

# Potential targets for Ovarian Clear Cell Cancer therapy

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## **Abstract**

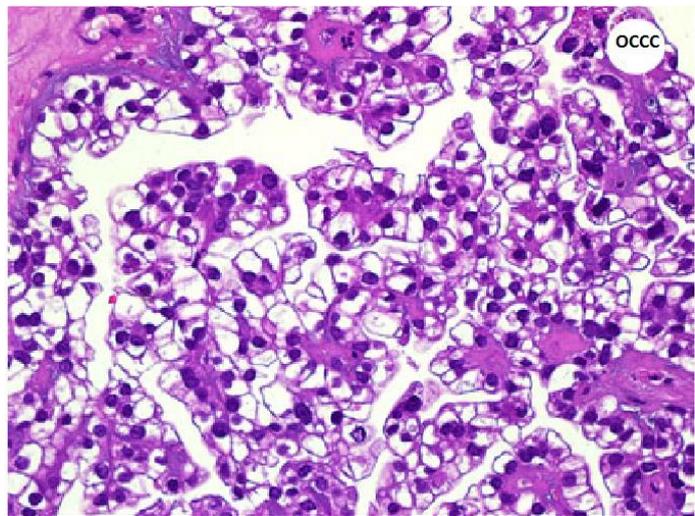
Ovarian clear cell carcinoma is a subtype of epithelial ovarian cancer. OCCC has a low response to platinum based chemotherapy, so new therapeutic strategies are desired. ARID1A is a subunit of the SWI/SNF complex and mutated in 50% of the OCCC cases. ARID1A regulates gene expression by controlling the gene accessibility. HNF1 $\beta$ , ER and WT1 are markers which can be used to diagnose OCCC. A potential target for OCCC therapy is the reduction of HNF1 $\beta$ . Another possibility is to target the ARID1A mutation. Since ARID1A is a loss of function mutation it is not directly targetable, but restoration of ARID1A inhibits cell survival and cell growth. ARID1A mutations often coexist with mutations in *PIK3CA* and *PTEN*, inhibition of PI3K/AKT pathway in which these transcription factors are involved also decrease cell growth and cell survival. Inhibition of KRAS is also interesting, but KRAS inhibition in low grade serous ovarian carcinoma did not show a decrease in tumorigenic effects. Between EZH2 and ARID1A an antagonistic link is found. This means that inhibition of EZH2 with GSK126 induces apoptosis in ARID1A mutated cells. PIK3IP1 is a target gene of ARID1A and EZH2, PIK3IP1 induces apoptosis. PIK3IP1 only becomes activated when EZH2 and ARID1A are not present in the cell. ARID1B is quite similar to ARID1A; presence of ARID1B in ARID1A mutated cells results in cell survival, which makes ARID1B an interesting target. Between TP53 and ARID1A a mutational exclusive relation is described. P53 stabilization in OCCC cells can induce apoptosis. So, several possible therapeutic targets are mentioned. Although for all of them further research is necessary.

## **Introduction**

Epithelial ovarian cancer (EOC) is the fifth deadliest cancer among women in the United States of America.<sup>1</sup> EOC is a heterogeneous cancer. There are five main subtypes of EOC; high-grade serous carcinoma (HGSC), endometrioid, mucinous, low-grade serous carcinoma (LGSC) and clear cell. The tumors are classified to subtypes based on histopathology and molecular genetic alterations.<sup>2</sup> These tumors subtype account for 98% of all ovarian

carcinomas.<sup>3</sup> Hormone replacement therapy and obesity are risk factors for EOC.<sup>4</sup> 61% of the cases present the cancer in a late stage when it is already metastatic.<sup>5</sup> 75% of these patients diagnosed with late stage ovarian cancer experience recurrence after surgery and chemotherapy, despite majority of the patients respond well to first line platinum-based chemotherapy. Eventually patients with recurrent EOC develop chemo resistance.<sup>6</sup> As a consequence the 5 year survival of ovarian cancer is 40%.<sup>3,7</sup> There is also a variety in response to platinum-based chemotherapy between subtypes, ovarian clear cell carcinoma (OCCC) and mucinous carcinoma do not respond well in comparison with HGSC.<sup>8</sup> Therefore the prognosis of OCCC is worse than the more common HGSC. Even though about 60% of all OCCC are stage 1 disease and stage 1 prognosis is usually favourable.<sup>9</sup> OCCC accounts for almost 10% of all ovarian carcinoma. The presence of clear cells alone is not enough to

