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REVIEW

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Current understanding of adenoid cystic carcinoma in the gene expression and targeted therapy

Quan-Quan Lin[1,2†], Jin-Long Sun[2†], Feng Wang[2†], Hai-Zhong Zhang[1], Ge Zhou[3] and Qing Xi[1*]

Abstract

Adenoid Cystic Carcinoma (ACC) has been considered as a "quiet" tumor. It is typically malignancy arising from exocrine glands with poor long-term prognosis due to high rate of recurrence and distant metastasis. It is characterized by perineural infiltration, distant metastasis, and positive incision edge. Surgery is the first line treatment for ACC, followed by cytotoxic chemotherapy and/or radiotherapy as adjuvant treatments to avoid recurrence. But recurrence or metastasis still occurs in more than 50% ACC. Recurrent and/or metastasis (R/M) ACC is usually incurable, and no systemic agent has been found effective. With the widespread use of whole exome sequencing (WES) and whole genome sequencing (WGS), its internal oncogenic mechanism is gradually revealed, which involving molecular mutations such as the MYB family gene translocation, Notch signal pathway, DNA damage repair (DDR) pathway and epigenetic molecular mutations. The review helps us to understand the linkage among the pathways and targeted genes in diagnosis and related treatment of ACC till now.

Keywords Adenoid cystic carcinoma (ACC), MYB, Notch, DNA damage, DNA damage repair (DDR), Epigenetics related gene

1 Introduction

Adenoid Cystic Carcinoma (ACC) is rare malignant tumor arising in exocrine gland with poor long-term prognosis due to high rate of recurrence and distant metastatic tendency [1]. It is mainly found in salivary glands with the highest incidence, accounting for 1% of head and neck malignancies, 4% of salivary gland tumors, and 7.5% of all epithelial salivary gland malignancies [2]. It shows that biological characteristics such as perineural infiltration, distant metastasis. Surgery is the first line treatment for ACC, followed by cytotoxic chemotherapy and/or radiotherapy as adjuvant treatments to avoid recurrence. But recurrence or metastasis still occurs in more than 50% ACCs [3]. Recurrent and/or metastasis (R/M) ACC is usually incurable, and no effective systemic agents have been identified to be effective. Therefore, the overall prognosis is poor, with 5 years,10 years, and 20 years survival rates of 68%, 52%, and 28% respectively [4].

ACC has been considered as a "quiet" tumor. However, with the widespread use of whole exome sequencing (WES) and whole genome sequencing (WGS), its internal oncogenic mechanism has been gradually revealed, which involves molecular mutations such as the MYB family gene translocation, the Notch signal pathway, and the DNA damage repair and epigenetic molecular mutations [4, 5]. Understanding the linkage among these pathways in ACC will help us fully understand and figure out

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the "true features" at the genetic level and the potential targets (Fig. 1).

1.1 The MYB family gene (Fig. 2)

1.1.1 MYB (V-Myb Avian Myeloblastosis Viral Oncogeneand) MYBL1 (V-Myb Avian Myeloblastosis Viral Oncogene Homolog-Like 1)

Myb, located on chromosome 6q23.3, is a proto-oncogene encoding c-MYB transcription factor [3, 6]. *Myb* was first identified in leukemia and later observed in ACC of breast [7], lung [8] and systemic secretory glands, indicating that MYB activation is mostly common in exocrine gland tumors [9]. In salivary ACC, MYB expression ranged from 65 to 85% [10]. As the hallmark gene, MYB and NFIB (Nuclear Factor I B) gene involved in chromosome translocation at t (6;9). This rearrangement has been identified in most 60% of ACC samples [6, 10].

In addition, *mybl1*, located on chromosome 8q22, rearrangement occur in approximately 35% of MYB-NFIB negative cases [10]. MYBL1 shows the same DNA domain with MYB. And it is mainly expressed in the central nervous system, B lymphocytes, breast, and testis and is verified in low-grade gliomas in children and adolescents [11]. Comparing the MYB and MYBL1 expressions in ACC samples from the same site suggested that myb and mybl1 transcripts were mutually exclusive [12]. However, both proto-oncogene transcription factors MYB and MYBL1 produce oncoproteins that display similar gene expressions and correlate with the recurrence and invasionof peripheral nerves [10–12]. Combined with survival research studies, MYBL1 is mainly expressed in

