific therapy in endometrioid related cancers such as OCCC and uterine endometrioid carcinomas. How therapy focused on ARID1A may be effective will be discussed in the next chapter.

PP2A

Another possible target for OCCC therapy is the serine/threonine protein phosphatase (PP2A) holo enzyme. This protein is member of a family of holo enzymes with a variety of activities. It exists of a core component containing a heterodimer, which can be divided in a catalytic (PPP2CA or PPP2CB) and a constant regulatory subunit (PPP2R1A or PPP2R1B). The regulatory subunit PPP2R1A functions as a binding site for one of the 15 regulatory subunits where after a heterotrimeric holo enzyme is formed. PP2A is involved in the inhibition of cell growth and mitosis. It is specially required for chromosome segregation (4, 32).

For these reasons it can be identified as a tumor suppressor gene. In OCCC mutations in PPP2R1A have been found in 7% of the cases. The mutations are all located in the α-helix repeats near the contact point between the catalytic and regulatory subunit of the enzyme complex. Evidence is provided that PPP2R1A mutations participate in the development of OCCC and other endometrioid related carcinomas. Although the occurrence of PPP2R1A mutations in OCCC is lower than in other endometrioid related carcinomas, this mutation is typical for endometrioid related carcinomas and can therefore be a good target for specific therapy in these cancers (33, 34). For further discussion, see the next chapter.

HNF-1β

Hepatocyte Nuclear Factor-1β (HNF-1β) is a transcription factor which is overexpressed in OCCC. The overexpression is caused by hypomethylation of CpG islands and is seen in the later development of endometriosis and OCCC. HNF-1β is expressed in normal epithelial cells in the kidney, liver, lung and urogenital tract. Its expression is involved in loss of mesenchymal phenotype and acquisition of the epithelial phenotype. Reduction of HNF-1β expression in experiments induced apoptotic cell death in OCCC cells, what suggests that expression of this gene is necessary for cell survival in OCCC cells (35).

This factor is important for the development of the kidney and is involved in the accumulation of glycogen in OCCC cells (36). Furthermore low HNF-1β levels are related to strong apoptotic activity and chemoresistance caused by a lower cell proliferation. Mutations in this gene are related to causation of diabetes type 2 on higher age. It is related to a variety of oncogenes involved in intracellular signaling (2, 6). Occurrence of the oncogenic overexpression of HNF-1β is high in OCCC, what could make it a useful biomarker to detect the disease. Because over expression of this gene is so oncogenic, it could be a good therapeutic target. Only the signaling routes that are induced by HNF-1β are unknown, what makes it hard to interpret the consequences of functional inhibition of this protein.

Emi1

Over expression of Early mitotic inhibitor-1 (Emi1) is particularly seen in OCCC in more than 80% of the cases. In endometrioid carcinomas it is overexpressed in 20% of the patients. Emi1 promotes entry of cells into S-phase and M-phase by inhibiting the anaphase promoting complex. Emi1 is activated via phosphorylation by Polo-like kinases (PLK) and in this way PLK makes sure that mitosis is started. Emi1 causes genomic instability and mitotic deregulation. For example in a recent study, overexpression of Emi1 in cell cultures led to tetraploidy and genomic instability (37). This makes Emi1 an important oncogenic factor that perhaps could be a good target for OCCC specific therapy. For sure this gene could act as a good biomarker for identification of OCCC in patients because it is over expressed 80% of the OCCC cases.

