The SWI/SNF complex a potential target against ovarian cancer

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Abstract

The switch/sucrose nonfermentable (SWI/SNF) complex is a chromatin remodeling complex with distinct functions regarding cellular fate and multicellular development. This complex consists of multiple protein subunits, of which some are mutually exclusive and could have opposite effects when incorporated into a complex. The SWI/SNF complex acts as a bona fide tumor suppressor. Mutations that lead to inactivation of the protein subunits BRG1 and AT-rich interacting domain containing protein 1A (ARID1A) have been linked to two types of ovarian cancer namely ovarian clear cell carcinoma and small cell carcinoma of the ovary (hypercalcemic type), respectively. These two types of ovarian cancer are one of the deadliest gynecological cancers and lack good treatment options. However, recent studies showed results, which can provide new treatment options for these two types of ovarian cancer. A synthetic lethal interaction is revealed because some cells, which harbor a mutation in one of the SWI/SNF subunits, are depending on the presence of other subunits for disturbed cell growth. These results are important for all cancers in general because in 20% of all the cancers, one or more mutations are found in one of the SWI/SNF subunits. This review will focus on the two types of ovarian cancer and possible new treatment options due to synthetic lethal interactions within the SWI/SNF complex will be discussed. Literature indicates that, inhibition of other pathways, like PI3K/AKT or MAPK pathways also has its effect on SWI/SNF subunits which can lead to alteration of SWI/SNF subunits expression or deactivation of the pathway in general.

Keywords: SWI/SNF, Synthetic lethality, ARID1A, Ovarian clear cell carcinoma, BRG1, Small cell carcinoma of the ovary (hypercalcemic type).

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Introduction

Ovarian cancer is one of the most deathly gynecological cancers in the world (1) and is ranked as 5th deadliest cancers in the United States (2). The SWI/SNF complex is a chromatin remodeling complex and therefore it can increase the accessibility of DNA for transcription factors. The complex consists of a 10-12 proteins and some has mutually exclusive subunits, which cannot be incorporated into the same complex. The most important ones are BRG1 and BRM, which are the core subunits and have a catalytic ATPase site (3). Other important subunits are ARID1A and ARID1B, which are variant subunits involved in cell differentiation (4). The complex is involved in development, gene regulation and lineage specification (5). Therefore, recent studies scanned for mutations in this complex and found that in 20% of all the human cancers, this complex harbored one or more mutations (6). Using whole exome sequencing, mutations in BRG1 have been linked to small cell carcinoma of the ovary (hypercalcemic type) (7-9) and ARID1A have been linked to ovarian clear cell carcinoma (2, 10). The current treatment of ovarian clear cell carcinoma is platinum based chemotherapy. However, the patients lack sensitivity towards this treatment (1, 2). Similar for small cell carcinoma of the ovary (hypercalcemic type) the treatment now is multi-drug chemotherapy, yet it is unknown if this treatment improves patient survival (9). Additionally, a recent study speculated that this disease is related to rhabdoid tumors, which is an aggressive malignancy frequently found in children (11). Therefore, more research is necessary to improve the treatment options for these types of ovarian cancer. Recent studies used screening methods to show that cells with a defect in one of the SWI/SNF subunits are depending on other SWI/SNF subunits for abnormal cell growth, which suggests synthetic lethal interactions. Synthetic lethality is a phenomenon that is based on cell dependencies. Therefore, the inactivation of two genes is mostly enough to cause cell death. The first one is often a mutation in a tumor suppressor gene and the second one is chemically inhibited (12). This will be more thoroughly discussed later in this review. This was also found for BRG1 and ARID1A, which are mutated in small cell carcinoma of the ovary (hypercalcemic type) and ovarian clear cell carcinoma, respectively (13-15). These synthetic lethal interactions can especially provide new treatment options for the two ovarian cancer subtypes, and for more types of cancer, because it is proposed that almost one-fifth of the cancers harbors a SWI/SNF complex subunit mutation.

In this review the current knowledge about the SWI/SNF complex will be discussed in relation to BRG1 and ARID1A and their role in two types of ovarian cancer. Additionally,

the synthetic lethality between different SWI/SNF subunits will be discussed as this may improve treatment options for patients with defects in the SWI/SNF complex.