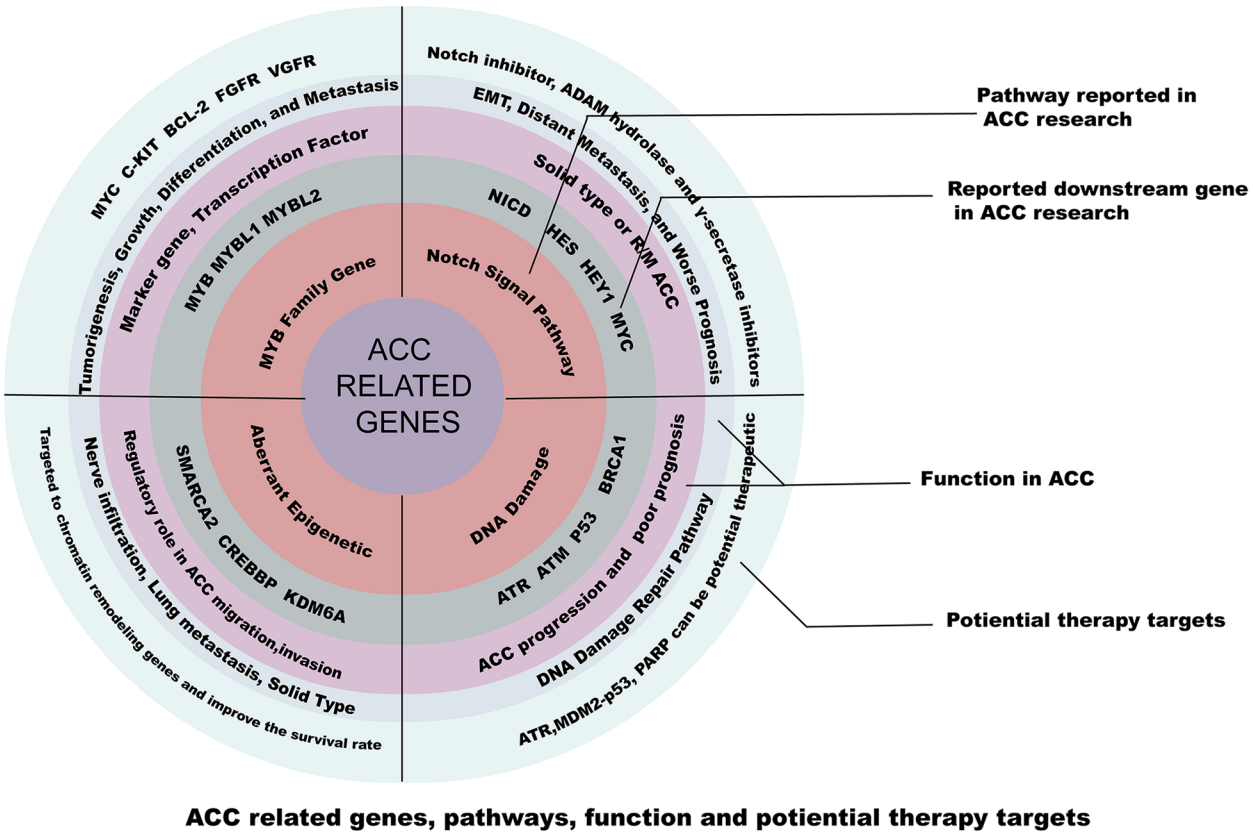

Fig. 1 The related genes, function, and the potential target in ACC. The related gene and pathway in previous ACC molecular reports. MYB family gene is the marker gene in ACC, including MYB, MYBL1, and MYBl2. It acts as a transcription factor in regulating tumorigenesis, growth, differentiation, and metastasis in ACC. MYC, C-KIT, BCL-2, FGFR, and VGFR is the downstream gene of MYB. The Notch signal pathway is activated in most solid types and R/M ACC. NICD is the product of the notch signal pathway. HES, HEY1 and MYC can be regulated by notch signal pathway. The Notch signal pathway is associated with EMT, distant metastasis, and worse prognosis. The notch, ADADM hydrolase, and γ-secretase inhibitors can be used for potential therapy in ACC. The related gene of the DDR pathway including the ATR, ATM, P53, and BRACA1 can be observed in ACC samples. ATR, PARP, and MDM2-p53 inhibitors can be potential targets. The related gene of aberrant epigenetics includes SMARCA2, CREBBP, and KDM6A. They are involved in ACC tumor migration and invasion, which lead to nerve infiltration, lung metastasis, and solid type. Targeting the chromatin remodeling genes can be a potential direction for improving the survival rate

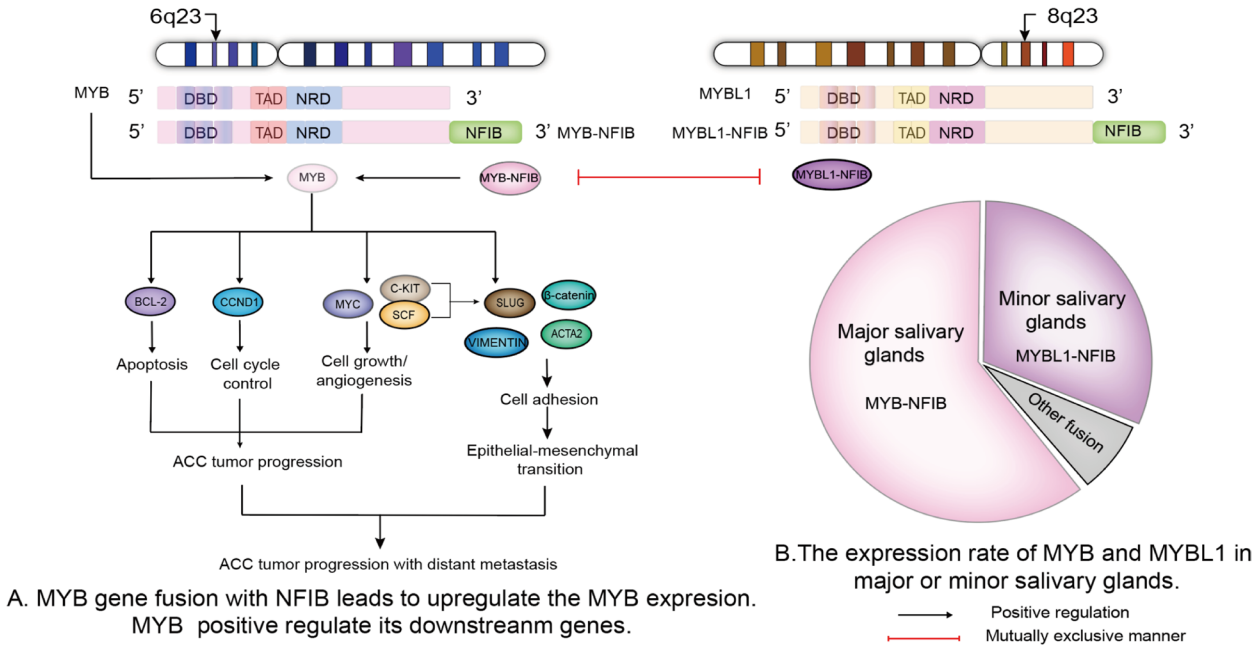

Fig. 2 How the MYB family gene works in ACC. **A** Schematic representation of MYB-NFIB and MYBL1-NFIB translocation in adenoid cystic carcinoma. MYB and MYBL1 oncogene is located at chromosomal band 6q23 and 8q23, respectively. NFIB is a transcription factor, occur the fusion with MYB or MYBL1 at the 3'UTR. The fusion results in overexpression of MYB and MYBL1. MYB family gene can upregulate the related gene involved in apoptosis, cell cycle control and cell adhesion. **B** MYB is mainly expressed in major salivary glands about 65%. MYBL1 is expressed in minor salivary glands about 30%. Other fusion were reported in few research

the minor salivary gland and its overexpression was significantly associated with further negative developments of prognosis [3, 10, 12].

Myb is a complex oncogene with frequent alternative RNA splicing. Previous research identified that different transcripts are associated to alternative splicing [11, 13]. In ACC, it was found that the location of the breakpoint of *myb* exon determined the level of mRNA expression. And the breakpoint in exons 8 and 15 seemed to be the most common [14]. The aberrant regulation of *myb* is due to its 3' untranslated region (UTR) binding to microRNAs such as miR-15a, miR-16, and miR-150. As a result of the translocation, *myb* lost its 3'-end target and upregulated the 5'-end expression. Similarly, to *myb* transcriptions, NFIB overexpression is due to negative regulations of *nfib* transcriptions by miR-21. Multiple variable breakpoints occur in the MYB gene between exon 8 and 15 and the NIFB gene in exon 8 and 12, or the 3' UTR result in the short or long fusion [14, 15]. No matter what occurs in the breakpoints mentioned, the MYB-NFIB translocation finally leads to MYB overactivation and overexpression in the MYB protein level [16].

It has been evidenced that the alternative promoter drives MYB as the major oncogenic gene in ACC. The alternative promoter transcripts produce N-terminally truncated MYB proteins lacking a highly conserved and phosphorylated domain. It can disrupt the expression of regulating proteins and lead to aggressive progression in ACC [17].

