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The ARID1A Gene Mutation and Cancer

Abstract

The gene ARID1A has sparked recently interest to researchers as having a major role in the suppression of cancers within humans. The gene codes for a protein on the SWI/SNF chromatin remodeling pathway, which when mutated shows a tendency for the development of tumors. **ARID1A shows a connection with a variety of cancer types and intersection with other suppressor pathways that are vital in further comprehension of this gene.** In this review, the role and function of ARID1A and the mechanistic and clinical effects of ARID1A mutation. ARID1A is compared to other important suppressor genes such as p53, p21 PIK3CA, and ARID1B.

Introduction

The ARID1A suppressor gene is being studied for its role and prevalence Chromatin remodeling is a form of gene expression in cells in which chromatin structure is adapted, letting DNA-modifying proteins to attach to genes during transcription. This process of chromatin structure detachment and subsequent reconnection has a large role in determining which genes are activated and which are not (Torigoe et al. 2013). The implications of chromatin remodeling extend to many levels of DNA function, and have a significant role in the developing of certain types of cancers. The incorrect activation or repression of even a single gene can lead to the creation of carcinoma cells.

Transcription in eukaryotes via the DNA of the nucleosome is initiated by two classes of chromatin remodeling complexes. One such method uses histone-modifying complexes that modify histones on the nucleosome by various processes including acetylation, deacetylation, phosphorylation, and methylation. The primary target of these processes is to free the chromatin DNA from their respective histones and open them up for modification. The other class of chromatin regulation and remodeling occurs in the presence of ATP (Lorch et al. 2010). This process uses ATP hydrolysis as a source of energy to facilitate the rearrangement of histones attached to DNA in the nucleosome.

There are many different known types of known ATP-based chromatin remodeling complexes, including the SWI/SNF complex (Shih et al. 2005). AT-rich interactive domain-containing protein 1A, also known as ARID1A, is a cancer

suppressor protein that is encoded by the ARID1A gene. The failed expression of this gene is linked to mammary and ovarian cancer. This review will be focused on the role of ARID1A as a chromatin remodeler and the link between ARID1A mutations and cancer, specifically ovarian carcinomas. The review will also discuss the value of ARID1A expression as an indicator for the prognosis of these specific diseases.

The Importance of ARID1A

ARID1A's main importance is as a cancer suppressor that is linked with SWI/SNF. As The Switch/Sucrose Non Fermentable complexes (SWI/SNF) are classed as ATP-based chromatin remodelers. Consisting of the protein products of SWI and SNF genes along with ATPase-type enzymes, this complex has been linked to the patterning of nucleosomes and the high occupancy of nucleosomes at promoters (Lu et al. 2013). ARID1A mutations have been observed in a wide variety of cancers, including 8.9% (Lee et al. 2016) and 39% (Cajuso et al. 2014) in colorectal cancer, 47% in endometrial carcinoma (Nagymanyoki et al. 2014), 40% in gastric cancers (Kang et al. 2016) 55% in breast cancer (Zhang et al. 2011), and 11% in pediatric neuroblastoma (**Sausen et al.**). Immunohistochemical analysis of Hepatocellular carcinoma revealed a decreased level of ARID1A protein expression in 64.1% of tumor tissues (He et al. 2015). Loss of ARID1A expression also correlated with other conditions such as lymphovascular invasion (Lee et al. 2016, Kang et al. 2016) but statistics of the age, gender, and level of relapse in subjects were either contradicting or inconclusive. The consensus of studies link ARID1A mutations as a tumor suppressor, however, no direct

evidence or mechanism of this activity has been observed (Guan et al. 2011). Furthermore, the wide variety of cancers, which include lymphomas, blastomas, sarcomas, and carcinomas leads to the conclusion that ARID1A has function as at least some effect on tumor suppression throughout every cell type.