The SWI/SNF Complex

The SWI/SNF complex was originally found in yeast and remained conserved from yeast to mammals. However, the complex that resides in mammals consists of more proteins than the complex that was found in yeast and therefore the conservation of the exact mechanism remains obscure (16). The SWI/SNF complex (further referring to the mammalian SWI/SNF complex unless mentioned otherwise) plays a role in gene regulation, cell lineage specification and development, and can bind to one-third of all the genes (13). This is mainly accomplished by chromatin remodeling. The nucleosome architecture can be changed by sliding of nucleosomes, by ejection/insertion of histone octamers (energy obtained by ATP hydrolysis) or by modification of histone tails (6). The sliding of nucleosomes is presumably proceeding in a stepwise manner. First, the SWI/SNF complex binds on the nucleosomal DNA. Second, histone-DNA contacts are disrupted and the ATPase subunits cause DNA translocation, following by a DNA loop formation. These can propagate around the nucleosome to develop sites that are more accessible for transcription factors. Histone specific chaperones regulate the process of insertion/ejection of histones octamers. However this is not happening to nucleosomes that are bound by SWI/SNF complexes but to nucleosomes adjacent to these complexes (17, 18).

The SWI and SNF genes encode proteins that are physically associated in a multisubunit complex, which consists of several core and variant subunits (3). These complexes consist either of BRG1 or BRM, which are mutually exclusive catalytic ATPase subunits and thus cannot be incorporated both in the same complex. These catalytic ATPase subunits provide the energy that is necessary for nucleosome remodeling. Some variant subunits also contain mutually exclusive partners, and give the SWI/SNF complex a more specific function (16). Figure 1 shows two different mammalian SWI/SNF complexes. The difference between the two complexes shown in Figure 1 is that one contains a BRG1 associated factor (BAF) and the other a polybromo BRG1 associated factor (PBAF). The function of every complex depends on the variety of the incorporated subunits, which are also different for the two complexes (yellow and red subunits).

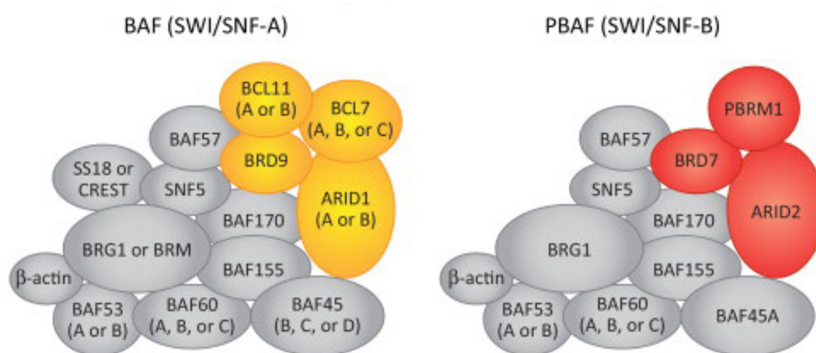

Figure 1 Two mammalian SWI/SNF complexes, BAF contains a BRG1 associated factor were PBAF contains a polybromo BRG1 associated factor. The functions of these complexes are partly depending on the incorporation of the different subunits. (Adapted from Hohmann A. & Vakoc C. 2014. (5))