1.2 Downstream genes of MYB

Myb can regulate the genes related to cell growth or angiogenesis (MYC, KIT, VEGFA), apoptosis (BCL2, API5, BIRC3, HSPA8, SET), cell cycle control (CCNB1, CDC2, MAD1L1) and cell adhesion (β-CATENIN, E-CATENIN, Vimentin, and ACTA2) [18].

In ACC research, *Myc* (V-Myc Avian Myelocytomatosis Viral Oncogene Homolog) is activated by *myb* amplification and translocation. It leads to *myc* trigger transcriptional dysregulation in the S phase activity [19]. *Myc* is significantly associated with myoepithelial cell loss and highly expressed in ACC solid type. It correlated with clinicopathological staging, bad prognosis and high-grade transformation (HGT) [20].

The expression of C-kit (Tyrosine-Protein Kinase Kit) is 80%-100%, commonly found in minor salivary and stage III-IV and significantly associated with poor prognosis [21]. *C-kit* activates the SCF/C-KIT signal pathway

by binding to its ligand SCF (Stem Cell Factor, SCF). Research has reported that the activation of SCF/C-KIT signal pathways can upregulate the expression of SLUG (Snail Family Transcriptional Repressor 2) and *myb* regulate the transcription in the binding domain located at the 5' end in the first intron of Slug gene [22]. In ACC, high expression of SLUG is significantly correlated with solid type and lung metastasis [22, 23].

To apoptosis regulator, *myb* upregulates the BCL-2 (B-cell lymphoma-2) overexpressed and diagnosed as a feature of worse prognosis [24]. *Myb* may promote cell proliferation by upregulating ccnd1 and downregulating *p16* (Cyclin Dependent Kinase Inhibitor 2A) to promote cell cycle progression [18, 25].

Myb have closely correlated with the genes associations with the occurrence of epithelial-mesenchymal transition (EMT) [26]. In addition, *myb* induces EMT by downregulating *e-cadherin* (Cadherin 1, Type 1, E-Cadherin (Epithelial)) and upregulating the expression of the mesenchymal marker VIM (Vimentin) and ACTA2 (Actin alpha 2, smooth muscle) [27]. *β-catenin* (Catenin Beta 1) was observed to be upregulated *by myb* to disrupt intercellular adhesion thereby activating the Wnt signal pathway leading to EMT occurrence [28].

1.3 Targeted gene therapy related with MYB

MYB is an upstream of some target genes, such as the VEGF (Vascular Endothelial Growth Factor), FGF (Fibroblast Growth Factor), and KIT [29].

VEGF receptor has already considered as a potential therapeutic target for ACC in several research [30]. In clinical trials, part of VEGFR-targeted drugs shows no objective response like sunitinib, regorafenib, and nintedanib [31]. The later clinical trials of lenvatinib showed it had a significant clinical improvement in objective response rate, time to progression and progression-free survival compared to sorafenib [32].

Bcl-2 is upregulated by *vegf* or *myb* directly. In the PDX (Patient-derived tumor xenograft) model of ACC, the small molecule inhibitors of BCL-2 were found to control the tumor progression. This may be a new direction in the treatment of ACC in the future [33].

FGF, the similarly to VEGF, has been indicated to be associated with overexpression of MYB [34]. The tumor cells were inhibited by the FGF receptor 1 inhibitor and cisplatin. The result showed a lower cell proliferation rate and cell migration compared to the single cisplatin group [35].

Since MYB was reported to be transcription factor, the structural disorder and lacking binding pockets have made design of small molecules for transcription factors challenging [36]. Previous studies have shown that it may be possible to inhibit the MYB expression by regulating the interaction with coactivators CBP/p300 (CREB Binding Protein/ E1A Binding Protein P300) with a natural low-molecular-weight compound Celastrol. But it seems to inhibit non-selective cell viability [37].

All-trans Retinoic Acid (ATRA) [38], was clinically available for the treatment of C (APL) but few were reported in ACC. In the MYB translocation-positive ACC PDX model, it was shown that ATRA can induce tumor cell death. A phase II trial of ATRA in Advanced Adenoid Cystic Carcinoma (NCT03999684) has been initiated to verify these results in humans [39].

Another Immunomodulatory therapy phase I clinical trial for ACC is currently active (NCT03287427), which is by using a full-length MYB fusion product cloned into the FDA-compliant DNA vaccine vector pVAX1 to create the pVAX1-Tet-human MYB DNA vaccine (PMC6804811) [40].

1.4 Notch signal pathway

The Notch signal pathway is involved in cell differentiation, proliferation, apoptosis, adhesion, migration, and angiogenesis. It acts as an oncogene in most human cancers, including lung cancer [41], glioma [42], colon cancer [43], breast [44], and head and neck tumors [45]. In 2010, Lin et al. found that the Notch family genes expressed and associated with distance metastasis in ACC [46]. Comparing the molecular differences between primary and R/M ACC, the Notch signal pathway-related genes were enriched in R/M ACC [4, 5, 16].

When the Notch signal pathway is activated, the Notch receptor releases the active notch intracellular domain (NICD) into the cytoplasm. The released NICD is transferred into the nucleus to directly regulate the functions of transcription factors (CBF-1, Suppressor of hairless, Lag / CSL) and activate a series of downstream target genes [47].

1.5 Notch signal pathway related gene (Fig. 3)

1.5.1 Notch-Hey1 signal pathway

Hey1 (Hes Related Family BHLH Transcription Factor with YRPW Motif 1) is a downstream regulatory gene of CSL-NICD transcription [48]. *Hey1* plays a critical role in the development of various tissues and organs as well as the occurrence and development of tumorigenesis and progression [49]. *Hey1* increases cell invasion and metastasis by driving the EMT-related genes and MMPS (Matrix Metalloproteinases).