PI3K/Akt
The last addressed possible target in the OCCC gene signature is the PI3K route. Phosphatidylinositol-3-kinase (PI3K)/Akt signaling is involved in proliferation, apoptosis resistance, metastatic capacity and angiogenesis. There are three classes of PI3Ks. Class 1 PI3Ks consists of heterodimers containing a catalytic subunit and one of two families of regulatory subunits. This first class processes the phospholipid component of the plasma membrane phosphatidylinositol(4,5)biphosphate (PI(4,5)P2) in PI(3,4,5)P3, which can be reversed by PTEN from which the function was described in the introduction. Class 2 PI3Ks have the ability to phosphorylate PI and PI-4-P. Class 3 PI3Ks only phosphorylate PI. In OCCC, just as in more types of cancer, the PI3K/Akt signaling route is up regulated, what can lead to altered induction of the described targets of this signaling route. Uncontrolled signaling could be initiated by dysfunction of pathways upstream or downstream of the PI3K route, for example mutated receptors. Examples are the loss or inactivation of the tumor supressor gene PTEN and mutations in the PH domain of Akt that could lead to PI3K/Akt pathway activation (38). It is found in studies that complete knockdown of the PI3K/Akt pathway is lethal. At this moment inhibitors of the first class in the PI3K/Akt pathway are available on the pharmaceutical market, but progression in specifity of the drug is desired. It is very important to maximize the effect of the drug on the deregulated isoform of PI3K in the carcinoma and minimize the effect on other PI3K's. in this way toxic changes like increased blood glucose and other metabolic disturbances can be prevented. Currently a variety of inhibitors with different targets within the signaling pathway are available and designed (39).

A target of the PI3K /Akt pathway is the mammalian target of rapamycin (mTOR). This factor is phosphorylated and thereby activated in OCCC and other endometrioid cancers. It can lead to enforced mRNA translation of cell cycle progression and proliferation genes (6). In renal cancers it is proven that inhibitory therapy of mTOR resulted in compensatory PI3K/Akt activation. When also the PI3K/Akt pathway was inhibited it led to modulation of pro angiogenesis pathways (40). Overexpression of the PI3K/Akt pathway could be a good target for therapy in OCCC because this pathway is involved in so many processes that could be carcinogenic. However this pathway is also over expressed in other types of cancer than OCCC, what makes it not a good biomarker for detection of OCCC in patient.

Discussion

In this discussion, the development and found characteristics of the disease are described and the possible targets for specific OCCC therapy are discussed on basis of their effectiveness to inhibit the disease and their presence. *Implications are marked in the text in cursive and numbered in superscript.* The research quote of this review was formulated:

In this review the molecular characteristics of epithelial ovarian clear-cell cancer are identified and possible targets for OCCC specific therapy are discussed.

General characteristics of OCCC were identified in histopathological view and more important the general gene profile of OCCC. As shown in this review, most OCCC cases develop from endometriosis, a process that can be promoted by oxidative stress. Oxidative stress induced by iron during menstruation could initiate endometrioid cells to become resistant to oxidative stress and therofore malignant. When available, therapeutics that eliminates free iron in the blood could help to prevent OCCC development from endometriotic tissue.

Since OCCC cells are slowly growing and therefore overall resistant to chemotherapy it is important to focus therapy on genes that are mutated in this cancer type, especially oncogenic mutations. Typical mutations of genes in cancer such as p53 are not the case in OCCC, but still some more common mutations are also found in this disease. The gene profile consists of mutations in genes involved in stress responses such as the mentioned Mitogen-activated protein kinase pathway. Also genes involved in cytokine, coagulation and glycogen biogenese are altered in OCCC. Mentioned mutations of the genes KRAS, p21, HIF-1a, IL-6, STAT3, Oct and PLK, are possible targets from which of their role in OCCC development is little known, the specificity in OCCC is too low, or it are targets that have a low prevalence in OCCC. Still IL-6 and STAT3 can be inhibited with respectively IL-6 blocking antibodies or STAT3 inhibitors . The accounted gene that could be altered, PTEN, is not such a good target because studies on the prevalence of this gene in OCCC are very contradictive. From a lot of the genes mentioned is also little known, what makes it hard to design therapeutics that inhibit the function of oncogenes or somehow promote or mimic the function of tumor suppressor genes in this group. *Research on the function of these genes should continue in order to design therapeutics*[1].