The core subunits of the SWI/SNF complex are expressed in all cell lineages, while the variant subunits are more cell lineage specific expressed (3). This is important in the genesis of different cell types (neurogenesis, adipogenesis, myogenesis etc.). Both variant subunits and core subunits can be mutated in different types of cancer. With ARID1A, SNF5 and BRG1 being the most common ones. It is thought that integration of different variant subunits into SWI/SNF complexes, allow the formation of probably several hundred distinctive complexes (19). Each complex can initiate chromatin remodeling or recruit transcription factors, histone modifiers or co-activators/repressors and is therefore capable to regulate many different genes in all kinds of pathways (16). For example, SWI/SNF complexes regulate the retinoblastoma (Rb) tumor suppressor pathway by binding Rb directly and cause modulation of E2F, an Rb target gene. This gene regulates G_1-S transition, DNA synthesis, DNA repair and mitosis, while aberrations in these factors can cause tumorigenesis (20). The MYC transcription factor is also regulated by the SWI/SNF complex and is involved in cell cycle progression, apoptosis and differentiation. During development, SWI/SNF complexes are capable of repressing MYC expression directly, which results in less proliferation. However MYC is often amplified in cancers and therefore it is an important pathway. In tumors containing an inactivation of SNF5, a core subunit of the SWI/SNF complex, MYC expression is constantly increased (21). Rhabdoid tumors where SNF5 is often mutated are extremely aggressive, despite the low rate of mutations. Therefore it is plausible that SNF5 mutations are not capable of causing cancer due to defects in DNA repair but rather due to epigenetic alterations. SWI/SNF complex subunits are acting as tumor suppressors, because many of the subunits are heterozygously mutated. However, often a loss of protein expression is observed so one allele is insufficient to suppress tumorigenesis. This raises the probability of

haploinsufficiency in these tumor suppressor subunits (3). The SWI/SNF complexes are active in stem cells and regulate the self-renewal or pluripotency properties in stem cells. Inactivation of either BRG1 or ARID1A in embryonic stem cells causes differentiation and loss of self-renewal capacity. This can be of interest because cancer stem cells are probably partly responsible for metastasis. Therefore it is thought that mutations in the SWI/SNF complex could also promote metastasis, given that inactivation of SNF5 increases Ras homolog gene family, member A (RHOA) activity (22). This increases migration because RHOA is responsible for the cytoskeletal structure and therefore responsible for cellular motility. Similar to inactivation of SNF5, reduced levels of BRG1 causes elevated levels of actin stress fibers, which stimulates cellular migration. Additionally, loss of CD44, a transmembrane glycoprotein that is associated with metastasis, correlates with loss of BRG1 (23).

In conclusion the SWI/SNF complex is involved in chromatin remodeling through ATP hydrolysis. The complex consists of multiple subunits with a special role within the complex and within different cell types, which are responsible for development, lineage differentiation and gene regulation. Subunits in SWI/SNF complexes are acting as bona fide tumor suppressor genes and have been linked to various types of cancer.

SWI/SNF complex subunits ARID1A (BAF250a) and BRG1 (SMARCA4)

ARID1A is located at chromosome position 1p36.11 and ultimately transcribes a protein that has a molecular weight of approximately 250kDa, which is probably only located in the nucleus. ARID1A potentially plays a role in the cell cycle because its expression varies highest in the G_0-G_1 phase and decreases significantly during the rest of the cell cycle. ARID1A not only coimmunoprecipitate with the SWI/SNF complex but also with p53, histone modifying enzymes and transcription factors. This suggests that ARID1A can also interact with other proteins, which may give ARID1A additional functions. In addition, posttranslational modifications cause ARID1A to regulate protein-protein interaction, such as with other SWI/SNF complex subunits or p53, and protein expression. However, interaction with the SWI/SNF complex is most widely studied. ARID1, a subfamily with the members ARID1A and ARID1B, share a 50% amino acid homology throughout the structure. In their DNA binding domain containing 100 amino acids, the homology is even 80% (4). Interestingly, despite the high homology in amino acids, ARID1A has complementary roles compared to ARID1B. As described earlier, SWI/SNF complexes can bind directly to Rb and regulates E2F, this also occurs during differentiation. However, the outcome is different and depends on whether ARID1A or ARID1B is incorporated in the complex, which causes repression or has no effect

