Protein-loss of SWI/SNF-complex core subunits influences prognosis dependent on histological subtypes of intra- and extrahepatic cholangiocarcinoma

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Abstract. Cholangiocarcinoma (CCA) is an aggressive malignancy with a 5-year-survival rate of <10%, mainly due to diagnosis in advanced stages and limited therapeutic options in case of progressive disease. Recently, evidence has indicated that alterations in the SWI/SNF-complex (SWI/SNF) may have an important role in the tumorigenesis of CCA. SWI/SNF-related chromatin remodeling has been reported to be crucial for differentiation and tumor suppression, and loss-of-function mutations of SWI/SNF are present in 20% of human malignancies; however, at present, little is known about its relevance in CCA. In the present study, a cohort of 52 patients with the diagnosis of primary CCA was retrospectively collected. All patients underwent surgery with curative intent. Tissue microarray analysis was performed on each tumor for immunohistochemical loss-of-protein analysis of the SWI/SNF core subunits ARID1A, INI-1, BRG1, PBRM-1 and BRM, corresponding to the following CCA subtypes: Extrahepatic CCA (ECCA), small duct or large duct intrahepatic CCA (ICCA). Kaplan-Meier analysis was used to determine survival distribution and survival differences were evaluated by log-rank test. In total, 14 of 52 patients (~35%) exhibited protein-loss of any tested SWI/SNF core subunit. Notably, 17% of patients exhibited a loss of ARID1a; this was the protein loss with the highest frequency. Patients with small and large duct ICCA with protein-loss of any tested SWI/SNF subunit exhibited significantly worse survival compared with the wild-type cohort with proficient protein expression (P=0.013 and P=0.002), whereas no significant survival difference was detected for patients with ECCA. SWI/SNF and its core subunits may be considered promising predictive and therapeutic targets, and require further investigation in patients with CCA.

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Abbreviations: CCA, cholangiocarcinoma; CTX, chemotherapy; ECCA, extrahepatic cholangiocarcinoma; ICCA, intrahepatic cholangiocarcinoma; IHC, immunohistochemistry/immunohistochemical; n, number of patients; RTX, radiotherapy; SWI/SNF, SWI/SNF-complex

Key words: SWI/SNF-complex, small duct type intrahepatic cholangiocarcinoma, large duct type intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, ARID1a, PBRM-1, BRM

Introduction

Cholangiocarcinoma (CCA) are epithelial cell malignancies arising from various locations within the biliary tree, showing markers of cholangiocyte differentiation. Most CCA are adenocarcinomas, which are anatomically classified as either of intrahepatic (ICCA) or extrahepatic (ECCA) origin (1). In general this heterogeneous group of CCA are aggressive malignancies with poor survival rates (5-year-survival of <10%) (2). Resectability is a critical factor associated with a better outcome. However, the majority of cases is diagnosed at the time of progressive disease, without the opportunity of surgical resection in curative intent (3,4). These data emphasize the need of new therapeutic options besides surgery, chemo- (CTX) and radiotherapy (RTX). So far, there are only very limited options of available targeted therapies, which are based on specific biomarkers of individual tumors like e.g. FGFR2-inhibitors (5). For this reason new biomarkers are urgently required, that might contribute to a better understanding of tumor biology in specific subgroups of the different CCA types. ICCAs are subdivided into large duct type and small duct type, according to their occurrence and cell of origin (6-8). Large duct ICCAs arise in the large intrahepatic bile ducts near the hepatic hilus and resemble ECCAs, whereas small duct ICCAs typically develop in the hepatic periphery (7,9). The ICCA subtypes, which were included into the WHO classification of 2019, go along with different

clinical, histomorphological and molecular features, diverse risk factors and prognosis (10,11).

Recently increasing interest has been paid to nucleosome remodeling complexes in many different cancers, e.g. esophageal-, pancreatic-, ovarian-, renal cell and hepatocellular carcinoma and they appear to be promising new opportunities for novel prognostic markers and therapeutic targets (12-14). The mammalian SWI/SNF-complex (SWI/SNF) functions as tumor suppressor in many human malignancies and plays an ATP-dependent chromatin-remodeling role, contributing to transcriptional regulation by altering chromatin structure and controlling the accessibility of DNA (15,16).

SWI/SNF are heterogeneous complexes of 12-15 protein subunits with diverse and variable functions required in different cellular and developmental contexts. Mammalian SWI/SNF complexes always contain two mutually exclusive ATPases: BRM (SMARCA2) or BRG1 (SMARCA4). Three additional subunits [INI-1 (SMARCB1), SMARCC1 and SMARCC2] form the core complex (17-19). The mutation frequency of the complexes is ~20% in cancer and AT-rich interactive domain-containing protein 1A (ARID1A) is the most frequently mutated gene subunit (12). Moreover, mutations of SWI/SNF might provide a potential target for therapy (13).

To date, inactivating mutations of ARID1a and PBRM-1 have been detected in 17-19% of ICCA by exome sequencing and have been correlated with worse survival (20). ARID1a and PBRM-1 gene mutations have even been described as predictors for poor prognosis in ICCA, however histomorphological subtypes were not considered (21). Simbolo *et al* demonstrated that specific molecular SWI/SNF alterations, including ARID1a, PBRM-1 and INI-1, were associated with different CCA subtypes and that potentially actionable pathways for small molecule inhibitors are evident in 68% of cases (16).