Clear-cell ovarian carcinoma (OCCC) is an uncommon cancer that is usually malignant and difficult to treat. Because of its extremely high mortality rate, there has been a recent surge of studies surrounding this disease in order to aid its prognosis. ARAD1A-based mutation occurs at relatively high levels in ovarian carcinoma, prompting researchers to treat the ARID1A gene as having a meaningful role in the suppression of this carcinoma. In immunohistochemical staining for ARID1A, 55 out of 119 (46%) (Wiegand et al. 2010) and 63/112 (56.2%) (Ye et al. 2016) had prominent ARID1A mutations. Likewise, PCR amplification and Sanger DNA sequencing identified 57% of OCCC cells as containing ARID1A mutations (Jones et al. 2010). The clinical and prognostic aspect of the ARID1A gene might be the key to further understanding for the treatment of ovarian carcinomas.

ARID1A Structure and Role as an SWI/SNF Complex

The structure of ARID1A is vital to its role as a tumor suppressor and other functions. ARID1A is a gene that encodes the similarly named ARID1A protein. The main feature of this protein is the ARID (AT-rich interaction domain), a binding site that is encoded by approximately 100 amino acids. This binding site is present in over 14 different proteins, with ARID1A classified in the ATP-using subfamily ARID1. The ARID

domain allows ARID1A to initiate the chromatin remodeling complex. A protein containing the ARID domain of ARID1A bonded to the pyrimidine-rich beta-globin locus (Nie et al. 2000), where it can be recognized by SWI/SNF. ARID1A's second important complex is located at its C-terminus, which was proven to associate with glucocorticoid receptor-dependent activation, which is an essential process in the SWI/SNF chromatin remodeling complex (Pico et al. 2016). This is evidenced by the results of Nie et al., where the removal of this C-terminus resulted in a 70% decrease in GR-dependent activation. Given the glucocorticoid receptor's importance as a gene regulator and as a hormone receptor, its presence in ARID1A and other subunits of the SWI/SNF complex is notable. Additionally, ARID1A and SWI/SNF may be connected by their impact on the cell cycle, with mutations causing impaired cell growth and tumor formation during the G phases of the cell cycle (Inoue et al. 2014). The connection between ARID1A and endocrine signaling throughout cells is unknown.

Comparing ARID1A to Other Tumor Suppressors

The p53 protein is a much more well known tumor suppressor, and is naturally compared to other suppressors like ARID1A for its role as a transcriptional activator. Gene p53's role in cell cycle regulation and apoptosis exhibits similarities to the role of the ARID1A gene in chromatin remodeling and regulation. Both genes are also linked to the preservation of telomeres: p53 initiates apoptosis when telomeres are deteriorated or rendered defective, while ARID1A has been shown to negatively regulate a telomeres reverse transcriptase enzyme that maintains the telomeres of cancer cells

(Rahmanto et al. 2016, Artandi and Attardi 2005). Through immunoblotting and CRISPR/Cas9 cell generation, it was found that ARID1A also negatively regulates the promoter of telomerase reverse transcriptase (TERT) by 55%-83% reduction (Rahmanto et al. 2016). The presence of TERT increases the likelihood of tumor cell survival when activated.

Like the link to many other genes related to cancer suppression, the link between ARID1A and p53 is not fully understood. The level of correlation between the two genes, and other tumor suppressors like CDKN1A, and SMAD3 genes. When the transcriptomes of control and ARID1A knockdown ovarian epithelial cells were compared, many pathways in cell cycle regulation were correlated with the decreased levels of ARID1A. Co-immunoprecipitation and ChIP analysis exhibit an interaction between p53 and ARID1A via the binding of ARID1A to p21 and SMAD3 promoter regions (Guan et al. 2011). ARID1A and p53 may collaborate or participate in similar processes as transcriptional regulators that activate genes such as p21 and SMAD3. There is also evidence that recombinant p53 and ARID1A are directly bonded into a single mechanism that partakes in transcriptional control via SWI/SNF chromatin remodeling. In this case, tumor cells in cancers like OCCC may use ARID1A mutations to alter p53 production levels and downregulating downstream targets of p53.