respectively. Another complementary action is observed in MYC expression during differentiation, ARID1A represses MYC expression whereas ARID1B promotes MYC transcription (3). This suggests that tumors, which contain a MYC amplification can possibly be treated by upregulation of ARID1A. The expression of ARID1A can possibly be altered due to inhibition of the PI3K/AKT pathway as this pathway interacted with ARID1A. Additionally, when ARID1A is epigenetically inactivated, it can probably be reactivated due to histone deacetylase inhibitors because this increases the accessibility of promoters. However, this is probably not specific for ARID1A. Furthermore, there may be a synthetic lethal interaction between MYC and ARID1B because ARID1B promotes MYC transcription. However, additional research has to be done to support this. In a non-transformed osteoblast model ARID1A is required for cell cycle arrest, however knockdown of ARID1B has no noticeable effect. An opposite effect was observed during serum deprivation, ARID1A knockdown caused a delayed cell cycle arrest and knockdown of ARID1B had no effect. ARID1A is also involved in the interaction with nuclear hormone receptors because the ARID1A C-terminal has multiple nuclear hormone receptor binding sites. However, the role of ARID1A in tumorigenesis must still be unraveled, namely that ARID1A is often mutated in hormone responsive tissues such as the ovary (4). Even though ARID1A and ARID1B have different functions, both can be mutated in the same type of cancer. However, one functional allele in ARID1B is always present in ARID1A deficient tumors (13). ARID1A plays a role in three factors that are important for tumor suppression, namely proliferation, differentiation and apoptosis. The results are inconsistent in different cancer types, knockdown of ARID1A in normal ovarian surface epithelial cells enhance proliferation and reintroduction of ARID1A decreased cellular proliferation in gastric and breast cancer cell lines. Knockdown of ARID1A causes inhibition of Fas mediated cell death in Jurkat leukemia cells (3). The loss of ARID1A causes uncontrolled cellular proliferation in cancer cells, probably by involving MYC to enhance cell cycle progression (24). The mutated version of ARID1A alters expression or stability of other SWI/SNF complex subunits and therefore can also alter the assembly of different SWI/SNF complexes, which can lead to limited availability of a couple SWI/SNF complexes (25).

BRG1 is located at chromosome position 19p13.2 and encodes a large protein with a molecular weight of 200 kDa. It has a catalytic ATPase subunit and is mutually exclusive with BRM. However, they are both adequate to change nucleosome architecture. Both proteins share an Rb and a bromodomain binding motif, which makes binding to acetylated histones possible (26). BRG1 has a unique domain, which can bind to zinc

finger proteins, whereas BRM lacks this domain. BRM can target a differential promoter because it can interact with ankyrin repeats. Though both share 75% of the amino acid sequence and several domains like the ARID1 subfamily, these two subunits also have opposite functions. Osteoblast differentiation is promoted by BRG1, while BRM negatively regulates this differentiation. Even more interesting is that homozygous deletion of BRG1 in embryonic mice causes lethality while deletion of BRM gives viable mice. However, BRM deficient mice have defects in the control of cellular proliferation and are 15% heavier compared to controls (3).

BRG1/ARID1A in Ovarian Carcinoma

Ovarian clear cell carcinoma

Endometriosis is a gynecological disease and affects 6-10% of the women in the reproductive age. This gynecological disease is both molecular and epidemiologically linked to ovarian clear cell carcinoma (OCCC). OCCC is in 46-57% linked to truncated mutations, distributed evenly over the ARID1A gene (27). Mutation in the ARID1A gene is followed by loss of ARID1A protein expression analyzed by immunohistochemistry (2, 10). Importantly, mutations in ARID1A are also linked to endometriosis, which suggest that ARID1A is mutated early in the onset of the disease (24). This is supported by the observation of decreased levels of ARID1A protein levels and mutations in ARID1A in preneoplastic lesions (2). However, the exact time point that the mutation occurs in tumorigenesis remains elusive (24). Interestingly, in all cases of ARID1A mutated OCCCs, which, 73% showed loss of protein expression without loss of heterozygosity. This supports the haploinsufficient tumor suppressor role for ARID1A, mentioned earlier (27). OCCCs have some characteristics, such as genetically stable and low mitotic rate, which can explains that OCCCs lack sensitivity towards platinum based chemotherapy, still the most common line of treatment as effective alternatives have not yet been developed (2). However, another study revealed that patients who have lost ARID1A expression and were treated with platinum based chemotherapy had a decrease in progression free survival. Therefore the ARID1A status should be measured in OCCC patients before treating the patients with platinum based chemotherapy. However, more research must be done to identify the molecular mechanism behind chemotherapy resistance in OCCCs. Another subset of ovarian carcinoma, serous and mucinous carcinoma were also analyzed for ARID1A expression. However, expression of ARID1A remained normal (1). OCCCs are frequently linked to mutations in ARID1A and