After the knockdown of the *Hey1* gene, the expression of the transcription factor Twist1 (Twist Family BHLH Transcription Factor 1) and MMPS was observed to be

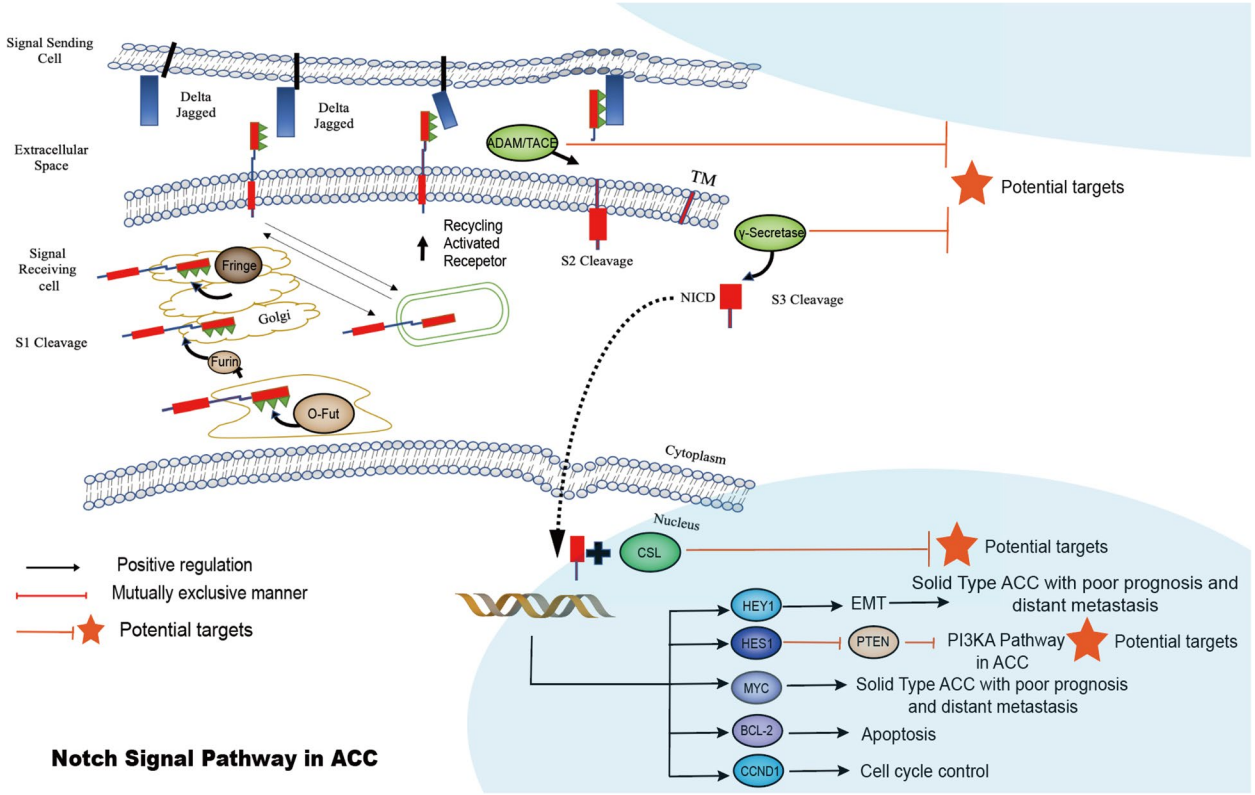

Fig. 3 How the Notch signal pathway works in ACC. It is accompanied by 3 hydrolytic enzymatic shearing of Notch receptors, and immature Notch receptors translocate to the cellular Golgi apparatus, where furin convertase is hydrolytically sheared at the S1 site at the extracellular end of the Notch transmembrane region to form mature Notch receptors translocated to the cell membrane. The NEC of the extracellular region of the mature Notch receptor binds to the ligand and is hydrolyzed by ADAM (a disintegrin and metalloprotease) metalloprotease at the S2 site located in the TM of the transmembrane region of the Notch receptor. The remaining TM region, hydrolyzed by γ-secretase, cleaves its S3 site and releases soluble NICD, which eventually enters the nucleus to bind to DNA and form a complex with other enzymes such as transcription factor CSL and to activate the notch signal pathway. HEY1, HES1, MYC, BCL-2, and CCND1 is the downstream of the notch signal pathway. HEY1 leads to the EMT and solid type with poor prognosis. HES1 inhibits the expression of PTEN. It is a potential way to treat ACC patients with PI3KA pathway activated. MYC is close to the solid type ACC with poor prognosis and distant metastasis. BCL-2 and CCND1 are associated with apoptosis and cell cycle control

reduced. Recently, targeted NOTCH1-HEY1 inhibitors provide a new direction for ACC target therapy [50, 51].

1.6 Notch-Hes1 signal pathway

Hes1 (Hairy and enhancer of split homolog-1) is a gene downstream of Notch pathway. It is highly expressed in R/M samples [52]. HES1 has the potential to induce self-transformation of cancer stem cells and to trigger apoptosis resistance and tumorigenic progression. In SACC LM cell experiments, the ability of metastasis and invasion was suppressed by knockdown the expression of HES1 [52, 53].

HES inhibits the expression of PTEN (Phosphatase and Tensin Homolog). PTEN, as a tumor suppressor, can dephosphorylate PIP3 to generate PIP2, thus achieving negative regulation of the PI3K signal pathway and exerting the function of inhibiting cell proliferation and promoting cell apoptosis [54]. The presence of PTEN mutations in ACC activates the functional activation of the PI3K pathway for promoting tumorigenesis [3]. When HES expression is downregulated by inhibition of Notch, HES inhibition of PTEN is diminished and PTEN expression is upregulated, inhibiting PI3K-Akt-mediated survival signal and ultimately tumor cell apoptosis [55].

1.7 Notch-Myc signal pathway

Notch induces Myc expression to promote cell proliferation. *Myc* is a direct downstream target of Notch signal and a key component of transformation and cell growth [56]. The oncogenic role of NOTCH1-MYC was well demonstrated in T-cell acute lymphoblastic leukemia (T-ALL) studies [57]. Notch was found to be positively correlated with MYC expression by immunohistochemical staining. MYC expression was found to be suppressed using Notch-related inhibitors in subsequent experiments [58]. In ACC, MYC significantly associates with

activation of Notch signal pathway. Increased expression of MYC and NICD can be found in solid tumors with poor prognosis and distal metastasis [59].

1.8 Clinical significance of Notch signal pathway activation in ACC

Notch and MYB can drive different regulatory programs in alternate cell lineages [60]. The high expression of NICD is a product of the activation of Notch signal pathway [61]. In 63 ACC cases, NICD was found to be positive in 47/63 cases, and 43 cases were solid type ACC. It was inferred that the Notch pathway was activated and NICD is positively correlated with solid type ACC with bad prognosis [62].

In addition, the expression of MYB protein was significantly higher in Grade1 and Grade2 cribriform and tube type. To further predict prognosis, molecular subgroups of + MYBNOTCH1 +, MYB/other +, WTMYBTERT + and WTMYBNOTCH1 + ACC tumors were classified according to alteration of MYB, NOTCH1 and TERT (Telomerase Reverse Transcriptase). The + MYBNOTCH1 + status had the worst prognosis [5, 62].