diagnose OCCC. Besides the characteristic clear cells with rounded eccentric bulbous nuclei, the presence of multiple complex papillae and expanded cores of the papillae by densely hyaline basement membrane material is essential. Also hyaline bodies are present in approximately 25% of the cases (figure 1)<sup>3</sup>. There is an association with OCCC and endometriosis, this correlation is positive for the prognosis.<sup>9</sup> Mutations in



**Figure 1:** A representative sample of ovarian clear cell carcinoma

*ARID1A*, *PIK3CA*, *KRAS* and *PTEN* are frequently occurring genetic alterations in OCCC. Of which *ARID1A* and *PIK3CA* are the most frequent mutated genes. In about 50% of the OCCC cases the AT rich interactive domain 1A (*ARID1A*) is mutated. Phosphatidylinositol-45-biphosphate 3-kinase catalytic subunit  $\alpha$  (*PIK3CA*) is mutated in approximately 40% of the patients with OCCC.<sup>10</sup> Because of the frequent occurring of these mutations they are interesting targets for targeted therapy. Moreover loss of *ARID1A* in OCCC leads to a shorter progression free survival and a lower response to chemotherapy compared with *ARID1A*-positive OCCC.<sup>11</sup> In this thesis I will discuss the effects of *ARID1A* and *PIK3CA* mutations in OCCC. How we can use *ARID1A* and *PIK3CA* as possible molecular target for OCCC. And if there are other pathways we can use as targets for OCCC treatment.

### ***HNF1B, ER and WT1***

Hepatocyte nuclear factor-1beta is upregulated in over 90% of the OCCCs. It is a transcription factor which regulates a majority of biological functions in OCCC. Reduction of HNF-1 $\beta$  induces apoptosis in OCCC cell lines.<sup>12</sup> This suggests that the survival of OCCC cells is depending on the presence of HNF-1 $\beta$ . This makes HNF-1 $\beta$  an interesting target for therapy. Moreover since HNF-1 $\beta$  is present in more than 90% of the OCCC it is an ideal marker to diagnose OCCC.<sup>13</sup> Other markers to diagnose OCCC are ER and WT1; OCCC cells are negative for these transcription factors in more than 95% of the cases.<sup>3</sup>

### ***The SWI/SNF chromatin remodeling complex***

The Switch/Sucrose Non-Fermentable (SWI/SNF) chromatin remodeling complex regulates transcription by hydrolysing ATP to remodel the chromatin structure.<sup>14</sup> Therefore the SWI/SNF complex is involved in essential cellular processes such as transformation, DNA damage repair, cell cycle regulation and development. The complex exists out of three types of subunits, a catalytic (ATPase) subunit, several core subunits and variable subunits. This results in a diversity of SWI/SNF complexes, which contributes to specific gene transcription.<sup>15</sup> In approximately 20% of all human cancers a mutation is found in one of the at least nine subunits of the SWI/SNF complex. *ARID1A*, *PBRM1*, *SMARCA4* and *SMARB1* are some of the most frequent mutated subunits. A mutation in one of these subunits causes inactivation of the SWI/SNF. This inactivation is thought to drive tumorigenesis by altered gene expression.<sup>16</sup>

### ***ARID1A***

ARID1A is a subunit of the SWI/SNF complex. ARID1A mutations are found in 46-57% of the OCCC, in 30% of the ovarian endometrioid carcinomas, but not in HGSC.<sup>17</sup>

ARID1A is encoded by the *ARID1A* gene which is located on chromosome 1p36.11.<sup>18</sup> Most *ARID1A* mutations are nonsense or frame- shift, these mutations result in deactivation of transcription of the protein product. *ARID1A* is a cancer inhibiting gene, so a mutation in this gene can cause onset and/or progression of cancer.<sup>19</sup> There are two types of hotspot mutations. Firstly, mutations which cause reduced nuclear export of ARID1A. Secondly,

mutations that affect the interactions between ARID1A and other SWI/SNF complex subunits.<sup>20</sup> Many reports already suggested a link between ARID1A mutation and tumor growth. Suppression of wild type ARID1A in ARID1A mutated ovarian cells inhibits cell proliferation, whereas silencing of ARID1A in normal cells induces proliferation and tumorigenicity.<sup>21</sup> ARID1A is involved in carcinogenesis through several mechanisms.