also a co-occurrence is found with activating PIK3CA mutations. PIK3CA was mutated in 46% of the tumors that lack expression of ARID1A (27). Also *in vitro* knockdown of ARID1A caused elevated levels of AKT phosphorylation. More results were linked to the PI3K/PTEN pathway. Knockout of ARID1A mice were compared with double knockout of ARID1A and PTEN. This revealed that mice with only ARID1A knockout were not capable of developing tumors, whereas in the double knockout, 40% of the mice developed ovarian tumors. These results suggest that ARID1A deficiency is not individually responsible for tumorigenesis (27). Collectively, mutation in ARID1A, which cause reduced protein expression, correlate with PIK3CA mutations. Hence, it is suggested that ARID1A somehow is involved in the PI3K signaling pathway. Therefore it can help to sequence for mutations in ARID1A and PI3K to validate OCCCs. In conclusion, endometriosis, which is also linked to ARID1A mutations, is a predispose to ovarian cancer. This suggests that an ARID1A mutation occurs early in the onset of OCCCs. However, current treatment for OCCC patients is platinum based chemotherapy, despite the lack of sensitivity. Therefore new treatment options are necessary and a synthetic lethal interaction can be a viable option due to the crosstalk of ARID1A with other proteins and especially proteins in the PI3K pathway.

Small cell carcinoma of the ovary, hypercalcemic type

Small cell carcinoma of the ovary, hypercalcemic type (SCCOHT), is a rare undifferentiated ovarian malignancy and is distinct in its kind by several aspects such as early age appearance (the mean age of diagnosis is 23 years), lively mitotic hyperchromatic cells with almost no cytoplasm and the presence of hypercalcemia. However, the latter is only present in two-thirds of all cases. Additionally, the hypercalcemic type was introduced to separate the malignancy from the neuroendocrine or the pulmonary type. The diagnosis of SCCOHT is challenging because no specific markers are known, however markers that are commonly expressed in ovarian malignancies can be used to exclude histological mimics. Despite the diagnostic difficulties, Robert Scully already described SCCOHT in 1979 (28). This aggressive malignancy is in general lethal if it metastasizes. Even if it remains in the ovary, the patient is likely to die within 2 years. The treatment option is most often multi drug chemotherapy, although it remains uncertain if this improves disease outcome which can probably be explained by the tumor aggressiveness (7-9). Strikingly a recent study discussed the origin of SCCOHT due to the following facts: hypercalcemic is only found in one-third of the patients, the morphology of SCCOHT mimics the morphology of malignant rhabdoid tumors (MRT) and the results that whole-exome sequencing

revealed a link between SCCOHT and BRG1 inactivation. Additionally, somatic and germline mutations in SNF5 are often linked to MRT, including loss of protein expression in the tumor cells. Interestingly, MRT cases with a normal SNF5 protein expression occur, hence the search towards another germline mutation. Remarkable this was found for BRG1, which is also linked to SCCOHT. Conclusively, SCCOHT resembles MRT, in a clinical, morphological and a molecular manner and is therefore suggested as malignant rhabdoid tumor of the ovary. The same treatment as for MRT can now also be considered for SCCOHT because it has been suggested that these malignancies are family members. However these are conventional treatments, which may not result in enough DNA damage to cause cell death. Therefore new treatments that regulate gene expression are likely to have more effect. Additionally, the cell of origin for MRTs is unknown, there is evidence that MRTs arise from mesenchymal cells (11). This might explain the metastatic abilities of MRTs because mesenchymal cells are probably more mobile compared to epithelial cells. More research has to be done towards the origin of MRTs and of ovarian cancers in general.