Despite advances in the analysis of SWI/SNF, so far little is known about its relevance in CCA. In our study we concentrated on the analysis of the ATPase-dependent core subunits of SWI/SNF (ARID1a, INI-1, BRG1, PBRM-1 and BRM) which, if mutated, all separately result in loss-of-function of SWI/SNF (22). These mutations can be detected by immunohistochemistry (IHC), demonstrating the loss of the nuclear protein.

Cases of large- and small duct ICCA as well as cases of ECCA were included in order to analyze the frequency of mutations of SWI/SNF in correlation to the subtype of CCA and survival. Simultaneously we assessed *FGFR2*-translocations, *HER2*-amplification status and P53 mutations to exclude other underlying alterations, which may bias our survival data.

Due to the abundance of actionable mutations and the absence of effective systemic therapy options against CCA, the aim of our study was, to reveal the influence of SWI/SNF core subunits protein-loss on overall survival of CCA patients corresponding to its subtype and further to identify possible new promising therapeutic targets.

Materials and methods

Case selection. Between 2000 and 2019, a cohort of 52 patients with the diagnosis of primary CCA was collected. All these patients underwent surgical treatment with curative intent within the surgical department of the University Hospital of Cologne (Cologne, Germany). All patients gave their written informed consent for the procedure. The current retrospective study was conducted with the approval of the Ethics Committee of the University of Cologne, utilizing clinical follow-up data that were collected retrospectively according to a standardized follow-up within the oncological outpatient clinic (application 18-269). The following exclusion criteria were defined: i) Administration of systematic therapy prior to surgery to avoid any bias affecting survival analysis. ii) Survival <14 day after surgery, to exclude short-term deaths due to surgical complications. iii) Age <42 or >89 years. Detailed patient cohort information is displayed in (Table I).

Classification and pathological features. CCAs were divided in ECCA (n=17) and ICCA (n=35) corresponding to their location in the biliary tree. Moreover, ICCA were subdivided into small duct (n=27) and large duct type (n=8) corresponding to the WHO classification of 2019 (10). This subdivision was based on standard histomorphological and cellular criteria (HE and PAS staining), as well as immunohistochemical staining (see below).

Immunohistochemical study (IHC). Tissue microarray analysis (TMA) construction was performed as previously described (23,24). In brief, four tissue cylinders from each tumor center with a diameter of 1.2 mm were punched out from the tumor center of selected tumor tissue blocks using a semi-automated precision instrument and embedded in empty recipient paraffin blocks. Four-micrometer sections of the resulting TMA blocks were transferred to an adhesive-coated slide system (Instrumedics Inc.) for further staining. IHC was performed on TMA slides using primary antibodies specific for ARID1A (Abcam, clone EPR13501, 1:1,000 citrate buffer), BRG1 (Abcam, clone EPNCIR111A, 1:300 citrate buffer), BRM (Cell Signaling, Inc., clone D9E8B, 1:50 citrate buffer), INI1 (Dako, clone DO-7, 1:1200, citrate buffer), PBRM-1 (Abcam, BAF180, EPR15860, 1:1000, EDTA buffer) and P53 (Dako, clone DO-7, 1:800, citrate buffer) with a Bond Max automated system (Leica). All these markers showed a nuclear staining pattern. For subdivision of ICCA into small duct- and large duct type the primary antibodies S100P (Cell Marque, 16/f5, 1:1600, enzyme buffer), N-cadherin (Novocastra, IAR06, 1:100, EDTA buffer), CD56 (Thermo Fisher Scientific, Inc., 123C3, 1:500, EDTA buffer) and MUC5ac (Abcam, MUC5AC/917, 1:500, EDTA buffer) were used in the same manner. S100P and MUC5ac showed a cytoplasmatic -, N-cadherin and CD56 a membranous staining pattern. The expression frequency of these markers corresponding to the CC subtypes is depicted in Fig. 1. Lymphoid tissue served as an internal control. Two pathologists (either U.D. or A.Q. and B.J.W.) manually performed IHC analysis. ARID1A-, BRG1-, BRM-, PBRM-1and INI-1 staining was assessed according to a three-tier scoring system (score 0, 1 and 2). A score of 0 associated with the loss of protein and defined as unequivocal clean absent staining in the nuclei of viable tumor cells for the SWI/SNF-complex subunits was interpreted as an underlying mutation, deletion or promotor alteration. Score 1 was determined as nuclear

Table I. Specifications of the patient cohort.

Characteristics	Total	ECCA	ICCA	ICCA-large duct	ICCA-small duct
Total patients	52	17	35	8	27
Sex					
Male	31	10	21	4	17
Female	21	6	15	3	12
Portal vein embolization	8	3	5	1	4
Postoperative chemotherapy	22	2	20	5	15
Postoperative radiotherapy	2	1	1	1	0
Alive at time of investigation	15	3	12	2	10
pT-statius					
pT1	12	0	12	2	10
pT2	25	12	13	1	12
pT3	9	2	7	1	6
pT4	6	2	4	3	1
pN1	23	10	13	2	11
M1	3	1	2	0	2
L1	44	14	30	5	25
V1	22	4	18	3	15
Pn1	34	15	19	3	16
R-status					
R0	38	10	28	3	25
R1	13	6	7	4	3
R2	1	0	1	0	1
Grading					
G1	1	1	0	0	0
G2	30	11	19	6	13
G3	20	4	16	1	15
G4	1	0	1	0	1
Operation					
Hemihepatectomy right	8	0	8	1	7
Extended hemihepatectomy right	8	1	7	2	5
Hemihepatectomy left	9	1	8	1	7
Extended hemihepatectomy left	4	1	3	0	3
Liver segment resection	5	0	5	1	4
Atypical liver resection	2	0	2	1	1
Pancreas operation (Whipple or Traverso-modification)	2	2	0	0	0
Trisegmentectomy	4	3	1	0	1
Extrahepatic bile duct resection	5	5	0	0	0
Hemihepatectomy right + extrahepatic bile duct resection	4	2	2	1	1
Hemihepatectomy left + pancreas operation	1	1	0	0	0