### ***The role of BAF250***

BRG-associated factor 250a (BAF250) is a protein encoded by the tumor suppressor gene *ARID1A*. BAF250 is an essential member of the SWI/SNF chromatin remodelling complex. It is responsible for regulating target specificity of the SWI/SNF complex and the ATPase activity of the complex.<sup>22</sup> BAF250 binds to DNA in a nonspecific way and then starts mobilizing other components to build the SWI/SNF remodeling complex. In this way BAF250 confers to specificity of SWI/SNF complex. The coordinated activity of the proteins in the complex contributes to the chromatin structure which is essential for cellular processes like, transcription, DNA syntheses and DNA damage repair.<sup>23</sup> Loss of BAF250 is more frequently found in clear cell carcinoma than in uterine HGSC. This is in accordance with the percentage of ARID1A mutations in OCCC and HGSC mentioned before.<sup>17 23</sup> Previous studies did not find a correlation between BAF250 loss and clinical pathological features, such as pathologic stages, grades, overall survival and epidemiological parameters of OCCC. That because researchers did not look for correlations. They either looked for mutations or clinical pathological features.<sup>24</sup> Except for one study, this study found a relationship between loss of BAF250 and response to chemotherapy. Tumors with loss of BAF250 were less likely to respond to chemotherapy than the once who did not lose their BAF250 expression.<sup>25</sup>

### ***PI3K/AKT pathway***

ARID1A mutations often coexist with mutations in PIK3CA and PTEN, two components of the phosphatidylinositol-3-kinase (PI3K)/AKT pathway. Guan *et al.* even suggested that inactivation of ARID1A alone is not enough to cause cancer; a combination with an aberration in the PI3K/AKT pathway is required.<sup>20 26</sup> Activation of this pathway induces cancer related mechanisms, such as inhibition of apoptosis of cancer cells and proliferation

of cancer cells. This pathway becomes activated by activation of the tyrosine kinase receptors or through activating somatic mutations in PIK3CA and PTEN. PTEN dephosphorylates PIP3 into PIP2 and inhibits the PI3K/AKT pathway. When PTEN is not present as a result of somatic mutation this inhibition stops. PIK3CA becomes activated and encodes more catalytic subunit PI3K. The increased amounts of PI3K lead to activation of the PI3K/AKT pathway and stimulation of cell growth, proliferation and cell survival (figure 2).<sup>20</sup> Furthermore, loss of ARID1A also causes an increased phosphorylation of AKT.<sup>27</sup>