To further clarify more accurately the molecular and prognostic correlation of ACC, Ferrarotto R et al. [59] named as "ACC-I" the group with notch signal pathway activation, enriched of MYC protein expression, mostly found in minor salivary glands with solid type. This group involved the upregulation of transcription by the epigenetic-related *crebbp* and its paralogous gene *ep300*. It indicated that the *notch* may be related to the transcriptional regulation of epigenetic-related genes. A correlation analysis was performed by comparing NICD (+) and NICD (-), verifying that patients with NICD-positive tumors were more likely to be solid histology and lung metastasis [5, 59, 62].

1.9 Targeted Notch signal pathway

Activation of Notch signal pathway is associated with concomitant cleavage of three hydrolases. The Notch pathway and its downstream gene can be targeted as pan-notch inhibitors such as gamma-secretase inhibitors or notch1 inhibitors. ADAM (A disintegrin and metalloprotease domain) hydrolase and γ-secretase inhibitors lead to block the cell migration and invasion, suggesting that it is possible to treat ACC for future clinical therapeutic approaches, which were in current clinical trials [60, 61].

Clinical trial using Brontictuzumab, a kind of Notch1 inhibitors, for treatment showed that Brontictuzumab benefits in 2 partial response (PR) and 3 stable diseases (SD) among 12 patients [59]. In another pan-Notch inhibitor study enrolled 22 ACC patients, 1 patient had an unconfirmed PR (15%) while 15 patients showed SD [62]. However, the significant adverse symptoms such as nausea and diarrhea were found. Currently, another phase I/IIA clinical trial with CB-103 targeted to the Notch transcription is recruiting patients with advanced or metastatic solid tumors, including ACC (NCT03422679) [63].

1.10 DNA damage repair pathway (Fig. 4)

In ACC, overexpression of MYB and MYB-NFIB leads to increase replication stress (RS). DNA damage can cause genetic mutations, oncogene activation, and chromosome structural changes, triggering abnormal regulations of cellular senescence, apoptosis, and metabolism, ultimately promoting tumorigenesis [64].

In response, the DNA damage repair (DDR) pathway could initiate cell cycle arrest to either promote repair of DNA lesion to activate tumors programmed cell death and inhibit tumor development [65]. Many DDRpathway genes are activated in cell cycle [66]. Some mutated gene related to the repair of single and double strand breaks can be observed in ACC sample.

1.11 Double strand breaks (DSBs)

Homologous recombination repair (HRR) is the most accurate mechanism among the DSBs during S—G2 phase [67]. It requires chromosome or a sister chromatid as template to precisely repair the damaged DNA [68].

The close genes related to HRR in ACC are ATM, TP53, BRCAl, and ATR [69]. *Atm* (ATM Serine/Threonine Kinase) encodes a protein belonging to the PI3/PI4 kinase family. It plays an oncogenic role in development by regulating the CDK2 (cell cycle checkpoint kinase) and its downstream proteins phosphorylation [70, 71].

Atm is directly involved in p53 phosphorylation. *P53* acts as cancer suppressor gene. The P53 protein is involved in the regulation of cell division, proliferation, and DNA damage repair. After TP53 phosphorylation, the abnormal function of downstream target genes eventually leads to tumor development [72]. *Tp53* (Tumor Protein P53) mutations were significantly correlated with the higher histopathological grade. TP53 positive expression was positively correlated with ACC solid type. Mutated *p53* showed a markable short overall survival as compared to *p53* wild type cases in ACC [73].

Similarly, to ATM, ATR (ATR Serine/Threonine Kinase) enzymes have been shown to be present around phosphorylate the CHK1(checkpoint kinase) and suppressor protein BRCA1 (Breast Cancer Type 1 Susceptibility Protein) [74]. *Brca1* is cancer suppressor gene and plays an important role in regulating the replication of human cells, repair of DNA damage of genetic material, and normal cell growth. The studies of mutations in the *brca1* involved in breast cancer, ovarian cancer, and prostate cancer [75]. The mutated BRCA1 in the DDR repair