ECCA, extrahepatic cholangiocarcinoma; ICCA, intrahepatic cholangiocarcinoma.

staining of tumor cells and interpreted as an intact, unmuted ARID1-, BRG1-, BRM-, PBRM-1 or INI1 gene with regular protein expression. Score 2 was used in case of heterogeneous expression, defined as loss and intact nuclear staining within different tumor areas of the same tumor sample and interpreted as partial underlying mutation, deletion or promotor alteration. Strong nuclear stainability of the surrounding non-tumor cells served as an internal control. Discrepant results were resolved by consensus between the reviewers. In case of PBRM-1 to proof absent nuclear staining certain tumor samples were reanalyzed on large tumor slides. S100P, N-cadherin, CD56 and MUC5ac staining was classed as positive if ≥10% of

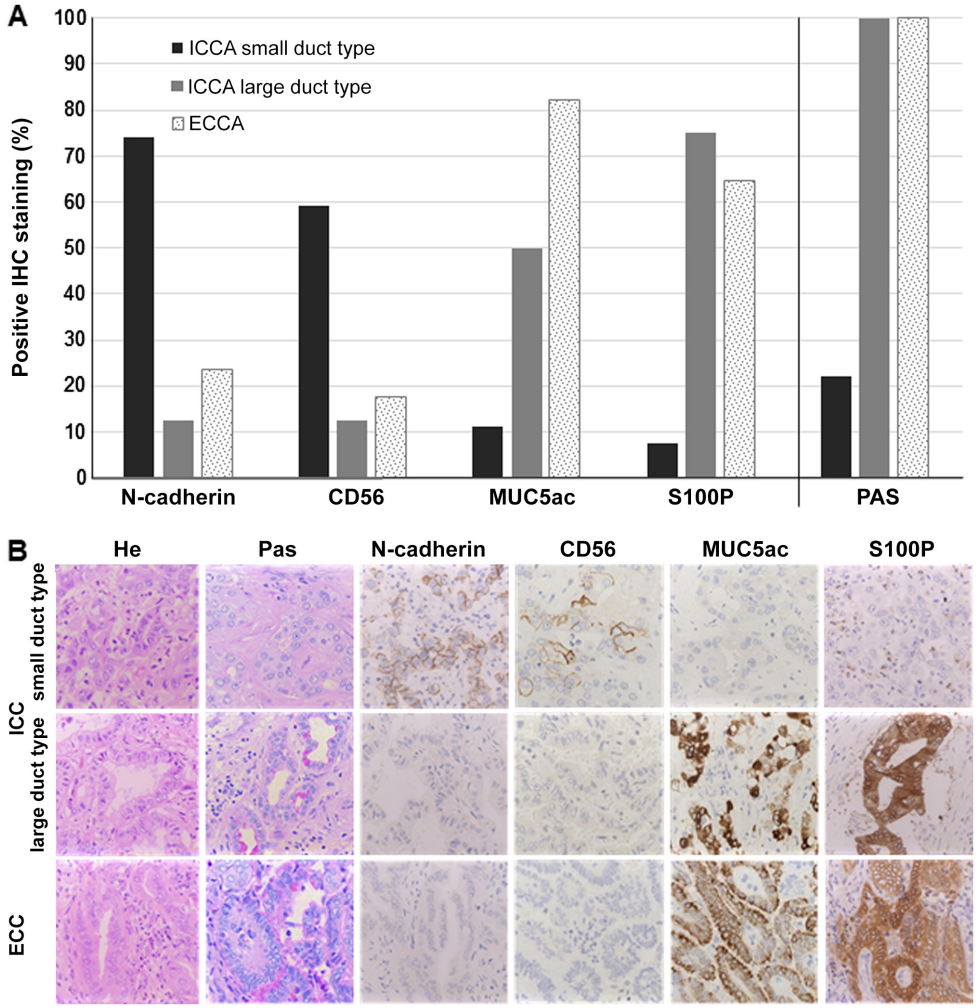

Figure 1. Subtyping of CCA. (A) IHC staining results. Positive IHC staining results for either N-cadherin, CD56, S100P or MUC5ac are depicted in percent (y-axes) compared to the CCA subtype (x-axes). The CCA sample was assessed as positive if staining was present in ≥10%. Small duct ICCA (n=27), large duct ICCA (n=8), ECCA (n=17). (B) Representative images of IHC staining, corresponding to the CCA subtype. CCA, cholangiocarcinoma; IHC, immunohistochemistry; CD56, cluster of differentiation 56; S100P, S100 calcium-binding protein P; MUC5ac, mucin 5AC; ICCA, intrahepatic cholangiocarcinoma; ECCA, extrahepatic cholangiocarcinoma.

tumor cells were positive. P53 was considered as altered in case of nuclear loss or homogeneous nuclear overexpression.