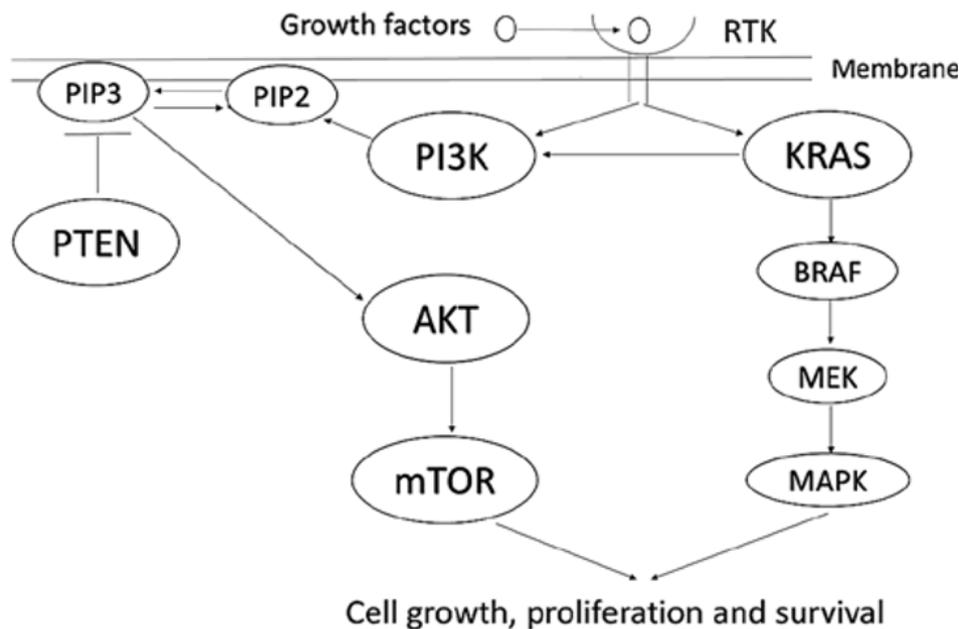


Figure 2: The PI3K/AKT pathway. Activation of receptor tyrosine kinase (RTK) leads to activation of KRAS and PI3K. KRAS also activates PI3K. PI3K phosphorylates PIP2 to PIP3, whereas PTEN dephosphorylates PIP3 to PIP2. PIP3 in turn activates AKT and then mTOR, which leads to stimulation of cell growth, proliferation and survival. KRAS also contributes to these processes through another pathway

PIK3IP1 (PIK3 interacting protein 1) is an inhibitor of the PI3K/AKT pathway. It inhibits the p110 PI3K subunit via its p85-domain.<sup>28</sup> Overexpression of PIK3IP1 causes a downregulation of the PI3K/AKT pathway and suppression of cell growth, survival and proliferation.<sup>29</sup> Inhibition of tumor cell growth via PIK3IP1 inhibition is an interesting target treatment because ARID1A mutations are related with a reduced amount of PIK3IP1.<sup>20</sup> MK-2206 is an AKT inhibitor. Inhibition of AKT leads to increased apoptosis in ARID1A mutated cell lines.<sup>30</sup> Another inhibitor, one which specifically targets the PI3K-AKT-mTOR pathway in OCCC cell lines is BEZ235. BEZ235 treatment reduces the expression of AKT. OCCC cells treated with BEZ235 showed cell cycle arrest when low concentrations were given and apoptosis with higher doses.<sup>31</sup>

## ***KRAS and BRAF***

The KRAS-BRAF-MEK-ERK-MAPK pathway is a frequently affected pathway in human carcinomas. RAS proto-oncogenes are mutated in 25% of all cancers. Mutations in KRAS or BRAF, they have the same tumorigenic effects, lead to over activation of the pathway and thus to increased cell proliferation.<sup>32</sup> The KRAS pathway activates the PI3K/AKT, which makes it likely that KRAS is involved in ovarian carcinoma (figure 2). KRAS or BRAF mutations in ovary carcinoma have already been reported in several reports.<sup>33,34</sup> Mutations in the KRAS pathway mostly occur in mucinous subtype of EOC, but in 5% of OCCCs a mutation in this pathway is found.<sup>10</sup> KRAS inhibition on OCCC is not yet tested, but in LGS ovarian carcinoma Selumetinib is tested as a potential target therapy. Selumetinib is a non-small molecule inhibitor of MEK 1/2, a downstream molecule of the KRAS pathway. It is a potent, selective, orally available and non-ATP competitive.<sup>35</sup> Despite the fact that 15 % of the patients treated with Selumetinib showed a response, Selumetinib did not seem to be related with KRAS and BRAF mutations in LGSC.

## ***EZH2***

EZH2 (Enhancer of Zeste Homolog 2) is a subunit of the polycomb repressive complex 2 (PRC2). PRC2 belongs to the polycomb group (PcG). Recently genetic studies in *Drosophila* first described antagonistic links between SWI/SNF and PcGs. Mutations in the subunits of the SWI/SNF complex contribute to suppress phenotypes associated with PcG gene mutations and PcG proteins are able to block the mobilization of subunits of the SWI/SNF complex.<sup>36,37</sup> Because of this antagonistic link EZH2 is an interesting therapy target for OCCC caused by mutations in SWI/SNF subunits. EZH2 is a catalytic subunit that trimethylates H3K27 (histone 3 lysine 27), which controls gene expression epigenetically, to repress the gene transcription.<sup>15</sup> EZH2 is overexpressed in various cancer types. Overexpression of EZH2 correlates with increased H3K27 trimethylation, cancer progression and poor survival chances. Also an EZH2 knockdown cell line shows a decreased cell proliferation, apoptosis, migration and angiogenesis which make EZH2 an interesting target for cancer therapy.<sup>38</sup> ZLD10A is a small inhibitor of EZH2. It inhibits the methyl transferase activity of EZH2 and therefore it decreases H3K27 methylation, tumor cell proliferation and induces apoptosis. In clinical trials in lymphoma it is reported as a potential and promising inhibitor to treat