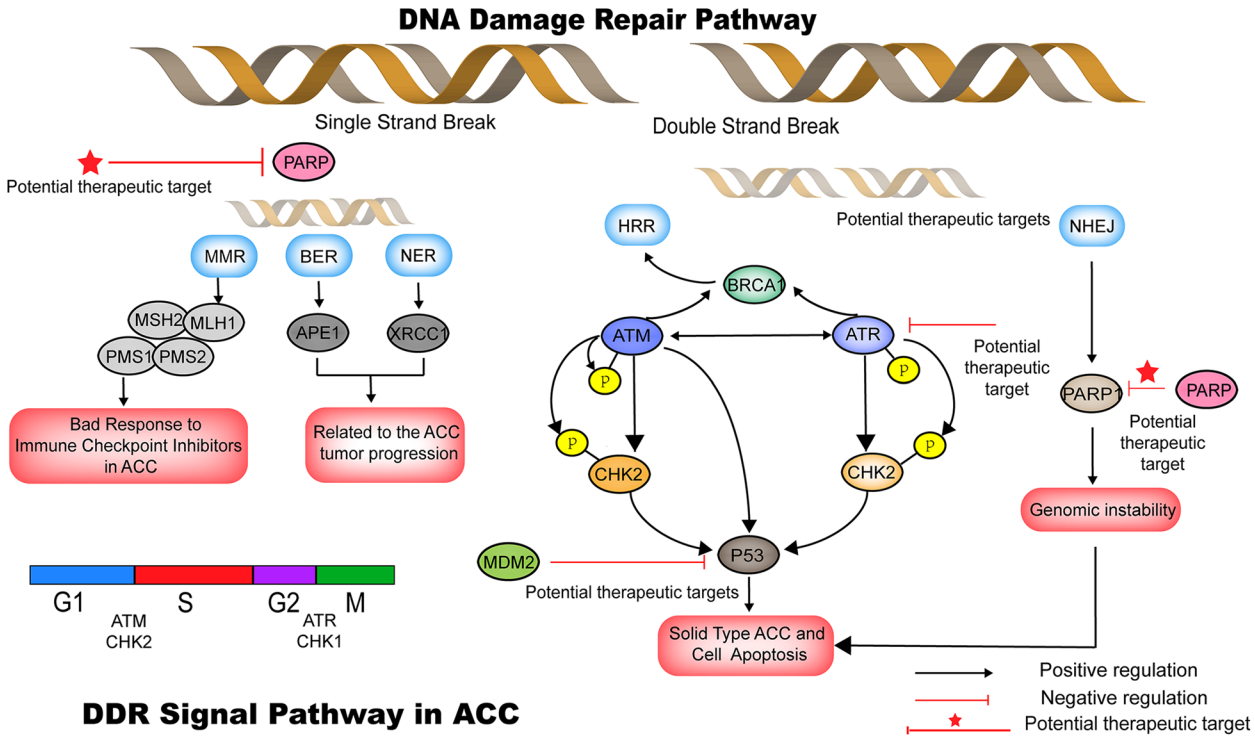

Fig. 4 How the DNA damage repair pathway works in ACC. DNA damage response pathways being observed in the ACC samples. Specific types of DNA damage — mismatches due to replication, single-strand DNA breaks (SSBs) or double-strand DNA breaks (DSBs) — result in the activation of DNA damage response (DDR) pathways. Poly(ADP-ribose) polymerase (PARP) enzymes are key to activating a host of downstream repair mechanisms and are primary proteins involved in SSB repair or base-excision repair (BER). The repair of DSBs occurs predominately through the rapid, error-prone non-homologous end joining (NHEJ) repair pathway in conjunction with the much slower higher-fidelity, error-free homologous recombination (HR) repair pathway. Loss of the BRCA1 can result in HRD. DNA replication is a necessary component of DNA repair and thus cell cycle regulation and replication stress responses are intertwined with DDR pathways. The kinases ATR and ATM have crucial roles in DDR signaling and in maintaining replication fork stability, while also working together via their downstream targets, CHK1 and CHK2, respectively, to regulate cell cycle control checkpoints

pathway was found in ACC samples [3, 5, 20]. When the mutated *brca1* loses its protein function, it leads to homologous recombination deficiency (HRD) function and genomic instability. DNA double-strand cannot be repaired with HRD [76]. PARP inhibitors block single-strand repair, resulting in a "synthetic lethality" effect, leading to the death of tumor cells [77]. Therefore, it could be one of the many potential clinical treatment approaches for the future treatment of ACC.

1.12 Single strand breaks (SSBs)

The BER repair pathway is one of major single strand breaks repair mechanisms that function to maintain genome stability and suppress tumorigenesis. It is associated with autosomal stealth familial adenomatous polyposis, primary immunodeficiency disorders, and neurological disorders [78, 79].

In ACC, the closely related gene in base excision repair (BER) repair pathway are *ape1* (Apurinic/Apyrimidinic Endodeoxyribonuclease 1), *xrcc* (X-Ray Repair Cross-Complementing Protein 1), *parp* (Poly (ADP-ribose) Polymerase) [80]. PARP is involved in the repair of DNA single strand breaks via the base excision pathway. In cell experiments, FKB (Flavokawain B, FKB) had a significant inhibitory effect on ACC cell proliferation, associated with the induction of apoptosis and the cell cycle G2-M block [81]. The PARP leads to a marked apoptotic effect on ACC cells. In addition, high XRCC1 expression is one of the factors that could be used as a predictor of poor prognostic 5-year survival in ACC [80, 81].

In the study of MMR (Macrophage Mannose Receptor 1-Like Protein 1) mutations and survival in multiple solid tumors, 30% (17 samples) were found in salivary gland tumors with MMR gene mutations [82], following: *pms2* (PMS1 Homolog 2, Mismatch Repair System Component), *mlh1* (MutL Homolog 1), *msh6* (MutS Homolog 6) *and msh2* (MutS Homolog 2) [83].

It was found that PD-L1 showed low expression, which did not correlate significantly with the cases of MMR mutations. That may be why treatment with immunotherapy may not be an effective treatment for the ACC patient [84].

1.13 Targeted DNA Damage Repair

Although the CNV of ATM, ATR, BRCA1, and TP53 is low in ACC, ATR is still MYB downstream target gene, which can be one of the future targets. Current stages of BRCA1 and HRR correlation studies indicate that when mutations occur in HRR-related genes, it will lead to HRD. PARP inhibitors can be a potential way to treat ACC in the future.

Murine double minute 2 (MDM2) is a major negative regulator of *p53* that promotes the degradation of *p53* upon direct binding [85]. Malignant tumors that survive may result of *p53* function an abnormality or an abnormality in the binding to MDM2. When the *p53* is mutated to *tp53*, it is released from the degradation mechanism of *p53* and accumulates rapidly in the cell to promote various transcription factors involved in senescence and apoptosis [86]. Therefore, the MDM2-p53 inhibitor may be effective in ACC treatment.

1.14 Gene mutations associated with epigenetics in ACC (Fig. 5)

Epigenetics is the study of heritable phenotypic changes that do not involve alterations in the DNA sequence with the processes. Epigenetic related genes are mainly concerned with DNA methylation [87], histone modifications [88], and chromatin remodeling [89].

1.15 DNA Methylation

DNA methylation is one of the most important epigenetic modifications, which occurs primarily in the CpG islands of the promoter region, resulting in reduced expression and affecting the normal proliferation and differentiation functions of cells leads to triggering tumorigenesis [87]. Studies focusing on aberrant DNA methylation occurring in ACC have shown that the following genes are closely associated: *p16, dapk, reck, pten and rassf1a.*

P16 is a tumor suppressor gene. It acts as a CDK inhibitor, which inhibits the binding of Cyclin-dependent kinase CDK4 to cyclin D function in transition from G1 to the S phase. In study of *p16* promoter region methylation expression [90], it shows that P16 is more common in recurrent high-grade solid types of ACC [91].

Dapk (Death Associated Protein Kinase 1) encodes a Ca + /calmodulin-regulated serine/threonine kinase