FISH-analysis. Fluorescence *in situ* hybridization (FISH) for *FGFR2*-translocation and *HER2*-amplification was performed on formalin-fixed, paraffin-embedded tissue specimens (TMA). Sections of 1.5 μm tumor material were cut and hybridized overnight. *FGFR2*-translocation was assessed by using a ZytoLight SPEC, *FGFR2* Dual Color Break Apart probe (Z-2169-200), *HER2*-amplification by using a ZytoLight SPEC ERBB2/CEN 17 Dual Color probe (Z-2015-200). Review of fluorescence signals was performed at x630 magnification with a Leica CTR 5500 fluorescence microscope. *FGFR2* was scored as positive, if separate Spectrum red and/or Spectrum green signals were present in >20% of nuclei throughout the tumor. *HER2* analysis was performed according to defined guidelines (25).

Data analysis and statistics. For statistical analysis and graphic presentation of the results, IBM SPSS v26.0 was used. Descriptive analysis included the frequency of nominal parameters, the median with lower (LQ) and upper (UQ) quartiles for numeric variables (ordinal or asymmetric distribution) and the mean for numeric variables with a normal distribution. Prognosis was calculated including all types of mortality beginning 14 days after the date of surgery. In this way, mortality associated to the surgical procedure itself was excluded. Kaplan-Meier univariate analysis was used to describe survival distribution, and log-rank tests were performed to evaluate survival differences. Significant differences between patient cohorts were defined as $P<0.05$ for all analyses.

Results

IHC analysis of SWI/SNF complex subunits. ARID1a-, BRG1-, BRM-, PBRM-1and INI-1 IHC staining shows a clear nuclear staining pattern, if proficiently expressed (Fig. 2). In case of protein-loss the nuclear staining turned negative. For ARID1a and PBRM-1 only complete protein-loss was detected whereas BRG1-, BRM and INI1 also showed a heterogeneous staining pattern. Assuming that a heterogeneous protein expression at

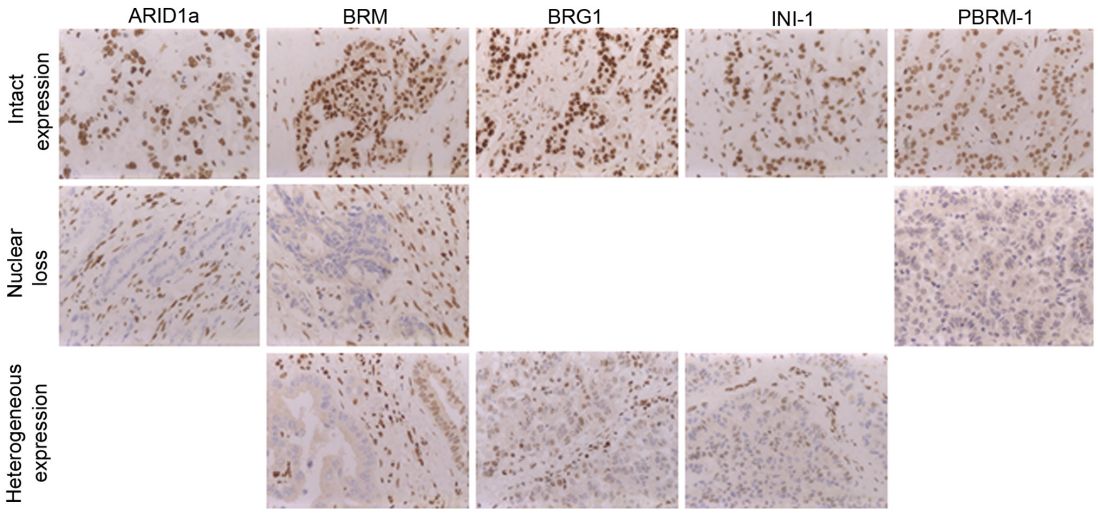

Figure 2. IHC analysis of SWI/SNF-complex subunits. All tested SWI/SNF subunits (ARID1A, BRG1-, BRM-, PBRM-1 and INI1) show a nuclear positive staining pattern, in case of IHC protein-loss nuclear staining was absent. A mosaic-like nuclear staining pattern was detected for BRG1, BRM and INI1 and was defined as heterogeneous expression (magnification, x40). IHC, immunohistochemistry; SWI/SNF, SWI/SNF-complex; ARID1A, AT-rich interactive domain 1A; BRG1, Brahma-related gene 1; BRM, Brahma, PBRM-1, polybromo-1; INI1, integrase interactor1.