From a number of genes that are specifically altered in OCCC, ARID1A, PP2A, HNF-1β, Emi1 and PI3K, more is known due to research in the last few years. This knowledge could help to design drugs that aim on the functionality of these genes. ARID1A is a gene which is very popular in OCCC research. Because it is involved in nucleosome methylation it has influence on the expression of many genes. It was already proven this gene is involved in regulation of HIF-1a and STAT3. Since ARID1A is a tumor suppressor gene and is involved in the development of the disease from endometriosis and not the progression, it can be hard to focus treatment on this gene. Restoring ARID1A function will be the first option. Genes that are silenced by methylation could be treated with DNA demethylating agents. In this way their function could be regained. ARID1A expression however, is not altered via methylation, but the gene is inactivated due to mutations. OCCC cells could be transfected with viral DNA encoding for the correct ARID1A gene. But in this way only a part of the cells will regain ARID1A function because transfection is never effective on all cells. Another option to focus on ARID1A treatment is to make use of synthetic lethality, a therapy in which a gene with a similar function as the mutated gene is also switched of what results in the inhibition of an essential process in the cell. Eventually this leads to cell death in those cells in which both genes are altered. *The point is to find another gene that is essential in nucleosomes methylation*[2]. If this gene can be found, the oncology field can try to find out whether inhibition of this extra gene is lethal to cells or if it is a better option to find way to perform nucleosome methylation in a ARID independent manner. *Researchers should study the effect of methylation therapy on OCCC patients with ARID1A loss. In this way it can be found out if the inhibited methylation due to ARID1A mutations can be corrected*[3].

The genes Emi1, HNF-1β and PI3K are up regulated in OCCC and could be used as targets for therapy. Emi1 could focus as a good target because it has a mitotic enhancing function and when over expressed is carcinogenic. Inhibition of HNF-1β in OCCC cells could induce apoptosis and therefore be a very good target for therapy, as is shown in vitro before HNF-1β reduction resulted in lethality of cells. *For Emi1 and HNF-1β it is necessary to research if down regulation of these genes can be achieved only in OCCC cells in some way, since down regulation in other organs could be toxic*[4]. Overexpression or activating mutations of PI3K are very oncogenic and deletion of this gene is shown to be lethal. *To specifically direct PI3K therapy on OCCC cells it would be useful to focus inhibition on the part of the pathway that is essential in OCCC development or progression and not the whole pathway*[5]. In this way toxicity to normal cells can be reduced. As mentioned for PI3K there already exist a number of inhibitors, but further specification of these genes is desired tot minimize the toxic effects on other normal functioning PI3K's. The tumor suppressor gene PP2A is involved in the inhibition of cell growth and mitosis and could be a good target because unless it's low prevalence (7%) it is highly specific for OCCC. *PP2A is proven to be a druggable target by activation of the protein, but more research on the pathways in which PP2A is involved could possibly lead to the discovery of genes with similar functions and a therapy based on synthetic lethality*[6].

Next the prevalence of mutations of these genes in OCCC has influence on the potency of being a good target. ARID1A down regulation has a prevalence of around 60%, Emi1 up regulation is involved in 80% of the cases, HNF-1β up regulation has also a high prevalence, while PP2A is down regulated in only 7% of the patients. PI3K is up regulated in a high percentage of OCCC patients but is not very specific for this cancer type. *It would be a good option to design a method to detect the disease by profiling genes of OCCC patients. In this way the disease can be discovered in an early stage what gives patients the best survival chance*[7]. Scanning for mutations in ARID1A, Emi1, HNF-1β would be suggested, and although PP2A has a low prevalence and PI3K is not very specific for OCCC, looking at mutations in these genes could help designing the method. *In the future the number of genes specific for OCCC that are known should be expanded to improve the gene set used to scan for OCCC in patients and enlarge the possibilities to target specific therapy in this disease on*[8].