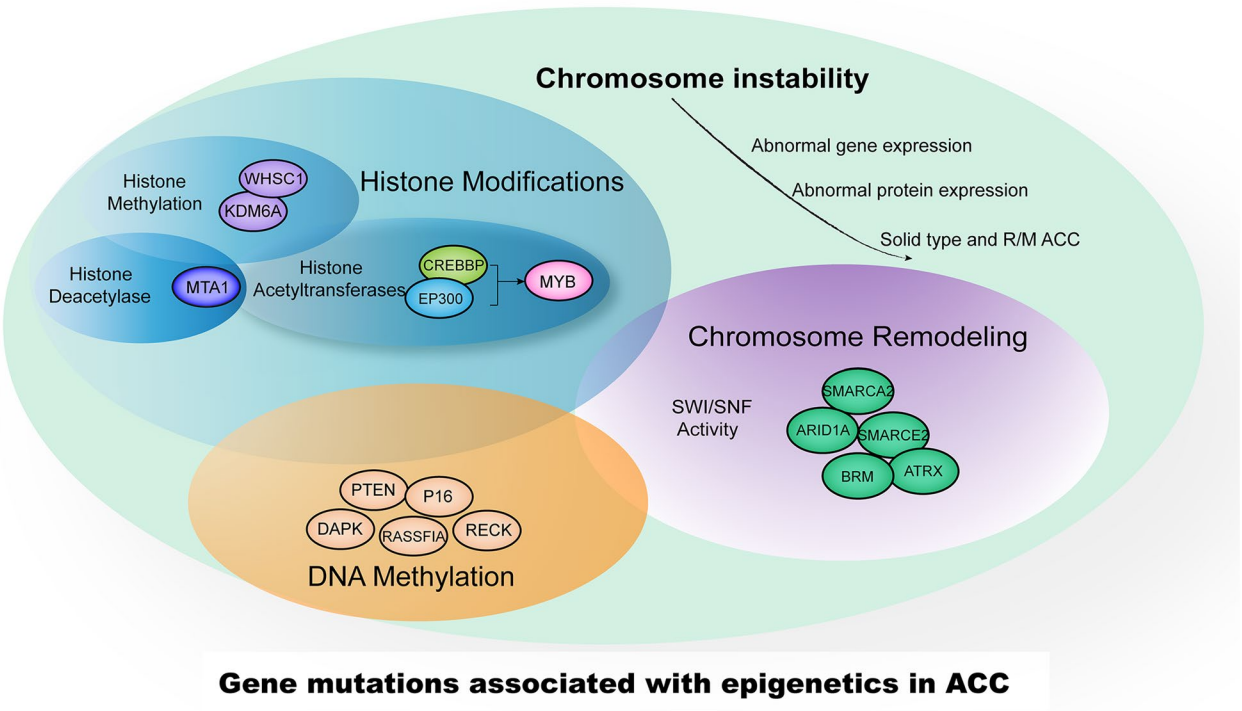

Fig. 5 How the related epigenetics gene mutations in ACC. Histone modifications, DNA methylation, and chromosome remodeling can cause chromosome instability. The related gene can be detected in the ACC samples. CREBBP and EP300 co-regulated the MYB expression. Chromosome instability is associated with the gene and protein expression, which may lead to the solid type ACC and a high rate of recurrence

that mediates apoptosis and inhibits tumor growth [92]. Methylation of DAPK promoter is strongly associated with TNM staging and pathological staging [92, 93]. The expression of DAPK promoter methylation was detected in 27% (16/60) ACC [93]. DAPK promoter methylation in high-grade tumors and in tumors with metastasis suggested a role for its progression function in ACC [93, 94].

Cytological studies in 83 ACC specimens reveals that *reck* (Reversion-Inducing Cysteine-Rich Protein with Kazal Motif) gene was significantly lower than normal tissues adjacent to cancer, with an expression of 25.3% (21/83) [95]. Later, the RECK protein expression was significantly increased by administration of different concentrations of methylation transfer inhibitors in cytological experiments, indicating that the low expression of RECK is associated with the advanced of its promoter in ACC. RECK demethylation may be a novel therapeutic tool to suppress ACC in the future [96, 97].

Pten is shown to be expressed in ACC lines with decreased expression compared to paracarinoma tissue. After the administration of methylation inhibitors, the expression level of PTEN was significantly upregulated, indicating a closely correlation between ACC development and methylation of the PTEN promoter region [98].

RASSF1A (Ras Association Domain Family Member 1) promoter methylation was significantly decreased in ACC samples. Results of qRT-PCR and Western blot in 48 ACC cases showed that 35 cases had higher, and 13 cases had lower RASSF1A expression levels, suggesting a negative correlation between RASSF1A methylation and ACC survival rate [99].

Oncogene methylation plays an important role in ACC and is significantly correlated with pathological staging, grading, and staging of tumors and prognosis. It may become a molecular marker for evaluating the prognosis of ACC patients.

1.16 Histone modifications

1.16.1 Histone methylation and histone demethylation

Histone methylation is an important process that regulates oncogenic gene expression and is controlled by histone methyltransferases and demethylases. WHSC1 (Wolf-Hirschhorn syndrome candiadate1) is a histone methyltransferase that mediates histone H3K36me2 methylation [88, 100].

Upregulation of *whsc1* activates many pathways like the RAC family, TWIST family, and NF-kB (Nuclear Factor Kappa B Subunit) in solid-type tumors [100]. It accelerates with tumor progression and metastasis. In ACC cell line experiments, a breakdown of *whsc1* revealed that reducing the modification of H3K36me2 in the MYC promoter results in more condensed chromatin in the MYC motif and represses the c-MYC transcription. It is hypothesized that WHSC1 may directly regulate c-Myc expression by mediating H3K36me2 modification, which could serve as one of the new targets for the future treatment of ACC [101].

Kdm6a (lysine (K)-specific demethylase 6A), also known as UTX, is located on chromosome Xq11.3. KDM6A is a histone demethylase expressed in high-grade solid-type triple-negative breast cancer and ACC [102]. It is commonly investigated in R/M ACC, suggesting that it plays an important role in the progression of ACC and predicts rapid proliferation and distant metastasis. It can be used as a biomarker to predict the prognosis of ACC patients [103].

1.17 Histone deacetylase and histone acetyltransferases

The functional balance of histone acetylation and deacetylation in cells is regulated by HDACs (histone Acetyltransferases and Deacetylases), which control the chromatin coiled state and alter gene expression appropriately in various malignancies. It can be deregulated in cancer cells, promoting carcinogenesis and tumor progression by altering histone and non-histone proteins affecting gene expression, cell cycle control, differentiation, and apoptosis [104].

CREBBP, a histone acetyltransferase, is located on chromosome 16p13.3 and is capable of transcription across multiple keys signal pathways. CREBBP mutation clusters serve important roles in the critical KAT11 histone acetylation domain. Ep300 binds CREBBP as a transcriptional coactivator, regulates cell proliferation and differentiation, and mutations in the KAT11 histone acetylation structural domain. The literature reports a more aggressive biological form of ACC in the recurrent/solid type enriched for mutations in the activating CREBBP and EP300 [105]. CREBBP and EP300 are known co-activators of MYB and regulate their function by binding to the TAD region of MYB to undergo transcription, ultimately leading to the malignancy of the tumor [106].

Mta1 (Metastasis associated genes 1) is a member of the nucleosome remodeling and histone deacetylation complex [107]. The deacetylates histones cause ATP hydrolysis leading to chromosome remodeling and regulates transcriptional genes. Overexpression of MTA1 occurs with enhanced invasion and metastasis. Comparing the expression of MTA1 in normal salivary gland tissues shows Pleomorphic Adenoma (PA), Mucoepidermoid Carcinoma (MEC), and ACC to be, 0% (0/23), 45% (9/23), 76.5% (13/23), 89.5% (17 /23) respectively. The expression was lowest in the normal group and highest in ACC. MTA1 may play an important role in the process of ACC [108].