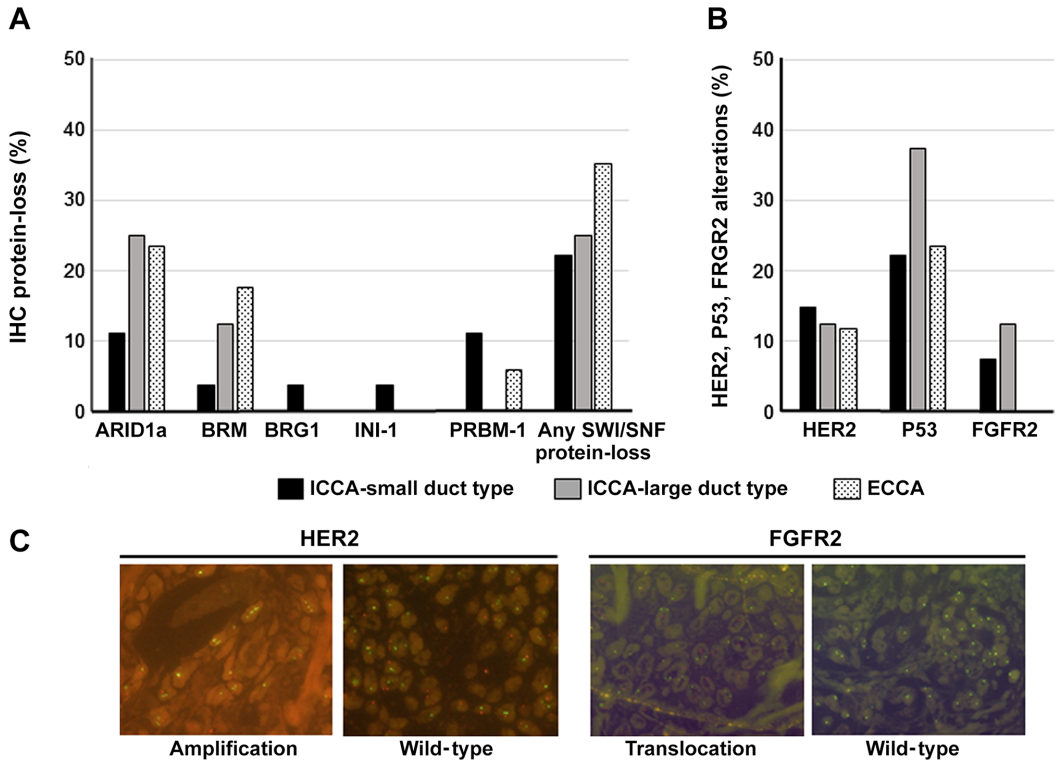

Figure 3. Frequency of SWI/SNF-complex-, *HER2*-, P53- and FGFR-2-alterations. (A) IHC proven protein-loss (heterogeneous expression included) of SWI/SNF subunits are depicted in percent (y-axes) compared to the CCA subtype (x-axes); (B) in the same manner *HER2*-amplification, *FGFR2* translocation and P53 alterations are illustrated. Small duct ICCA n=27, large duct ICCA n=8, ECCA n=17. (C) Fluorescence microscopy photographs of *HER2*-amplificated (left) and *FGFR2*-translocated (right) CCAs compared to wild-type each (magnification, x63). *HER2*, human epidermal growth factor receptor 2; P53, protein 53; FGFR-2, fibroblast growth factor receptor 2; SWI/SNF, SWI/SNF-complex; CCA, cholangiocarcinoma; ICCA, intrahepatic cholangiocarcinoma; n, number of patients; ECCA, extrahepatic cholangiocarcinoma.

least results in a reduced function of SWI/SNF, we summarized both immunophenotypes as protein-loss for further analysis. This approach was confirmed by the finding that heterogeneous protein expression and protein-loss, separately analyzed, both resulted in reduced overall survival (data not shown).

In total, 14 of 52 patients (~35%) showed a protein-loss of any tested SWI/SNF core subunit, 2 of them with heterogeneous expression, and 4 of them with protein-loss of more than one SWI/SNF subunits. The highest frequency showed ARID1a with 17%. The CCA subtypes showed different frequencies of the lost SWI/SNF subunits (Fig. 3). In small duct ICCA, all