In a study of hOsa2/BAF250a in HeLa cells, the level of p53 proteins increased after an increase in ARID1A-type expression (Inoue et al. 2014). The resulting decreased cell growth were consistent with increased p53 levels. Using RNAi, the decreased expression of hOsa2 led to increased C-myc mRNA, which is a protein

speculated to cause swift growth of tumor cells. Again, it is implicated that ARID1A has a direct effect with downstream targets of p53 like c-myc and p21, linking p53 with ARID1A furthermore. Similarly, ARID1A mutation has been shown to exhibit abnormally low p53 expression, as well as a loss of MMR protein. MMR protein deficiencies are shown to lead to carcinogenesis (Bernstein and Bernstein, 2015). Another study, this one involving immunohistochemical analysis of endometrial clear cell carcinomas, found no significant negative correlation between p53 overexpression and ARID1A. This study has a relatively large sample size, however, it could not find any clinical demonstration of a relationship between chromatin remodeling complexes like ARID1A and p53. However, the percentage of p53 and BAF250a (ARID1A) occurrence in these endometrial carcinomas were much lower (34% and 20% respectively) than other studies of the more commonly analyzed ovarian clear-cell carcinoma (Fadare et al. 2013).

ARID1A and the PIK3CA Protein

PIK3CA, or Phosphatidylinositol-4,5-biphosphate 3-kinase, is a gene that codes for the p110α protein in humans. Like ARID1A, it exhibits high mutation rates in a variety of cancers and is believed to be vital in tumor suppression. The comparison between samples of mice with injected ovarian tumor cells and control mice showed that the rate of tumors is dependent on the cooperation of ARID1A mutations and PIK3CA mutations. In addition, the mutation of ARID1A alone produces much less tumor formation. This interaction of ARID1A and PIK3CA mutations is suggested to lead

to excessive production of Interleukin - 6 and therefore theorizes a link between inflammatory cytokine signalling and clear-cell ovarian carcinoma (Chandler et al.). In the immunohistochemical analysis of clear-cell carcinoma samples and mutation assay of the PIK3CA gene, analysis found that although the two mutations coexist in tumor cells, they are not necessary statistically linked. For samples with PIK3CA mutations, ARID1A mutations were found in 12/17 (71%). This number dropped to 11/25 (44%) in PIK3CA-intact carcinoma cells. These findings do not suggest a statistical correlation, and further research is required to confirm a relationship between somatic mutations of the ARID1A and PIK3CA expression pathways (Yamamoto et al. 2012). Furthermore, another immunostaining study revealed that PIK3CA had no effect on clinical survival. This study agreed that the level of interaction between ARID1A and PIK3CA was unclear, as it could find no correlation between the two (Ye et al. 2016). PIK3CA is related to PI3K/ATK as a downstream activator of the pathway.The PI3K/AKT pathway is critical for many functions of the cell, including cell development, proliferation, and metabolism.

ARID1A and ARID1B Subunits

Along with the homologous ARID1B and ARID2 genes, ARID1A has been shown to bind at active genomic regions, however, ARID1A differs from other ARID subunits by the specific chromatin remodeling complexes that bind to the region (Raab et al. 2015). Mutations in ARID1A and ARID1B are related; loss of ARID1B is linked to loss of ARID1A expression as well as a decrease in cell count in both wild-type and tumorous

cells (Samartzis et al. 2016) Mutations of ARID1B, along with PIK3CA, scored as one of the two highest molecules needed for the the growth of ARID1A mutated tumor cells.

In another report, he effect of reducing ARID1A was shown to be an increased dependency on ARID1B within tumor cells. (Helming et al. 2014) It is shown that these two genes are mutually exclusive subunits of the SWI/SNF complex, however, either one or the other must be present for the complex to proliferate. Because of their common co-mutations in tumor cells and the large occurrence of ARID1B mutation in ARID1A cell lines, (Helming et al. 2014), it is possible that ARID1B has similar function to ARID1A in terms of tumor suppression. Future research could explore the level of dependency of mutations between each other.