lymphoma.<sup>39</sup> Also other highly specific EZH2 inhibitors have been developed.<sup>40,41</sup> These inhibitors are primarily based on targeting the Polycomb-repressive activity and use H3K27 methylation to determine the effects. These therapies have side effects because they also inhibit the wild type PRC2-repressive function.

However, EZH2 seems to have more functions. Besides the repressive function of PRC2, EZH2 can also switch to a transcriptional coactivator of androgen receptor (AR) and potential other factors.<sup>42</sup> As said before, EZH2 seems to have an antagonistic link with mutations in the SWI/SNF subunits SMARCB1 and ARID1A. The loss of SMARCB1 (also known as SNF5, INI1 and BAF47) causes tumor formation.<sup>43</sup> Wilson et al reported that inactivation of EZH2 is enough to stop tumor formation through SMARCB1 mutation.<sup>44</sup> This antagonistic relation makes SMARCB1 dependent of EZH2. This dependence of SWI/SNF subunits is largely dictated by non-enzymatic contribution of EZH2 instead of enzymatic activity. Kim et al suggest that mutations in the SWI/SNF complex create dependence of both functions of the EZH2. Therefore EZH2 inhibitors which only inhibit the PRC2 repressive function are not capable to fully suppress the oncogenic function, unless they are also able to disrupt the protein interactions.<sup>15</sup>

### ***ARID1A and EZH2 inhibition***

As said before ARID1A is mutated in almost 50% of all OCCC. The ARID1A mutation is a loss of function mutation and therefore not directly targetable by conventional targeted approaches such as antibodies. It has been proven that restoration of ARID1A wild type in ARID1A mutated cells suppresses the tumor cell growth in OCCC cell lines.<sup>21</sup> ARID1A is a subunit of SWI/SNF and probably sensitive for EZH2 inhibition. GSK126 is an EZH2 inhibitor which inhibits the growth of ARID1A knockdown cells. GSK126 is small-molecule inhibitor of EZH2 that decreases the level of trimethylated H3K27 and reactivates the PRC2 genes.<sup>45</sup> GSK126 treatment leads to a reduced amount of ARID1 knockdown cells, but it does not affect ARID1A wild type cells.<sup>11</sup> It induces apoptosis in ARID1A mutated cells. ARID1A wild type restoration affects the sensitivity to GSK126 negatively.<sup>46</sup> In short, ARID1A wild type restoration suppresses ARID1A mutated cell growth and inhibition of EZH2 activity by GSK126 suppresses the growth of ARID1A mutated OCCC cells. A combination of both strategies does not improve results of the therapy.

### ***PIK3IP1 as ARID1A/EZH2 target gene***

Between EZH2 and ARID1A the phenomenon synthetic lethality is observed, which means that cell death only becomes induced when you simultaneously disrupt 2 factors. There must be an underlying mechanism which elucidates the synthetic lethality between the EZH2 methyltransferase activity and ARID1A mutated cell growth in OCCC. The EZH2 inhibition of ARID1A mutated cells must be regulated by a set of

genes that targets both ARID1A and EZH2.<sup>11</sup> Bitler et al. revealed antagonistic roles of ARID1A and EZH2 in regulating a significant number of genes. Of which PIK3IP1 was the most interesting ARID1A/EZH2 target gene. Both ARID1A and EZH2 are present on the PIK3IP1 promoter, but ARID1A dominates the gene expression of PIK3IP1. (Figure 3)<sup>11</sup> When ARID1A is absent in ARID1A mutated OCCC cells, EZH2 takes over the gene regulation and suppresses PIK3IP1 expression. If EZH2 methyltransferase activity is also suppressed, PIK3IP1 was expressed.<sup>46</sup> As said before PIK3IP1 triggers apoptosis. In conclusion EZH2 inhibitors

as GSK126 only inhibit cell growth of ARID1A mutated cells and not wild type, because EZH2 inhibition only affects PIK3IP1 expression when ARID1A is absent.