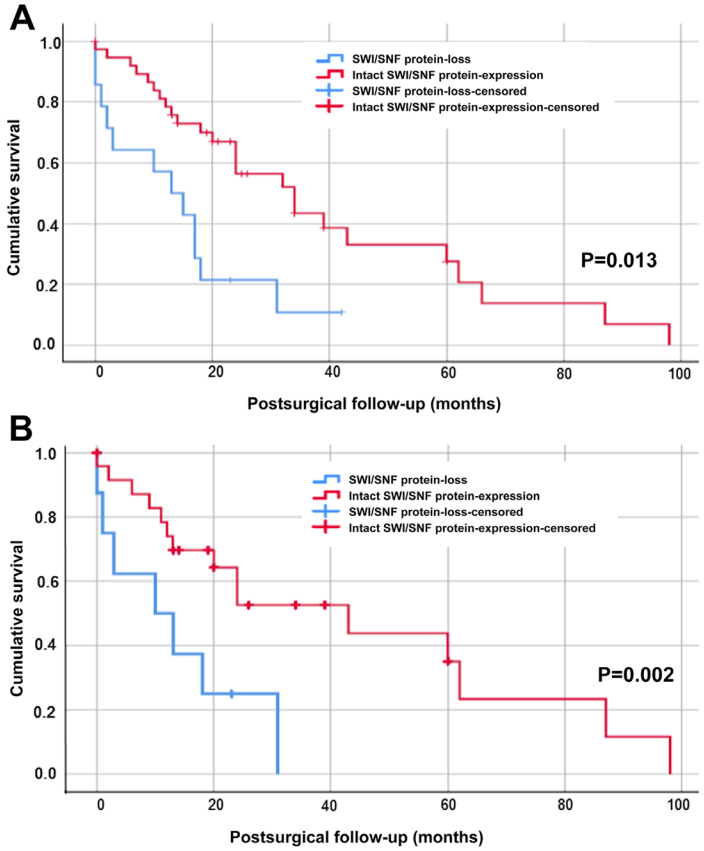

Figure 4. Kaplan-Meier survival analysis of the SWI/SNF subunit protein-loss cohort versus patients with proficient expression. (A) Overall survival of the SWI/SNF altered patients (n=14, blue line) compared to the cohort with intact expression pattern (n=38, red line). (B) Overall survival of SWI/SNF altered patients (n=7, blue line) compared to the cohort intact expression pattern (n=25, red line) after exclusion of *HER2*-, *FGFR2*- or P53 alterations. With or without exclusion of *HER2*-, *FGFR2*- or P53 alterations, the SWI/SNF-subunit protein loss cohort showed a highly significant worse survival than the SWI/SNF wild-type subgroup [log-rank test: (A) P=0.013 and for (B) P=0.002]. In each section the x-axes shows cumulative survival, postsurgical follow-up in months is depicted on the y-axes. SWI/SNF, SWI/SNF-complex; n, number of patients; *HER2*, human epidermal growth factor receptor 2; FGFR-2, fibroblast growth factor receptor 2; P53, protein 53.

tested subunits showed protein loss. The most frequent alterations were detected for ARID1a and PBRM-1 with 11% each. In opposite, in large duct type ICCA only protein-loss of ARID1a (25%) and BRM (12.5%) were revealed. With 6 of 17 ECCA patients this CCA subtype showed the highest frequency (35%) of any lost SWI/SNF subunit. ARID1a, BRM and PBRM-1 alterations were found in decreasing frequency. Besides these findings, alterations of *HER2*, P53 and *FGFR2* were observed as follows: *HER2* amplification in 13.46%, P53 alterations in 25% and *FGFR2* translocation in 5.77% (Fig. 3). Whereas HER2- and P53 alterations were found in each CCA subtype, *FGFR2* translocations were only observed in ICCA. Further, 7 of the 14 patients showed protein-loss of SWI/SNF subunits parallel to *HER2*, *FGFR2*- or P53 alterations.

Overall survival analysis. Kaplan-Meier analysis (log-rank test) was used for overall survival. We combined IHC proven protein-loss of ARID1A, BRG1, BRM, PBRM-1 or INI-1 as SWI/SNF altered cohort (n=14) and compared it to patients with intact expression pattern (n=38) (Fig. 4A). The median survival of the SWI/SNF subunit protein-loss cohort was 13±4.677 months versus 34±7.200 months in the wild-type CCA patients, which was statistically significant (P=0.013). Further, we analyzed the effect of molecular alterations (*HER2*-amplification, *FGFR2*-translocation or P53 alteration) on the overall survival and excluded CCA patients with these alterations from the cohorts (Fig. 4B). Again, the difference in overall survival (P=0.002) was statistically significant: The median survival of SWI/SNF subunit protein loss cohort (n=25) was 10±7.071 months compared to 43±13.835 months for the cohort with intact expression pattern (n=7).

As we did not see an effect of HER2, P53 and FGFR2 alterations on overall survival in ratio SWI/SNF altered patients and patients with intact expression pattern, we analyzed the overall survival corresponding to the histological CCA subtypes in the total patient cohort (Fig. 5). Only for small duct (Fig. 5A) and large duct ICCA (Fig. 5B) a significant worse survival for the SWI/SNF subunit protein-loss cohort was detected (small duct ICCA P=0.031, large duct ICCA P=0.010), but not for ECCA (Fig. 5C). For small duct ICCA the median overall survival of the cohort with intact expression pattern was 43.911±7.356 months, whereas SWI/SNF altered patients only survived 13±7.088 months in average. Similar results were detected for large duct ICC: overall survival of the cohort with intact expression pattern was 46.5±6.759 months compared with 16±8.667 months in SWI/SNF altered patients. General overall survival of small duct ICCA was the longest (39.709±6.849 months), followed by large duct ICCA (33.571±7.480 months). The shortest overall survival was detected for ECCA patients (21.486±6.381 months). Each