1.18 Chromosome remodeling

Alterations characterized by chromatin remodeling are also seen in ACC samples [109], where chromosomal infrastructure is critical for gene control and determines gene expression regulating the future trajectory of cells, and these alterations play a key role in malignancy [110]. Chromosomal remodeling alterations occur mainly in somatic mutations, and genes directly involved in chromosomal mutations include single mutations in SMARCA2 (5%) and SMARCE1 (2%), ARID1A (2%) and ATRX (2%). All these genes belong to the SMARC family of genes [109, 110].

The SMARC family of genes, known as the SWI/SNF-related, matrix associated, actin dependent regulator of the chromatin (SMARC) family, located on chromosome 22q, has been significantly associated with the development of tumors and genetic diseases [110]. The SMARC genes encode proteins that are members of the SWI/SNF family. SWI/SNF mutations and subsequent abnormal function of the SWI/SNF complex are the most common genetic alterations in cancer. SMARCA2 encodes the core catalytic subunit of the SWI/SNF complex, which is involved in the regulation of gene transcription [5]. Chromatin remodeling, chromosome segregation and cell cycle checkpoints are dependent on BRM, which is located at 9p24 [110].

Previous studies have found that ACC may be characterized by mutations in genes encoding regulators of chromatin state [3, 5]. SWI/SNF expression was found to be significantly higher in ACC compared to normal salivary gland tissue by performing BRM immunohistochemical analysis. The elevated levels of BRM protein suggest the existence of a stable mechanism to produce BRM protein in ACC cells, which is involved in tumor growth processes such as proliferation, intracellular translocation, stress responses and drug resistances. SMARCA is also associated with ACC tumor cell proliferation and drug resistances [110], which are clustered within the helicase C family domain, and mutated uncoupling enzymes may increase cancer susceptibility [111] by disrupting core repair mechanisms, in turn may explain the susceptibility to recurrence in the clinical features of ACC.

In addition, *arid1a* (AT-Rich Interaction Domain 1A) in the SMARC family, with the specificity to alter genome-wide nucleosome remodeling and to attract the complex to its target region through protein-DNA or protein–protein interactions [112]. ARID1A possesses two regions important for its function. One binds specifically to the DNA sequence-specific domain known to be recognized by the SNF/SWI complex at the β-bead protein site. The second is a C-terminal region that stimulates glucocorticoid receptor-dependent transcriptional activation. In ACC, it was shown that in 28 samples, about 18% of them were found to have KD6MA mutations and 14% of them had ARID1A mutations by NGS sequencing [113].

It is an evident that ACC involves a variety of different chromatin genes, suggesting that different approaches to chromatin dysregulation may contribute to the development of ACC through different pathways. The future may be promising for cases of ACC with chromatin dysregulation.

1.19 Targeted the epigenome

Promoter methylation levels can be further validated in ACC samples. Aquaporin 1 (AQP1) showed the most significant hypomethylation. In 5-aza-dC/TSA-treated SACC83, AQP1 was upregulated and promoted cell proliferation and colony formation. AQP1 is a promising oncogene candidate for ACC and is transcriptionally regulated by promoter hypomethylation [114].

Mutations in genes involved in histone modification and chromatin remodeling, such as KDM6A, ARID1A and CREBBP have also been identified in 35–50% of all cases of ACC, suggesting that progression of ACC may be associated with epigenetic regulation. In the current clinical trial of Vorinostat et al., a histone deacetylase (HDAC) inhibitor, in 30 patients with incurable ACC, two patients (6.7%) recorded partial remission and 75% had stable disease at 6 months [113].

Several studies have demonstrated an association between PRMT5 and carcinogenesis [115]. Preclinical studies showing a relationship between salivary gland carcinoma and PRMT5 are inadequate, but clinical trials have shown some promise for ACC. It is also possible that PRMT5 inhibitors can have a therapeutic effect on ACC patients. By knocking down PRMT5 enhances drug sensitivity. PR was confirmed in 3 out of 14 patients in the ACC patient group who were treated with GSK3326595 [116], which inhibits PRMT5 (NCT02783300). Further, a PRMT5 inhibitor (PRT543) is also currently being tested in phase I studies in patients with ACC (NCT03886831) [63, 116].

1.20 Perspective

In conclusion, ACC seems not to be as a "quiet" tumor in genetic level. It involves in different mutated gene and pathway. MYB and NOTCH positive mutation samples can be further subdivided into the molecular type of ACC. It can be used as one of the criteria for diagnosis. MYB as a transcription factor may serve as a potential therapeutic direction in the future by altering the structure of small molecule proteins and its DNA vaccines are now under investigation. Several research Notch inhibitors are undergoing clinical trials.

A comparison of published reports shows that Notch inhibitors have higher response rates in Notch mutated samples. For DDR-associated mutations, the use of PARP inhibitors may be effective in ACC, but this has not been reported. Epigenetics-associated mutations, it is used as a diagnostic criterion for poor prognosis in ACC. And its inhibitors are under clinical trials.

In our research, the result of our ACC patients WGS sequence showed that ACC is characteristic of a mutation landscape with wide range and low mutation rate. And no prominent positive mutation has been found yet. With the artificial intelligence (AI) analysis, it is possible that AI deep study is a new tool of diagnostic and therapeutic criteria for ACC by quantifying and scoring low and wide mutation and assessing their malignancy. It could assist us to find the new potential or combined targets can be detected and lead to some progress in treatment.

In perspective of the single cell sequencing analysis, we profiled the transcriptomes of 49,948 cells from paracarcinoma and carcinoma tissues of three patients using single-cell RNA sequencing [117]. Three main types of the epithelial cells were identified into myoepithelial-like cells, intercalated duct-like cells, and duct-like cells by marker genes. And part of intercalated duct-like cells with special copy number variations which altered with MYB family gene and EN1 transcriptomes were identified as premalignant cells. Developmental pseudo-time analysis showed that the premalignant cells eventually transformed into malignant cells. Although there is less report about the single cell transcriptome analysis in ACC, it is a new direction for future research. Moreover, the poor immunotherapeutic effect of ACC may be related to the immune escape of ACC tumors. In the future, we will try to analyze the immune microenvironment from the perspective of single-cell sequencing and then provide new findings and progress in the future diagnose and treatment in ACC.

Supplementary Information

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Additional file 1: Supplementary Table 1. Abbreviation. **Supplementary Table 2.** Gene Associated with ACC.

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Authors' contributions
XQ and ZG designed the review. LQQ, SJL, and WF drafted the manuscript. LQQ, SJL, and WF prepared the figures. LQQ, SJL, ZHZ, ZG, and XQ participated in its coordination and modification. All authors read and approved the final version of the manuscript.

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Declarations

Ethics approval and consent to participate
The present study was approved by the Ethics Committee of Chinese PLA General Hospital (Approval No. of Ethics Committee is S2018-281–02) and was performed according to the guidelines of the Declaration of Helsinki.

Consent for publication
All authors read and approved the final manuscript.

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