### ***ARID1B***

ARID1B is another subunit of the SWI/SNF complex. ARID1B is quite similar to ARID1A; they are about 60% identical. ARID1A and ARID1B are mutual exclusive in the SWI/SNF complex. They seem to have opposing effects on cell cycle arrest.<sup>47</sup> ARID1B containing SWI/SNF complexes are present in ARID1A mutated and wildtype cells.<sup>48</sup> ARID1B knockdown does not affect the composition of SWI/SNF complexes in wildtype cell lines, but it does affect mutant ARID1A cell lines. This mutant cell lines show dissociation of SMARCA4 and reduced incorporation of several other subunits to the SWI/SNF complex. Deactivation and depletion of ARID1A and ARID1B affect proliferation strongly, but loss of either ARID1A or ARID1B affects it modestly. A similar effect is observed in the formation of the SWI/SNF complex. So

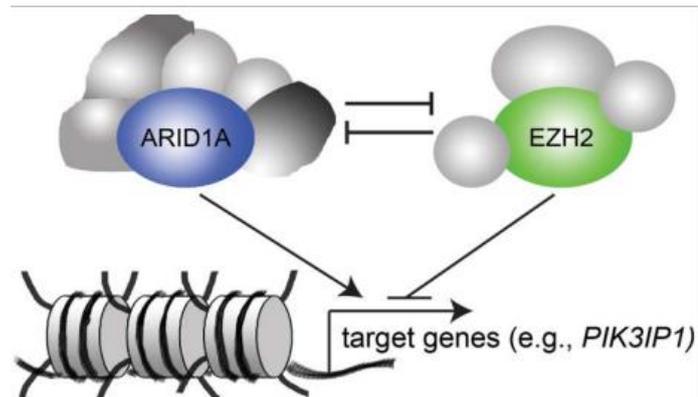


Figure 3: Antagonistic regulation of PIK3IP1 expression through ARID1A and EZH2. ARID1A dominates over EZH2 and drives the expression of the target genes. If ARID1A is absent, EZH2 silences the PIK3IP1 expression. Subsequently when EZH2 methyltransferase activity is also suppressed PIK3IP1 is expressed.

there must be a synthetic lethal relationship between this mutual exclusive pair of subunits. Surprisingly, ARID1B mutations co-occur with ARID1A mutations, 38% of the ARID1A mutated cell lines also contain ARID1B inactivating mutations. This is significantly higher than the 3% co-occurrence of ARID1B with wildtype ARID1A. However, all the cell lines contained at least one allele of ARID1A or ARID1B, which suggests that the presence of ARID1 is essential for the survival of the cell.<sup>49</sup> ARID1B is a specific vulnerability in ARID1A mutated cancers. Presence of ARID1B in ARID1A mutated cell lines is essential for the formation or stabilization of SWI/SNF complexes. Consequently, the presence of ARID1B is essential for the survival of ARID1A-mutated cancer cells.<sup>50</sup> This suggests that ARID1B is a potential therapeutic target of ARID1A mutated cancer.