ARID1A's and Tumor Suppression

Tumor suppressors are one of the most important genes preventing the synthesis of cancer. Their many functions include the control of cell cycle checkpoints, signaling pathways, DNA damage and repair, initiating apoptosis and protein turnover (Sherr 2004). ARID1A demonstrates a large capability for all of these functions, and fits the definition of a tumor suppressor thoroughly. One of the most common studies features the ARID1A mutations' effect on the proliferation of the cell. In immunochemical MTT proliferation assay of colorectal carcinoma, levels of ARID1A expression were found to be lower than the control (Xie et al. 2014). Overexpressed ARID1A in these colorectal carcinoma cells revealed a higher rate of proliferation. When ARID1A is added to ARID1A-depleted carcinoma cells, the level of cellular proliferation

was restored back to a controlled rate. This result and the results of similar carcinoma analysis studies support the deduction of ARID1A functioning as a tumor suppressor. In the same research, apoptosis staining was analyzed using FACS between an ARID1A knockdown plasmid, a tumor cell control, and the ARID1A-increased plasmid. As expected, the apoptosis percentages increased from 5.5% to 10.7% to 16.5% for the three samples respectively. The unmutated ARID1A gene's role as a tumor suppressor is supported by its activity in cell apoptosis.

Conclusion

Due to the improvement of oncogenetic technology, ARID1A has emerged as a crucial tumor suppressor. Mutations of chromatin remodeling are traced to the ATP-dependent SWI/SNF chromatin remodeling complex. ARID1A is a binding domain that provides the SWI/SNF complex with DNA needed by SWI/SNF. As tested by Sanger DNA sequencing, nearly all ARID1A mutations are somatic, and involve the production of a premature nonsense mutation due to insertion or deletion (Jones et al. 2014) At the clinical level, the mutation of this gene is involved in almost every single type of cancer, but there is still little connection between the types and ARID1A. Further research can be applied to the comparison of tumor types, and finding trends that would indicate higher instances of ARID1A mutations. These could include: the difference between hereditary and environmental cancers, the tumor's type of tissue, or the presence of molecules or hormones in different cancers relating to the glucocorticoid receptor in ARID1A c-terminus.

ARID1A has many connections with other tumor suppressing pathways and transcriptional complexes. However, much of the challenge behind studying ARID1A is the lack of knowledge concerning the specific mechanisms and pathways that the gene is associated with. Further research could solidify the link between more studied tumor suppressor genes like p53 or PIK3CA could lead to combined treatments that would add ARID1A mutation therapy. The continuation of research down these areas could lead to the development of treatments or therapy specific to the mutation deficiencies of ARID1A-based tumors.

Works Cited

Artandi C, Attardi LD. 2005. Pathways connecting telomeres and p53 in senescence, apoptosis, and cancer. Biochem Biophys Res Commun. 10,331(3): 881-90.

Bernstein C, Bernstein B. 2015. Epigenetic reduction of DNA repair in progression to gastrointestinal cancer. World J Gastrointest Oncol. 7(5): 30–46.

Cajuso et al. 2014. Exome sequencing reveals frequent inactivating mutations in ARID1A, ARID1B, ARID2 and ARID4A in microsatellite unstable colorectal cancer. International Journal of Cancer. 135: 611–623.

Chandler et al. 2014. Coexistent ARID1A–PIK3CA mutations promote ovarian clear-cell tumorigenesis through pro-tumorigenic inflammatory cytokine signalling. Nature Communications. 6: 6118

Fadare et al. 2013. The clinicopathologic significance of p53 and BAF-250a (ARID1A) expression in clear cell carcinoma of the endometrium. Modern Pathology. 26(8).

Guan et al. 2011. ARID1A, a factor that promotes formation of SWI/SNF-mediated chromatin remodeling, is a tumor suppressor in gynecologic cancers. Cancer Res. 71(21): 6718-6727.

He et al. 2015. Decreased expression of ARID1A associates with poor prognosis and promotes metastases of hepatocellular carcinoma. Journal of Experimental & Clinical Cancer Research. 34(47).