Recently, three studies have been published which, investigated this rare ovarian malignancy with whole-exome sequencing as primary tool (7-9). The first study examined family cases of SCCOHT, which proved the autosomal dominant transmission of the disease, is probably caused by a mutation in a single gene. In fact BRG1 was mutated in two individuals in each of the three families. The mutations were either germline or somatic. Additionally, 26 non-familial cases were sequenced and in 24 cases either a germline or somatic mutation in BRG1 combined with a loss in protein levels was found, suggesting biallelic alterations or epigenetic modifications of the BRG1 gene (7). In the second study, 12 SCCOHT tumors were examined and biallelic mutations were found in every tumor. Most of these mutations were found in the ATPase catalytic domains. Similar to the first study, in almost all cases protein expression was lost. Additionally, this study investigated the co-occurrence of BRM mutations, the mutually exclusive partner of BRG1. However, this was found only in one case. Reintroduction of BRG1 in a non-small-cell lung adenocarcinoma cell line, which is deficient for BRG1, induced suppression of cell growth and elevated levels of p21 (9). The third study also examined 12 SCCOHT tumors, 75% harbored a mutation in BRG1 and all samples with a BRG1 mutation lacked BRG1 protein expression. Additionally, this study checked if loss of BRG1 is specific for SCCOHT by performing immunohistochemistry on primary ovarian epithelial tumors, germ cell tumors and sex cord stromal tumors, 485 in total. In 0.4% of the cases, BRG1 staining was observed. This suggests that loss of BRG1 expression is specific for SCCOHT tumors (8). All studies contained low sample size,

however all the studies showed the same result, suggesting that a mutation in BRG1 is responsible for SCCOHT. Interestingly the epithelial-to-mesenchymal transition (EMT), which is thought to be an important process for metastasis and what makes SCCOHT so lethal, is correlated with loss of BRG1 expression. However this was only found in lung tumors (7). The mechanisms underlying these results must be unraveled for optimizing drug treatment. Nevertheless, the results indicate that synthetic lethality is a potential new treatment option for this devastating disease. Synthetic lethality can be established by inhibition of the catalytic ATPase site of BRG1, However, these inhibitors still need to be developed.

The SWI/SNF complex and synthetic lethality

The development of cancer is most often combined with alterations in molecular pathways, which in most cases is due to oncoproteins or inactivating tumor suppressor genes. The inhibition of oncoproteins was much easier and has been achieved several times, for example BCR-ABL inhibition by Imatinib. However, restoring tumor suppressor gene activity was much more difficult (29). Instead of developing a treatment to restore the original function of the tumor suppressor gene, researchers searched for cell dependencies. These cell dependencies are caused by the fact that the inactivation of two or more genes causes lethality. An inactivated tumor suppressor gene already has one gene inactivated, by inactivating another gene the cell loses its viability. This phenomenon is called synthetic lethality (12). However, the inactivation of the second gene must not cause a substantial lethality in cells, which do not have the inactivated tumor suppressor gene. Treatments based on a synthetic lethal interaction have already made it into clinical trails. In this case, BRCA1/2-mutated cells, which cause absence of homologous recombination, were treated with PARP1 inhibitors, which cause absence of another repair mechanism. Therefore PARP1 inhibition has much more effect in BRCA1/2-deficient cells compared to healthy cells. Therefore PARP1 inhibitors were developed for the treatment of BRCA deficient tumors (12). As mentioned before, most of the subunits in the SWI/SNF complex act as tumor suppressors and knowing that in almost 20% of all the human cancers SWI/SNF subunits are inactively mutated, it is worth looking for a synthetic lethal interaction (6). A few recent publications have described found a synthetic lethal interaction (13-15, 29). The first study published in 2009 was about tumors that lost expression of the SNF5 core subunit, which earlier has been found to be associated with the development of