### ***TP53***

*CDKN1A* and *SMAD3* are both downstream targets of ARID1A. They are also p53 target genes. This means that p53 and ARID1A function in the same pathway to regulate these genes. *CDKN1A* and *SMAD3* are known for the role they play in pathways related to the cell cycle. As mentioned before ARID1A forms SWI/SNF complex by interacting with other subunits. One of these complexes, the ARID1A/BRG1 complex promotes the expression of *CDKN1A* and *SMAD3*. Therefore in ARID1A knockdown cell lines *CDKN1A* and *SMAD3* are downregulated.<sup>21</sup> Also it is reported that p53 can interact with subunits of the SWI/SNF complex, for example *BAF60A* and *BRG1*.<sup>51,52</sup> Guan et al stated that the ARID1A/BRG1 SWI/SNF complex and p53 occupy the same promotor regions of *CDKN1A* and *SMAD3*. This suggests that loss of *TP53* or *ARID1A* can result in loss of expression of *CDKN1A* and *SMAD3*. Mutational analysis of OCCC revealed a mutually exclusive pattern of *ARID1A* and *TP53*, if *ARID1A* was mutated the cells contained wild type *TP53* and vice versa. This considered it might be possible to overcome the effects of *ARID1A* loss by restoration of wild type *TP53* in OCCC cells.<sup>14</sup> Nutlin-3 is able to increase the amount of p53 in OCCC cells. Nutlin-3 does not induce apoptosis in A2780 ovarian cancer cell lines, but it does suppress the growth of these cell lines. However Nutlin-3 treatment in combination with rhTRAIL or D269H/E195R did induce apoptosis in cancer cell lines.<sup>53</sup> So a therapy based on p53 stabilization alone will not be sufficient to treat OCCC, but a combination with another target inhibitor could be a solution.

## ***Discussion***

Mutations in *ARID1A*, *PTEN* and *PIK3CA* frequently occur in OCCC. *ARID1A* is a subunit of the SWI/SNF chromatin remodelling complex, which regulates gene expression and is involved in genomic stability. Loss of wild type *ARID1A* results in loss of essential cell growth regulating processes and contributes to cancer development. *PIK3CA* and *PTEN* mutations often coexist with *ARID1A* mutations. Mutations in these genes result in activation of the PI3K/AKT pathway which activates cell growth, proliferation and survival. Because of the significant role these mutations play in tumorigenesis, they are interesting targets for target therapy. Promising results have been described in inhibiting the PI3K/AKT pathway which results in downregulation of cell growth of *PIK3CA* or *PTEN* mutated OCCC cell lines. Despite the fact these inhibitors have been described as prospective new effective targets in OCCC cell lines, they have not been tested in clinical trials. Mainly because of the low incidence of OCCC (5% of all EOCs), which does not make it an interesting research topic.<sup>54</sup> EZH2 inhibition by GSK126 and *ARID1A* wild type restoration both are promising OCCC therapy strategies. Both therapies increase the PIK3IP1 expression. Overexpression of PIK3IP1 leads to inhibition of cell proliferation and induces apoptosis. However a combination of both could be even more effective. In fact, the effect of GSK126 is reduced when *ARID1A* is restored in *ARID1A* mutated cells. Both *ARID1A* and EZH2 respond on PIK3IP1 overexpression, but the *ARID1A* response is dominant over EZH2. Therefore the GSK126 inhibition of EZH2 does not have the same effect in a combination therapy.<sup>20</sup> In lung cancer patients EZH2 seems to be a downstream effector of the KRAS pathway. Depletion of PI3K/ATK decreased the EZH2 expression. This effect is interesting in cancers caused by EZH2 overexpression. The same study also showed that a combined inhibition of the PI3K/ATK pathway and EZH2 increased the sensitivity of KRAS mutated lung cancer cells to PI3K/ATK targeted therapy.<sup>55</sup> EZH2 overexpression is also common in OCCC, so a combination treatment of EZH2 inhibition and PI3K/AKT targeted therapy to treat OCCC is another possibility. All the possible targets named in this thesis are molecules that play an important role in OCCC, but none of them especially targets the genetic mutations of *ARID1A*. In this thesis I suggested several therapeutic targets to treat OCCC. All of them are in different stages of research. First, there are the strategies that only target one aspect of OCCC. For example ZLD10A which inhibits EZH2 overexpression and MK-2206 or BEZ235 which inhibit the PI3K/ATK pathway. Second I

mentioned several possible combination therapies. Namely, the inhibition of GSK126 in combination with ARID1A restoration. The inhibition of PI3K/AKT pathway and EZH2 at the same time or a combination therapy of p53 stabilization with another inhibitor. Thirdly, I described a possible target for OCCC targeted therapy, namely ARID1B. Finally, there is PIK3IP1. PIK3IP1 is an inhibitor which inhibits the PI3K/AKT pathway, but also plays an important role in the synthetic lethality between ARID1A and EZH2. In conclusion, there are several possible therapeutic targets for OCCC treatment. However more research is necessary, because none of these strategies is already a successful treatment for OCCC.

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