The writer's vision

Recapitulating, in the last decennia cancer treatment research has shown that just one approach to treat cancers is not sufficient. The so-called multitherapy method is proven to be more efficient to eliminate cancers. Also in OCCC a method in which therapy on multiple targets is performed, would be the best way to treat patients. The best way to prevent OCCC development could be treatment of endometriosis patients with iron eliminating therapeutics during menstruation periods and therapy that re activates the function of ARID1A. Treatment of existing OCCC should focus on a combination of targets from the 5 discussed OCCC specific genes, ARID1A, PP2A, HNF-1β, Emi1 and PI3K and existing operative and chemotherapeutic treatment. Although for PP2A and PI3K drugs are available jet it is hard to tell which genes will provide the best target since more research is needed to find this out. For ARID1A there is a special role because it is particularly involved in the development of OCCC from endometriosis, however it is unknown what result the reactivation of ARID1A in OCCC cells would give. Besides the discussed therapy targets, also this set of OCCC specific genes can be used as

biomarkers on which patients can be scanned for the disease. It could be very useful in finding OCCC in early stages, what would improve life expectancy.

References

1. **Tothill R.W. et al.**, Novel molecular subtypes of serous and endometrioid ovarian cancer linked to clinical outcome, Clinical Cancer Research 14:5, 198-208, 2008.
2. **Yamaguchi K. et al**., Identification of an ovarian clear-cell carcinoma gene signature that reflects inherent disease biology and the carcinogenic processes, Oncogene 29, 1741-1752, 2010.
3. **Kobel M. et al.**, Ovarian carcinoma subtypes are different diseases: implications for biomarker studies, Plos Medicine 5:12, 1749-1760, 2008.
4. **Jones S. et al.**, Frequent mutations of chromatin remodeling gene ARID1A in ovarian clear-cell carcinoma, Science 330, 228-330, 2010.
5. **Anglesio M.S. et al.**, Clear-cell carcinoma of the ovary: A report from the First ovarian clear-cell symposium, june 24th, 2010, Gynecologic Oncology 121, 407-415, 2010.
6. **Kobayashi H. et al.**, Molecular pathogenesis of endometriosis associated clear-cell carcinoma of the ovary (review), Oncology reports 22, 233-240, 2009.
7. **Kobayashi H.**, Ovarian cancer in endometriosis: epidemiology, natural history, and clinical diagnosis, International Journal Clinical Oncology 14, 378-382, 2009.
8. **Vlahos N.K. Kalampokas T. and Fotiou S.**, Endometriosis and ovarian cancer: a review, Gynecological Endocrinology 26, 213-219, 2010.
9. **Brinton L.A. et al.**, Cancer risk after a hospital discharge diagnosis of endometriosis, Am J Obstet Gynecol 176, 572-579, 1997.
10. **Olive D.L. and Pritts E.A.**, Treatment of endometriosis, N Engl J Med 345, 266-275, 2001.
11. **Ness R.B.**, Endometriosis and ovarian cancer. Thoughts on shared pathophysiology, Am J Obstet Gynecol 189, 280-294, 2003.
12. **Osuga Y.**, Novel therapeutic strategies for endometriosis: apathophysiological perspective, Gynecol Obstet Invest 66, 3–9, 2008.
13. **Nezhat F. et al.**, The relationship of endometriosis and ovarian malignancy: a review, Fertil Steril 90, 1559–1570, 2008.
14. **Otsuka J. et al.**, K-ras mutation may promote carcinogenesis of endometriosis leading to ovarian clear cell carcinoma, Med Electron Microscopy 37, 188–192, 2004.
15. **Kajihara H. et al.**, Clear-cell carcinoma of the ovary: potential pathogenic mechanisms (review), Oncology Reports 23, 1193-1203, 2010.
16. **Kimberly C. et al.**, ARID1A mutations in endometriosis-associated ovarian carcinomas, N Engl J Med 363(16), 1532-1543, 2010.
17. **Tavassoli F.A. Devilee P.**, Pathology and genetics of tumours of the breast and female genital organs, World Health Organization of classification of tumours Vol. 4, 2003.
18. **Storz G. and Imlay J.A.**, Oxidative stress, Curr Opin Microbiol 2, 188-194, 1999.
19. **Cerutti P.A.**, Oxy-radicals and cancer, Lancet 344, 862-863, 1994.
20. **Kabat G.C. and Rohan T.E.**, Does excess iron play a role in breast carcinogenesis? An unresolved hypothesis, Cancer Causes Control 18, 1047-1053, 2007.
21. **Zorn K.K. et al.**, Gene expression profiles of serous, endometrioid, and clear cell subtypes of ovarian and endometrial cancer, Clinical Cancer Research 11, 6422-6430, 2005.
22. **Tan D.S. and Kaye S.**, Ovarian clear cell adenocarcinoma: a continuing enigma, Journal Clinical Pathology 60, 355-360, 2007.
23. **Gao X. et al.**, ES cell pluripotency and germ-layer formation require the SWI/SNF chromatin remodeling component BAF250a, Proc Natl Acad Sci 105, 6656–61, 2008.
24. **Wu J.I. et al.**, Cell 136, 200, 2009.
25. **Maeda D. et al.**, Clinicopathological significance of loss of ARID1A immunoreactivity in ovarian clear cell carcinoma, Int. J. Mol. Sci. 11, 5120-5128, 2010.