malignant rhabdoid tumors in humans and deletion of SNF5 was lethal in embryonic mice. The study found that in absence of SNF5 and BRG1 cells stopped proliferating beginning five days after deletion. However BRM up regulation was observed after BRG1 deletion. This suggests that BRM can partly compensate for BRG1 loss. A more striking result was found when BRG1 and SNF5 were co-inactivated, since this was leading to rapid cell death. Additionally, mouse models supported these findings because conditional knockouts of BRG1 and SNF5 have impaired tumor formation and prolonged tumor onset, suggesting a role of BRG1 inhibition in treatment of SNF5 deficient tumors. This was tested with mouse models that lack either BRG1 or SNF5 compared with mouse models that lack both (15). Interestingly, another study observed tumors harboring a BRG1 mutation, having a synthetic lethal interaction when BRM the mutually exclusive homologue of BRG1, was inhibited with RNA interference. The treatment of BRG1-deficient cells with BRM RNA interference lead to suppressed growth relative to BRG1-positive cancer cells and senescence was induced due to activation of p21. Truncated mutations or deletions were most common in the BRG1-deficient cells and therefore suppression in colony formation was observed. This suppression was more prominent when BRM was also knocked down. However this was observed after 4 weeks of culture indicating that knockdown of BRM in BRG1-deficient cells have a suppressive effect, which takes some time to establish (14). Another study also found a synthetic lethal interaction between BRG1 and BRM, however here they searched for the epigenetic dependencies by screening a short hairpin RNA library across 58 different cancer cell lines. Such a screening method has a great value to find more selective cancer cell dependencies. Additionally to the previous study they found that inhibition of BRM in BRG1 deficient cells leads to increased levels of H3K9me3, which is a repressive histone marker representing heterochromatic gene regions (29). Both studies found no noticeable effect when BRM was inhibited in cells that had the BRG1 wildtype allele or were heterozygous for BRG1. Both studies also showed similar *in vivo* results supporting the potential of BRM inhibition in BRG1 deficient tumors (14, 29). ARID1A, another subunit of the SWI/SNF complex, is often mutated in OCCCs. Mutated ARID1A was found to have a synthetic lethal interaction with ARID1B, which is interesting because just like BRG1 and BRM, ARID1A and ARID1B are also mutually exclusive. Furthermore, this study found that inhibition of ARID1B in ARID1A-deficient cells, caused suppression of proliferation and colony formation (13). This probably occurs because of the depletion of ARID1B in ARID1A-deficient cells, which causes isolation of the catalytic ATPase subunit BRG1. In combination with the previous study this suggests that inhibition of BRM can also provide a synthetic lethal interaction in

ARID1A-deficient cells. The fact that knockdown of ARID1B in ARID1A-deficient cells results in diminished protein levels of SWI/SNF core subunits, including BRG1, further supports this hypothesis. Interestingly, is that mRNA levels remained normal, indicating that yet unknown factors cause posttranslational loss of these core subunit proteins (13).

Collectively, these results suggest that BRG1-deficient cancers can be treated with a BRM inhibitor due to their synthetic lethal interaction and that ARID1A-deficient tumors can be treated with ARID1B inhibitors. This can have great promise towards a sensitizing effect for current treatment or new treatment options for OCCCs and SCCOHTs. However the studies described above do not give a molecular explaining for the synthetic lethal interaction, so more research has to be done to explain this mechanism in molecular detail.

Discussion

The SWI/SNF complex is undoubtedly one of the most interesting complexes within the cell. This is supported by the diversity of processes in which this complex is involved, for instance, cell lineage specification, gene regulation, development and the fact that mutations in this complex are linked to all types of cancers. This complex can also increase our knowledge about cell fate due to crosstalks between different pathways, which are responsible for differentiation, cell cycle arrest, DNA repair, mitosis and G_1-S transition. However, additional research has to be done regarding the SWI/SNF complex because it has a lot of contradictory functions and the functions are also different between cell types and probably also between cancer types. Furthermore, estimations are made that in 20% of the human cancers, one or more defects are present in the SWI/SNF subunits (6). Therefore, the SWI/SNF complex must be considered as a relevant target in treatment options because it almost reaches a similar percentage as TP53, one of the most notorious tumor suppressor genes (6). Whole-exome sequencing has found more and more mutations in SWI/SNF subunits that are linked to different types of cancer, such as ARID1A and BRG1 for OCCCs and SCCOHT, respectively. Mutation in ARID1A probably occurs early in the process of tumorigenesis because these mutations are already present in preneoplastic lesions. This suggests that mutation in ARID1A alone is insufficient for tumorigenesis, and other hits are necessary (2, 27). Therefore it is important to validate the presence of mutations in ARID1A for early treatment. Consequently, patients with an ARID1A mutation should not be treated with platinum based chemotherapy because it correlates with reduction of progression