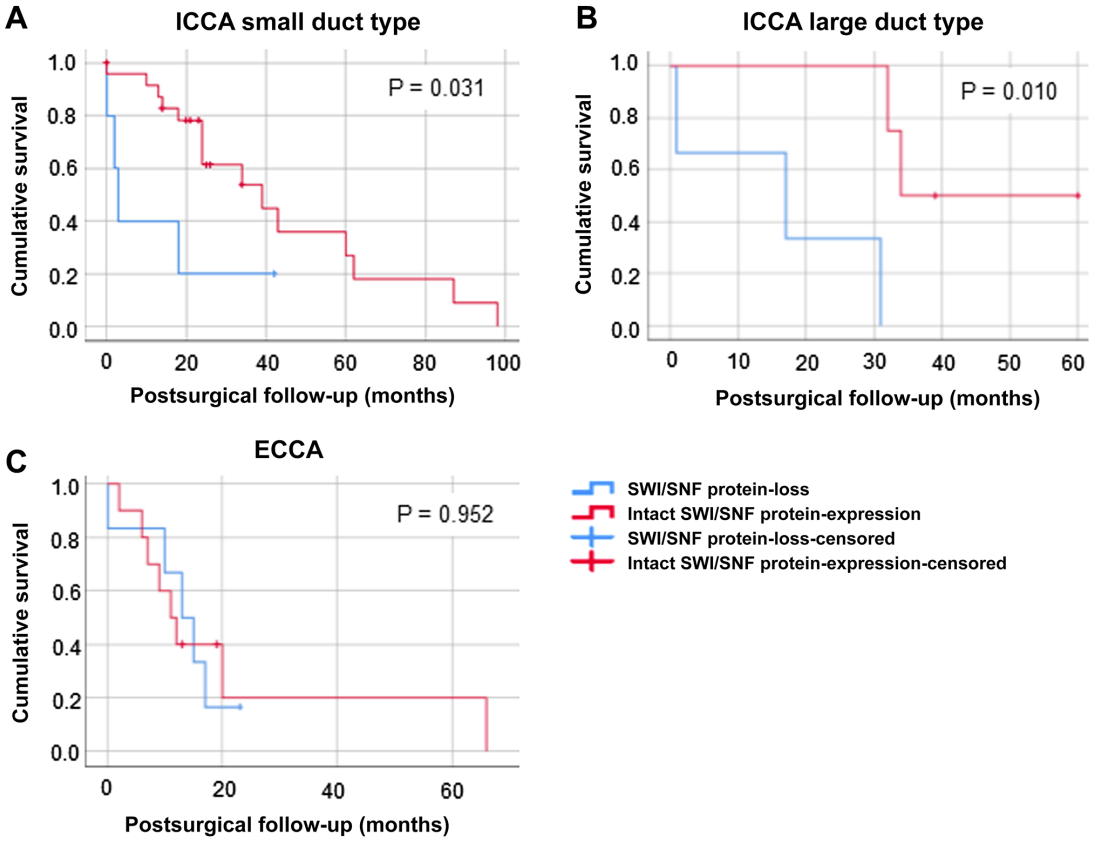

Figure 5. Kaplan-Meier survival analysis (log-rank test) for the patient cohort with SWI/SNF-complex- subunit protein loss versus proficient expression corresponding to CCA subtypes. (A) Small duct ICCA: Overall survival of the SWI/SNF protein-loss cohort (n=6, blue line) compared to patients with intact expression pattern (n=21, red line). (B) Large duct ICCA: Overall survival of the SWI/SNF protein-loss cohort (n=2, blue line) compared to patients with intact expression pattern (n=6, red line). (C) ECCA: Overall survival the SWI/SNF protein-loss cohort (n=6, blue line) compared to patients' intact expression pattern (n=11, red line). A significant worse overall survival of the SWI/SNF protein-loss cohort was detected for small duct ICCA (P=0.031) and large duct ICCA (P=0.010), but not in ECCA. In each section the x-axes shows cumulative survival, postsurgical follow-up in months is depicted on the y-axes. CCA, cholangiocarcinoma; ICCA, intrahepatic cholangiocarcinoma; SWI/SNF, SWI/SNF-complex; n, number of patients; ECCA, extrahepatic cholangiocarcinoma.

SWI/SNF subtype protein-loss in relation to overall survival and CCA subtype was analyzed separately (data not shown). For this analysis other underlying SWI/SNF alterations were excluded. ARID1a protein loss correlated with lower overall survival significantly (P=0.025). Median overall survival of the CCA patient cohort with intact expression pattern was 39.4±5.626 months versus 16.125±4.725 months of ARID1a altered patients. Corresponding to the histological subtypes, median survival of ARID1a altered patients was also shorter, whereas only significant for large duct ICCA (P=0.010). For PBRM-1 survival of the cohort with intact expression pattern was longer compared to the altered PRBM-1 patients. However, this finding was non-significant. BRM protein-loss also decreased overall survival in all CCA subtypes, whereas significant results were only detected for the large duct ICCA (P=0.046).

Discussion

In our study we established a cohort of 52 cases of resected CCAs. Consistent with prognostic data given in the literature (26), our patients had a poor median overall survival of 35 months, despite all of them underwent surgery in curative intent. Overall incidence of CCA has increased progressively worldwide over the past four decades (27,28). CCA are heterogeneous tumors with different risk factors and precursor lesions, clinical features and prognosis. Previous studies have documented that their origins form different parts of the biliary tree result in different histopathological and molecular pathological features (6). Within the last few years, new molecular alterations have been discovered in CCA, which are improving the pathological characterization and which might be transformed into personalized targeted therapy algorithms, which are imperatively needed.

We divided our cohort of tumors into the subtypes ECCA, large duct and small duct type ICCA according to a comprehensive analysis of macroscopic and histopathological aspects together with an immunohistochemical and molecular pathological characterization (ECCA, 17; small duct ICCA, 27; large duct ICCA, 8) (10).

In line with literature, our analysis of molecular pathological changes revealed in all CCA subtypes mutations of the tumor suppressor gene P53, with a maximum of 38% in ICCA, large duct type. FISH-analysis for *HER2* revealed amplification in all three tumor types with a maximum of 15% in small duct type ICCA. *HER2* amplification is a relevant marker, serving as putative therapeutical target with the availability of drugs.

According to other studies, *FGFR2* fusions are typically found in ICCA and are present in ~10-16% of patients (29,30).

The efficiency of *FGFR2* inhibitors is tested to date in several clinical trials in patients with advanced ICCA. Targeted therapies e.g. the pan-selective FGFR kinase inhibitor BGJ398 lately showed promising antitumor activity in a multicenter, open label phase II trial in CCA patients with *FGFR2*-fusions (26). In our cohort *FGFR2*-fusions were detected in ICCA with a frequency of 8% in small duct type and 15% in large duct type. As described earlier, *FGFR2* translocation were absent in ECCA (29,30).

Recent studies demonstrated somatic mutations of chromatin remodelers in a number of human cancers. Notably, Jiao *et al* documented mutations in at least one chromatin-remodeling gene in 47% of CCA (20). Mutations in the genes coding for ARID1a-, BRG1-, BRM-, PBRM-1and INI-1 are inactivating and result in a loss of protein expression. Previous studies demonstrated that loss of protein expression correlates strongly with a mutational status in these genes (31).