Helming et al. 2014. ARID1B is a specific vulnerability in ARID1A-mutant cancers. Nat Med. 20(3): 251-254.

Inoue et al. 2011. Target genes of the largest human SWI/SNF complex subunit control cell growth. Biochem J. 434(1): 83-92.

Jones et al. 2010. Frequent Mutations of Chromatin Remodeling Gene ARID1A in Ovarian Clear Cell Carcinoma. Science. 330(6001): 228-231.

Kang et al. 2016. Biomarkers of EBV-positive Gastric Cancers: Loss of PTEN Expression is Associated with Poor Prognosis and Nodal Metastasis. Annals of Surgical Oncology. 23(11): 3684-3692.

Koshiyama et al. 2014. Recent Concepts of Ovarian Carcinogenesis: Type I and Type II. Biomed Res Int. 934261.

Lee et al. 2016. ARID1A expression in early stage colorectal adenocarcinoma: an exploration of its prognostic significance. Human Pathology. 53: 97-104.

Lorch et al. 2010. Mechanism of chromatin remodeling. Proc Natl Acad Sci U S A. 107(8): 3458-3462.

Lu P, Roberts CWM. 2013. The SWI/SNF tumor suppressor complex regulation of promoter nucleosomes and beyond. Nucleus. 4(5): 374-378.

Nagymanyoki et al. 2014. ARID1A Is a Useful Marker of Malignancy in Peritoneal Washings for Endometrial Carcinoma. Cancer Cytopathy. 123(4): 253-257.

Nie et al. 2000. A Specificity and Targeting Subunit of a Human SWI/SNF Family-Related Chromatin-Remodeling Complex. Molecular and Cellular Biology. 20(23): 8879-8888.

Pico et al. 2016. Glucocorticoid receptor-mediated cis-repression of osteogenic genes requires BRM-SWI/SNF. Bone Reports. 5: 222-227.

Raab et al. 2015. Genome-Wide Transcriptional Regulation Mediated by Biochemically Distinct SWI/SNF Complexes. PLoS Genet. 11(12): e1005748.

Rahmanto et al. 2016. ARID1A regulates TERT transcription. The Journal of Biological Chemistry. 18(291): 9690-9699.

Samartzis et al. 2014. Loss of ARID1A expression sensitizes cancer cells to PI3K- and AKT-inhibition. Oncotarget. 5(14): 5295-5303.

Sausen et al. 2013. Integrated genomic analyses identify ARID1A and ARID1B alterations in the childhood cancer neuroblastoma. Nat Genet. 45(1): 12-17.

Sherr C. 2004. Principles of Tumor Suppression. Cell. 116(2): 235-246.

Shih et al. 2005. Amplification of a Chromatin Remodeling Gene, Rsf-1/HBXAP, in Ovarian Carcinoma. Proceedings of the National Academy of Sciences of the United States of America. 102(39): 14004-14009.

Torigoe et al. 2013. ATP-dependent chromatin assembly is functionally distinct from chromatin remodeling. eLife. 2: e00863.

Wiegand et al. 2010. ARID1A Mutations in Endometriosis-Associated Ovarian Carcinomas. N Engl J Med. 363(16): 1532-1543.

Xie et al. 2014. Decreased ARID1A expression facilitates cell proliferation and inhibits 5-fluorouracil-induced apoptosis in colorectal carcinoma. Tumor Biology. 35(8): 7921-7927.

Yamamoto et al. 2012. Loss of ARID1A protein expression occurs as an early event in ovarian clear-cell carcinoma development and frequently coexists with PIK3CA mutations. Mod Pathol. 25(4): 615-624.

Ye et al. 2016. Clinicopathologic Significance of HNF-1β, AIRD1A, and PIK3CA Expression in Ovarian Clear Cell Carcinoma. Medicine (Baltimore). 95(9): e3003.

Zhang et al. 2012. Frequent low expression of chromatin remodeling gene ARID1A in breast cancer and its clinical significance. Cancer Epidemiology. 36(3): 288-293.