free survival. In addition, ARID1A is also a marker for early tumor recurrence, thus another reason to validate mutations in ARID1A (1). To validate OCCC it will help to look for defects in ARID1A combined with mutations in the PI3K/AKT pathway, because mutational co-occurrence occurs (2). Therefore whole-exome sequencing will probably be used as a diagnostic tool to help diagnose different types of tumors in the future (3, 7). The mutations in the PI3K/AKT pathway, most often cause a loss of PTEN or an activation of PIK3CA, which cause activation of the PI3K/AKT pathway in general (27). There are several PI3K/AKT inhibitors and some already made it to clinical trails because they were used to treat other types of cancers, for instance, CH5132799 and Triciribine. Additionally, duo-inhibitors exist, such as, GDC-0980. These inhibitors inhibit PI3K and mTor, which is closely related to the PI3K/AKT pathway (30). SCCOHT has an overall poor prognosis, however metastases make it even worse, which counts for the majority of cancers. The SWI/SNF complex has been suggested to be involved in the process of metastases because it is involved cellular motility, stem cell regulation and EMT (3, 7). Especially BRG1 loss is associated with the process of metastases and is linked to SCCOHT.

Potential treatment options for ARID1A- and BRG1-deficient tumors can be based on synthetic lethality approach targeting ARID1B, which shows great perspective for ARID1A-deficient tumors. ARID1B has a BC-box that has interactions with for example elongin C, an E3 ubiquitin ligase components. May be inhibition of this component or other components can be used to alter ARID1B expression (13, 31). BRM, which is suggested for the treatment of BRG1-deficient tumors, has a catalytic ATPase site and a bromodomain, both targetable by small molecule inhibitors (29). The nucleotide binding domains from ATPases varies a lot between different ATPases even if they catalyze the same chemical reaction. Therefore it is possible to design specific ATP-competitive inhibitors, which fits exactly into the catalytic ATPase site (32). Therefore it is necessary to know the crystal and protein NMR structures, which are available for all SWI/SNF complex subunits (29). Bromodomains can be targeted by a small cell-permeable molecule, JQ1, and can bind onto the bromodomain therefore it prevents the recognition of acetylated chromatin (31). BRM expression can also be altered indirectly due to crosstalk with the MAPK pathway because inhibitors, such as PD98059, can induce BRM (33). However clinical resistance does occur in treatments based on synthetic lethality, for example resistance to PARP1 inhibitors in BRCA deficient tumors (34). Regarding towards the resistance, treatment with BRM/ARID1B inhibitors, which probably cause synthetic lethality must probably be integrated in a combination of treatments and cannot be effective as single treatment. However small molecules that specifically inhibit

BRM and ARID1B are not yet developed. Additionally, inhibition of a second protein can probably enhance the synthetic lethal effect. On the other hand, cell viability will decrease when more proteins are inhibited in both tumor and normal cells that do not have the inactivated tumor suppressor gene. Therefore the reasonable specificity, which is a characteristic of synthetic lethality, may eventually be lost. The screening methods that were used in two studies have great potential to find more synthetic lethal interactions between SWI/SNF subunits and should be done for other relevant subunits (13, 29).

This review shows that it is important to know the genetic back-ground of the tumor. Although the genetic background within a tumor can probably differ, it is still important to look at the genetics of a tumor to optimize the treatment. A promising method to reveal the genetic background of the tumor is whole exome-sequencing. However this method still need some innovation before it is relevant in clinical use. In the paper from van Allen, E. et al. this is thoroughly discussed (35). The call for personalized medicine in the field of cancer is growing and can be established by the rapidly decreasing costs of sequencing. Although, chemical inhibition of BRM/ARID1B is not yet possible, it is possible to engage other pathways, like the PI3K/AKT pathway, MAPK pathways or pathways that involves MYC because these pathways become active due to SWI/SNF complexes.

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