In our analysis, 35% of the cases showed protein loss in at least one of the complex-proteins, suggesting inactivating mutations in the respective gene. ARID1a was the most frequent lost SWI/SNF protein, followed by BRM. ARID1a- and BRM-negative cases were found in a small percentage of all tumor subtypes. In literature, alterations in the ARID1a gene were detected in 7.2-36% of ICCAs and 5%-12.3% of ECCAs (32). In this aspect, our results are in line with previous studies. The absence of ARID1a in the different subtypes of biliary carcinoma appears to reflect similar mechanisms of carcinogenesis in the different subtypes of CCA.

In our study, BRG1 and INI-1 protein loss was only seen in the group of small duct ICCA, while PRMB-1 protein loss was found in small duct type ICCA and ECCA. Luchini *et al* analyzed PBRM-1 loss in large duct and small duct ICCA and found a prevalence of 20-30% in both subtypes (33). In our study we did not find cases with PRBM-1 loss in the group of large duct type ICCA, which might be explained by the low number of cases of this subtype (n=8). Within the whole group 7.6% of cases showed protein loss of PBRM-1.

There is still controversy about the loss of proteins of the SWI/SNF complex subunits and its correlation to prognosis of CCA. In the study of Jiao *et al*, there was no correlation between overall survival and ARID1a alteration (20). This was in line with other studies (32,34). However, they subdivided ECCA and ICCA and did not look at small duct and large duct type ICCA separately, which might be an explanation for the varying results, compared to our data. We identified that median survival of ARID1a altered patients was reduced, but it was only significant for large duct ICCA. Sarcognato *et al* saw in their cohort of ICCA a correlation with retained protein expression of PBRM-1 and longer overall survival and disease-free survival (35). Other results presented by Misumi *et al*, demonstrated PBRM1 protein loss in both, small-duct type and large-duct type ICCA. However, it was not associated significantly with any specific characteristics, including prognosis (36).

Our current data give strong evidence, that IHC proven protein-loss of SWI/SNF core subunits (namely ARID1A-, BRG1-, BRM-, PBRM-1and INI1) is associated with a highly significant worse survival of small duct and large duct ICCA, whereas no significant change in survival for ECCA was detected. Furthermore, significant worse overall survival could also be shown for the SWI/SNF-altered cohort after exclusion of *HER2*-amplificated, *FGFR2*-translocated or P53 altered patients, which was partly observed in coincidence. These data underline the importance of this chromatin remodeler complex in tumorigenesis of CCA. Our results strengthen preceding data, that the SWI/SNF complex and its core subunits is worth further investigation for targeted therapy options as well as predictive marker for ICCA in future (16,20,21).

Furthermore, several studies suggest a possible increased therapeutic vulnerability of ARID1a-deficient carcinoma. Shen *et al* were able to show in cell culture analyses and in mouse models that therapeutic inhibition of the enzyme poly-ADP-ribose polymerase (PARP) is effective in ARID1a-deficient ovarian and colon carcinoma (37). ARID1a-deficient carcinoma cells also show activation of the PIk3 pathway. Therapeutic inhibition of this pathway could also be promising in this tumor subgroup. Lee *et al* successfully inhibited ARID1a-deficient gastric cancer cells with AKT inhibitors (38). Several studies indicate an increased expression of the programmed cell death-ligand 1 (PD-L1) in ARID1a deficient tumor cells. First results show a significantly better response to PD1-/PD-L1 blockade in this setting than in ARID1a intact tumors (39). The vast majority of studies focus on the loss of function of ARID1a. There are no reliable findings as to whether the loss of function of the other SWI/SNF subunits investigated offers comparable therapeutic intervention options. This should be the subject of future clinical studies.

However, our study has some limitations. As a monocentric study, the case number is limited. The statistical analysis gives interesting results and should be confirmed by the analysis of a larger, separate and prospective cohort.

In conclusion, our data prove that CCAs are heterogeneous tumors with different immunohistochemical and molecular marker profiles. The proteins of the SWI/SNF complex are lost in 35% of cases with an impact on prognosis. The core subunits of SWI/SNF, with ARID1A and PBRM-1 for small duct ICCA and ARID1a and BRM for large duct ICCA leading the way, may be possible promising predictive and therapeutic targets and worth further investigation in CCA patients.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

BJW, RB, AQ and UD designed the study, planned the study and were the primary writers. PSP, KA, MS, DS, DB and SB contributed to the study design. BJW, AQ and UD conducted and evaluated histopathological and immunohistochemical analyses. BJW conducted the FISH analysis. PSP performed statistical analysis. PSP, MS, DS, DB, and SB chose, collected and assembled the clinical data, including patient follow-up from 2000 to 2019. BJW, UD and KA chose and prepared the samples, and collected and assembled pathological data. BJW, AQ and UD performed clinical-pathological data analysis and interpretated the results related to the CCA subtype and clinical implications. BJW and UD confirm the authenticity of all raw data. All authors participated in writing, and read and approved the final manuscript.

Ethics approval and consent to participate

The present retrospective study was conducted with the approval of the Ethics Committee of the University of Cologne, utilizing clinical follow-up data that was collected retrospectively according to a standardized protocol (application 18-269). All patients gave their appropriate informed consent to the procedure.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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