26. **C. van Rechem et al.**, HIC1 interacts with a specific subunit of SWI/SNF complexes, ARID1A/BAF250A, Biochem. Biophys. Res. Commun. 385, 586–590, 2009.
27. **Weissman B. and Knudsen K.E.**, Cancer Res. 69, 8223, 2009.
28. **F. Banine et al.**, Cancer Res. 65, 3542, 2005.
29. **Wiegand, K.C. et al.**, ARID1A mutations in endometriosis-associated ovarian carcinomas, N. Engl. J. Med. 363, 1532–1543, 2010.
30. **Guan B. et al.**, Mutation and Loss of Expression of ARID1A in Uterine Low-grade Endometrioid Carcinoma, Am J Surg Pathol 35, 625–632, 2011.
31. **Wiegand K.C. et al.**, Loss of BAF250a (ARID1A) is frequent in high-grade endometrial carcinomas, J Pathol 224, 328–333, 2011.
32. **Tang. et al.**, Dev. Cell 10, 575, 2006.
33. **McConechy M.K. et al.**, Subtype-specific mutation of PPP2R1A in endometrial and ovarian carcinomas, J Pathol 223, 567–573, 2011.
34. **Shih I.E.M. et al.**, Somatic mutations of PPP2R1A in ovarian and uterine carcinomas, Am J Pathol 178(4), 1442-7, 2011.
35. **Kobayashi H. et al.**, The Role of Hepatocyte Nuclear Factor-1[beta] in the Pathogenesis of Clear Cell Carcinoma of the Ovary, International Journal of Gynecological Cancer 19(3), 471-479, 2009.
36. **Zaffanello M. et al.**, TCF2 gene mutation leads to nephro-urological defects of unequal severity: an open question, Med Sci Monit 14, 78–86, 2008.
37. **Gütgemann I. et al.**, Emi1 protein accumulation implicates misregulation of the anaphase promoting complex/cyclosome pathway in ovarian clear cell carcinoma, Mod Pathol 4,445-54, 2008.
38. **Ihle N.T. and Powis G.**, Take your PIK: PI-3-kinase inhibitors race through the clinic and towards cancer therapy, Mol Cancer Ther. 8(1), 1–9, 2009.
39. **Stein R.C. and Waterfield M.D.**, PI3-kinase inhibition: a target for drug development?, Mol Med Today 6(9), 347–57, 200.
40. **Holland W.S. et al.**, Evaluating rational non-cross-resistant combination therapy in advanced clear cell renal cell carcinoma: combined mTOR and AKT inhibitor therapy, Cancer Chemother Pharmacol, 2011.